MYASTHENIA GRAVIS

JOHN ALEXANDER SIMPSON

Glasgow University Department of Neurology
Institute of Neurological Sciences, Glasgow

Submitted for the Doctorate of Science
University of Edinburgh
May 1991

(C) JOHN A. SIMPSON, 1991
CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CONTENTS</td>
</tr>
<tr>
<td>2–9</td>
<td>LIST OF PUBLICATIONS ON MYASTHENIA GRAVIS</td>
</tr>
<tr>
<td>10–11</td>
<td>SUMMARY</td>
</tr>
<tr>
<td>12–19</td>
<td>INTRODUCTION AND DISCUSSION</td>
</tr>
<tr>
<td>20</td>
<td>PUBLICATIONS</td>
</tr>
</tbody>
</table>
PUBLICATIONS


(71) Simpson JA. Myasthenia gravis in pregnancy. (Submitted for publication.)

(72) Simpson JA. Recurrent intrauterine death associated with the anti-cardiolipin antibody in myasthenia gravis. (Submitted for publication.)
SUMMARY

The thesis is 72 publications describing the author's hypothesis of an autoimmune basis for myasthenia gravis (MG) and its validation. At the time of the first 24 papers many considered that MG was a syndrome rather than a disease entity, due to a biochemical disorder of the neuromuscular junction. Favoured models were a circulating "curare-like" substance released from the thymus gland, or a pre-junctional abnormality, possibly causing release of small quanta of acetylcholine at the motor nerve terminals. Endplate receptor substance was speculative. The immunological role of the thymus was unknown and autoimmunity was a new concept in medicine. The therapeutic value of thymectomy was controversial.

The controversy about thymectomy was resolved (papers 2,3) by re-analysing data separately for patients with a thymoma and the conclusions have proved definitive. Papers based on the cases reviewed in that survey led to an autoimmune hypothesis with a thymic disorder causing production of antibodies with loss of tolerance to self-tissue, mainly but not exclusively at the motor endplates of muscle. The first recognition of many associated diseases and a re-interpretation of the relationship with thyroid disorders are described, with the first evidence for a genetic predilection with alternative expression.

During the 35 years of this work the distinct nature of "carcinomatous" myasthenia has been identified, and the non-immunological congenital myasthenias defined by other workers.

A personal series of observational and theoretical papers argued against the contemporary views on the neuromuscular disorder in favour of blockade and/or damage of the putative acetylcholine receptors (AChR) at the neuromuscular junction by antibody. In the 1970s,
recognition of α-bungarotoxin as a ligand for the AChR enabled identification by other workers of the proposed antibodies and their attack site on the recently identified endplate receptors. Antibody raised against electroplaque tissue (with high concentration of AChR) caused a myasthenic model in experimental animals, supporting the proximate mechanism of my model. With colleagues (acknowledged in text) I have investigated the polyclonal nature of the immunological disorder of MG and in the final papers (submitted for publication) review evidence that MG is a limited manifestation of systemic lupus erythematosus. Clinical studies on the possible role of steroid hormones on insertion of new receptors in the post synaptic membrane had to be abandoned when I retired from medical practice.
INTRODUCTION AND DISCUSSION

This volume consists of my papers on myasthenia gravis and related syndromes written over a period of 35 years. As they cover all aspects of the disorder, a logical presentation could have been to group them under the headings used in the following paragraphs. As, however, I was working on all aspects throughout the whole period, I have decided to present the papers in chronological order to show how conceptions advanced. Much of the work is theoretical or speculative, only becoming factual as techniques and ideas evolved, notably in the field of immunology. Two advances in other areas of experimental biology made it possible to validate some of my hypothesis (44). These were the recognition that a snake venom, alpha-bungarotoxin, had a selective affinity for part of the acetylcholine receptors of the neuromuscular junction (the receptors were also hypothetical when this work started), and the chance finding that laboratory animals, used to raise antibody to acetylcholine receptors of the electroplaque of the electric eel in order to identify the protein structure, developed a neuromuscular disorder so similar to myasthenia gravis in man as to be accepted as an animal model of that disease (Patrick and Lindstrom, 1973).

For the first ten years or more, my own theory of the autoimmune basis for myasthenia gravis was disparaged because (a) it was confused with contemporary autoimmune theories with different heuristic value (6,10,18,25,44,46,54), (b) there was no statistical support for the clinical observations although they could be replicated by others with large series of cases. Since the acceptable animal model, discovered serendipitously, myasthenia gravis has been regarded as a paradigm of autoimmune disease. That being so, certain theoretical consequences (54,55,65,71) should be important for immunological theory. An
unconsidered question is the role of thymic neoplasia of a particular type and of penicillamine as "tolerance breakers" which appear to influence antireceptor autoimmunity in particular.

The chronological arrangement of papers inevitably leads to repetition, but it is hoped that this will be acceptable to the reader as each paper contains at least one new idea or fact to advance the argument. Some of these papers are the text of communications to Societies which, especially in the early days of small groups developing new sub-specialties, did not publish abstracts or communications. Nevertheless they are worth preserving as they were influential in encouraging others to accept my views on the mechanisms of myasthenia gravis. Three papers were included in my 1964 thesis for the MD (Glasgow) but are repeated here as they are essential to the understanding of the later papers. They are J Neurol Neurosurg Psychiat, 1956;19:224-231; Brain 1958;81:112-144 and Scott med J 1960;5:419-436. Unfortunately the advancing concepts and data were published mainly as chapters in books and so excluded from Index Medicus and abstracting journals. Particularly important were the chapter in the Biological Basis of Medicine (21) and the updated reviews in five editions of Walton's Disorders of Voluntary Muscle, 1964-1988. I have included the 2nd edition chapter (1969) (16) to represent the knowledge prior to alpha-bungarotoxin and the animal model, and the completely rewritten 5th edition (70) to represent the position when I retired from active work. The latter provides a bibliography to the most important work on myasthenia gravis. Additional unpublished papers (71,72) are added as they provide important new evidence supporting my contention that myasthenia gravis is closely linked with Systemic Lupus Erythematosus (SLE) rather than the so-called "organ-specific" autoimmune diseases.
I have been entirely responsible for the clinical, the electrophysiological, and the theoretical work but have had the valuable support of skilled immunologists in the laboratory immunological studies. All these contributors are acknowledged in individual papers, usually as co-authors.

The papers could be grouped under the following headings.

Clinical analysis
(3,4,9,11,12,13,19,20,23,27,29,30,37,45,46,53,57,62,67,71,72)

These papers describe the clinical features which differentiate myasthenia gravis from other myasthenic syndromes. The three clinical stages are not yet in general use, other authors preferring a classification system which I rejected at an early stage (3). The observation that in my stage III the clinical weakness may improve with effort is still unfamiliar. At first there was a widespread belief that "myasthenia" (progressive weakness on repeated contraction of skeletal muscle, with restitution after rest) was a syndrome of multiple aetiology. From the first paper I have consistently argued that a number of functional disorders of neuromuscular transmission may cause the symptoms of myasthenia but that Myasthenia Gravis is a disease entity recognizably different from other myasthenic syndromes. My electrophysiological studies on disorders of neuromuscular transmission in man are referred to in some of these papers but are bound in a volume on Clinical Electrophysiology. These include the first report of a syndrome resembling carcinomatous myasthenia but without neoplasia. In the 1960s it became clear that a myasthenic syndrome could accompany certain tumours, polymyositis, and systemic lupus erythematosus (7,14,16,37,63). My suggested mechanism for carcinomatous myasthenia is obsolete since Newsom-Davis and colleagues
(Lang et al 1981) have provided strong evidence for immunogenesis (70, p657). The belief that "congenital myasthenia" was a group of genetically determined diseases distinct from myasthenia gravis (1,16) is now confirmed by the important work of A Engel and E Lambert from the Mayo Clinic, described in 70, p654.

Type of autoimmune disorder

The status of myasthenic syndromes in polymyositis and SLE remains uncertain. Do these disorders damage the receptor surface of the neuromuscular junction inter alia? Is it a case of (true) myasthenia gravis being associated with other autoimmune disease? Is myasthenia gravis an incomplete version of SLE? Throughout these papers the latter view gradually dominates, culminating in the final paper but the decision must rest with later workers or until a definitive marker of SLE emerges. It is now certain that the abnormal antibodies in myasthenia gravis are polyclonal (54,60,64,72) and the common classification of that disease with the organ-specific autoimmune diseases has never been acceptable to this author. With collaborating immunologists I have tentatively suggested that myasthenia gravis may be one of the autoimmune diseases associated with immunodeficiency rather than hyperergy (38,41).

A strange and unexplained finding is that at least one of the (presumably non-immunogenious) congenital myasthenias is associated with reduced serum IgA. I had already noted this in one patient in 1968 but it was the report by Bundey et al (1972) of depressed levels of IgA in juvenile-onset myasthenia that alerted us to this (40,70 p635).
Genetic and other predisposing factors

My early statement that there was genetically determined predisposition for myasthenia gravis (4,9) was not supported by geneticists until there was acceptance of my suggestion that a gene activity may manifest in alternative ways. Linkage with HLA loci is now clearly established for non-thymoma myasthenia (33,34,43,64,70).

The psychological aspects (11,28) are difficult to assess - are they part of the SLE-like syndrome or are they primary triggers for an autoimmune disturbance mediated by the hypothalamus? This aspect has never been formally investigated though there is an extensive literature on hormonal influences on the thymus.

Role of the thymus

The role of the thymus is still obscure. When this work was begun the thymus was regarded as an endocrine gland associated with growth and tissue maturation. In 1960 I advocated (4) that it was an immunological organ (as distinct from Smithers (1959), White and Marshall (1962) and Goldstein and Whittingham (1966) who interpreted the histological changes as indicating immunological attack on the thymus). This was supported in the next year by Miller (1961) who first established that the thymus had an immunological function (64). My studies with Dr John Vetters (31,32) have helped to clarify the relationship between thymic histology and myasthenia but we have not been able to contribute to the nature of this involvement (22,55).

In the following thirty years there have been major advances in immunology. When the possibility of suppressor cells as well as "killer" cells and producers of antibody was first mooted the idea was derided. The roles of complement and of immune complexes and the importance of histocompatibility antigens have been recognised, the
latter indicating one possibility of the target for gene action (70). Nevertheless, the function(s) of the thymus remain debatable. At the most basic, it is difficult to suggest how surgical removal of the main regulatory organ for immunological homeostasis might reduce the severity of stage I myasthenia (23,35,47,48,55) but encourage "escape" of other autoimmune diseases (9,29). Clearly the work on the aetiology of myasthenia will not advance until thymic function and the nature of self-tolerance are better understood. If the primary causative factor is viral it is unlikely to be related to a single virus. Personal proposals to look for a retrovirus were not pursued by a collaborator. It seems more likely that one or more banal viruses activate a genetically defective thymus (54,55). Production of antibody against pathogenic antibody (anti-idiotypic) seems unlikely to be a practical procedure for therapeutic use (59). At present the main thrust of immunological research is directed to separation of the numerous anti-ACh receptor antibodies with a view to finding the important one - a project unlikely to influence clinical management.

Mechanism of transmission failure

The historical development of theories on the failure of neuromuscular transmission resulting from repetitive per-neural stimulation is fully discussed in 14,17,21. In the 1950s every author prefaced his account of myasthenia with a summary of possible biochemical disorders of the neuromuscular junction, the prime favourite being a circulating "curare-like" substance which was also capable of crossing the placental barrier to cause neonatal myasthenia (though the failure to paralyse the fetus was not discussed). My studies on neuromuscular transmission in the normal subject (not included here) and in disorders of the lower motor neurone (14)
indicated pre-junctional pathology in some myasthenic syndromes, but predominantly post-junctional disorder in myasthenia gravis, contrary to the pre-junctional model of Desmedt and the "small ACh quantum" theory of Elmqvist and his colleagues. My analyses of their data pointed to an alternative post-junctional disorder such as blockade or structural damage of ACh receptors (21) which I postulated to be caused by one or more anti-receptor antibodies. The model (26) was validated by Engel's outstanding ultramicroscopic studies using the recently discovered affinity of alpha-bungarotoxin to identify residual receptors. The relative roles of blockade, modulation, and destruction of receptors is argued by interested researchers but the general concept of antibody attack is now widely accepted.

Other researchers have shown little interest in the other aspect of my model to account for the relapsing course of myasthenia gravis and its eventual recovery if life can be maintained through stages I and II. This depends on regeneration of neuromuscular junctions (4,21,46) and particularly insertion of new receptors in the subsynaptic region (69). At the end of my clinical career I was investigating whether steroid hormones and especially androgenic steroids might promote these restorative changes.

Treatment of myasthenic syndromes

Ability to influence favourably the re-insertion of receptors into undamaged post-synaptic membrane would offer a better prospect of curing myasthenia gravis than further specification of the proximate attack mechanism and better understanding of the nature of disordered immune tolerance (54) could lead to better immunoregulating drugs, as distinct from immunosuppressive drugs which may cause greater morbidity than the disease itself (69). There is reasonable hope that
both of these developments will soon be reported. Meanwhile the treatment depends firstly on clear analysis of myasthenic syndromes for their mechanisms of loss of "safety factor" for transmission and then on the informed prescription of known drugs (5, 8, 16, 17, 24, 42, 48, 50, 57, 66, 69, 70). There is still a lot to learn about the myasthenic syndromes but the broad outline is now clear.
BUNDEY S, DONIACH D, SOOTHILL JT. (1972) Immunological studies in patients with juvenile-onset myasthenia gravis and in their relatives. Clinical and Experimental Immunology, 11, 321-332.


SMITHERS DW. (1959) Tumours of the thyroid gland in relation to some general concepts of neoplasia. Journal of the Faculty of Radiology, 10, 3-16.


Numerical references are to the list of personal papers in this volume.

20
BENIGN CONGENITAL MYOPATHY WITH MYASTHENIC FEATURES

BY
*JOHN N. WALTON, †NORMAN GESCHWIND, and ‡J. A. SIMPSON

From the Neurological Research Unit and the Biochemical Department of the Institute of Neurology, the National Hospital, Queen Square, London

It has been increasingly apparent in recent years that in addition to cases which fall into recognizable categories of muscle disease, a number of less common disorders occur from time to time which do not correspond to the accepted descriptions. Some of these appear to be metabolic in origin and can be elucidated, at least in part, by modern methods of investigation (McArdle, 1951) while others seem to fall into a borderland of either myopathy or myasthenia gravis. A case of the latter type is described and discussed below.

Case History

R. M. (N. H. case 4838), a female telephonist, was born in 1919; her mother was well during pregnancy, labour was normal, and the baby thrived well during the neonatal period. She seemed normally active and lively and sat up at 7 months; at 9 months she tipped over her pram by jumping vigorously and sustained a cut chin but no other injury. Shortly after this episode her mother noticed that the limbs and body tended to flop limply when she was lifted and the head lolled as if she were totally unable to support it. The limbs were unusually flexible, like those of a rag doll, and she lay in her pram almost immobile, without kicking her legs or waving her arms. Nevertheless, at the age of a year she was able to crawl a short distance when put on the floor; her crawling improved steadily, although her limbs remained rather loose and "flappy". However, this was her only means of locomotion until she reached the age of 5 years, when she began to pull herself up with her arms and to walk around the furniture. When she was 5½ years old she was able to walk a few paces unaided and the limbs, though somewhat weak, were not so loose. The patient's mother suggested that at this age she was very little stronger but had learned to overcome her weakness. At the age of 7 she was able to go to a school for disabled children and could walk about 20 yards, but would then have to rest for about a minute in order to regain her strength. She always tended to tire throughout the day and was much weaker in the evening than on waking. She had partial difficulty in climbing stairs or in rising from a low chair and showed a considerable tendency to trip and fall, after which she would find it difficult to get up again. Apart from her muscular disability the patient developed normally; the menarche occurred at 13 years and she had menstruated normally since.

As the patient grew older she was gradually able to extend her activities, although her muscular weakness was virtually unchanged. She attempted numerous occupations and finally worked (from 1948) for two years as a telephonist, but was compelled to give up this post because of her muscular disability; since 1950 she had helped her mother in the home. The patient had two sisters, one older and one younger than herself, both of whom were well, and there was no history of muscular disease in the family.

The patient was first admitted to the National Hospital in 1941 under the care of Dr. E. A. Carmichael, when generalized muscular hypotonia of moderate degree and diffuse atrophy of proximal limb muscles were discovered. She showed an accentuated lumbar lordosis and tended to waddle when she walked. There was also bilateral ptosis and weakness of the upper facial musculature. A diagnosis of atypical myotonia congenita was made. She was readmitted on several occasions during the ensuing years, when her symptoms and physical signs were virtually unchanged. In 1944 she was seen by Dr. Gordon Holmes, who suggested that she was suffering from an unidentified defect of muscle metabolism. In 1948 Sir Charles Symonds could demonstrate no myasthenic tiring of the eyelids, although there was pathological fatiguability of the deltoids; he agreed that the patient was suffering from an unusual metabolic disorder of muscle. On at least three occasions the effect of an intramuscular injection of 1.5 mg. prostigmine was tested. Each time the drug made the patient feel "queer" and dizzy, but nevertheless it produced some subjective improvement in muscular power, though there was little objective change. Twice the improvement in strength appeared to persist for two or three days after the injection and the drug was given by mouth in a dosage of up to 90 mg. daily. On each occasion there was a marked subjective improvement which, however, passed off after between one and two weeks and the treatment was discontinued. A similar improvement appeared to follow ephedrine, gr. ½, three times daily; the effect of

*King's College (Newcastle) Travelling Fellow in Medicine; aided by a grant from the Muscular Dystrophy Associations of America, Inc.
†Research Fellow, National Institutes of Health, U.S. Public Health Service, 1953-55.
‡Clinical Research Fellow of the Medical Research Council.
this drug was, in the patient's view, sustained, and she had been taking it continuously for several years. In 1952 the patient was admitted to the Clinical Research Unit at Guy's Hospital and was investigated by Dr. B. McArdle; the results of these studies are given below. At that time she seemed to show doubtful improvement on treatment with oral potassium (dosage 1 g. KCl t.d.s.) and had continued to take this remedy, as well as ephedrine, until she returned to the National Hospital in 1955. Assessment of therapeutic results in this patient was always difficult as she was a suggestive, nervous individual, who suffered numerous episodes of emotional instability, exaggerated by periods of conflict with her mother.

On readmission under the care of Dr. Carmichael on May 6, 1955, the patient's symptoms were virtually unchanged from those she had expressed on her previous admissions, save for the fact that she had experienced occasional dysphagia when tired. However, she was still able to do housework and to walk considerable distances (with many rest periods). She felt that the muscles of her legs seemed to "let her down" less often than they had done some years before, but there had been no striking change in the condition of the limbs for many years.

On examination (Fig. 1) the patient was thin and slightly built and walked with a distinct waddle and with a considerable increase in the lumbar lordosis. There was bilateral ptosis, with impaired ocular movement upwards, but not laterally or downwards; the ocular axes were parallel throughout and there was no diplopia. Both orbiculares oculi were strikingly weak, but the lower facial muscles, masseters, and temporales were strong. Palatal and pharyngeal movements were normal and the tongue showed no atrophy or fasciculation. The patient had a curiously long "swan-like" neck, but the sternomastoids were large and powerful as were the posterior cervical muscles; because of the ptosis she tended to hold her head backwards. There was undoubtedly atrophy of the sacrospinalis and other posterior spinal muscles, but those of the abdominal wall were good. The limbs were generally thin, particularly proximally, and there seemed to be a general moderate atrophy, with considerable weakness, of all girdle and proximal muscles in the upper and lower limbs. The extensors of the wrist and fingers were also weak; the finger flexors were stronger but, nevertheless, considerably weaker than would have been expected in a normal individual of the patient's age. In the lower limbs the anterior tibial and peroneal groups showed the same atrophy and weakness as the proximal muscles, although the calf muscles were more powerful. All deep tendon reflexes were present, though depressed, and direct muscle excitability was normal; the abdominal reflexes were brisk, the plantar responses flexor. The secondary sexual characteristics were normally developed and there was no abnormality to be detected on examination of the chest, abdomen, and cardiovascular system.

Electrodiagnosis.—In 1948, an intensity-duration curve from the right deltoid was normal; Dr. W. A. Cobb recorded an electromyogram from the same muscle, using a concentric needle electrode. He reported that there was no spontaneous activity and on voluntary contraction the motor unit action potentials were normal in amplitude and duration. In June, 1948, Dr. P. Merton found no decrement in the amplitude of a muscle action potential recorded with a surface electrode on the hypothenar eminence, on supra-maximal stimulation of the ulnar nerve at 3 per second.

Muscle Biopsy.—A specimen of muscle was removed from the right deltoid in 1950. There was no increase in perimysial connective tissue nor was there any accumulation of fat between the fibres. Some of the muscle fibres were slightly enlarged, measuring 85 μ in diameter, while very occasional atrophic fibres were seen. In a few fibres sarcolemmal nuclei had migrated into the substance of the fibre and one or two short chains of nuclei, subsarcolemmal in position, were seen. No segmental necrosis of fibres was evident and there was no cellular infiltration or evidence of muscle fibre regeneration. A number of nerve filaments were present in the section and appeared to be normal; two muscle spindles of normal appearance were also observed. Hence the histological changes were minimal. Although perhaps compatible with a mild myopathic disorder they were
much less than would have been expected considering the length of history and the comparative severity of the patient's weakness.

Dr. J. N. Cumings reported that the potassium content of the muscle was 11 g. % by dry weight, a normal figure.

Metabolic Studies.—Studies carried out in June, 1948, by Dr. J. N. Cumings yielded the following results:—

<table>
<thead>
<tr>
<th>CREATINE AND CREATININE EXCRETION</th>
<th>Day</th>
<th>Urinary Volume (ml)</th>
<th>Creatinine (g)</th>
<th>Creatine (g)</th>
<th>Inorganic Phosphate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>900</td>
<td>0.71</td>
<td>0.18</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>830</td>
<td>0.84</td>
<td>0.18</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>940</td>
<td>0.86</td>
<td>0.12</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>763</td>
<td>0.70</td>
<td>0.16</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>770</td>
<td>0.75</td>
<td>0.04</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>940</td>
<td>0.87</td>
<td>0.08</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

Ephedrine medication and all other medicinal treatment was discontinued from the third day of this test; a creatine tolerance test was performed on the fourth day and gave results as follows:—

**Creatine Tolerance Test.**—Urinary and blood estimations were carried out at the stated times before and after the oral ingestion of 1 g. of creatine.

<table>
<thead>
<tr>
<th>RESULTS IN URINE</th>
<th>Time</th>
<th>Volume (ml)</th>
<th>Creatinine (g)</th>
<th>Creatine (g)</th>
<th>Inorganic Phosphate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>35</td>
<td>0.07</td>
<td>0.01</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>2 hours</td>
<td>121</td>
<td>0.10</td>
<td>0.04</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS IN BLOOD**

<table>
<thead>
<tr>
<th>Time</th>
<th>Creatinine (mg. %)</th>
<th>Creatine (mg. %)</th>
<th>Inorganic Phosphate (mg. %)</th>
<th>Potassium (mg. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>1.0</td>
<td>0.38</td>
<td>4.3</td>
<td>19.4</td>
</tr>
<tr>
<td>1 hour</td>
<td>1.12</td>
<td>1.63</td>
<td>4.7</td>
<td>20.9</td>
</tr>
<tr>
<td>2 hours</td>
<td>1.25</td>
<td>0.87</td>
<td>4.7</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Response to Insulin and Glucose.—The patient was given 100 g. glucose by mouth and 25 units of insulin subcutaneously. Before the experiment was begun the serum potassium level was 20.0 mg./100 ml., the serum inorganic phosphate level 4.5 mg./100 ml., and the blood sugar level 100 mg./100 ml. Thirty minutes after the injection, the serum potassium level was 18.3 mg./100 ml., the inorganic phosphate level was unchanged, and the blood sugar level was 168 mg./100 ml.

Dr. Cumings remarked that the urinary creatine was low and almost absent when the patient was taking no drugs, while the creatine tolerance and the potassium response to insulin and glucose were all normal.

Metabolic Activity of Forearm Muscles.—In January, 1952, the following studies were carried out by Dr. B. McArdle in the Clinical Research Unit at Guy's Hospital, London. The patient had received no drugs for about a week before the test. Blood was taken from the left antecubital vein before the test and again following the release of an occluding cuff after a period of ischaemic work by the forearm muscles. The work consisted in raising and lowering (56 pulls) a 5 kg. weight by means of a gripping movement on an ergometer. A wrist cuff inflated to 200 mm. Hg ensured that blood taken from the antecubital vein came only from the forearm muscles. The results of this test were as follows:—

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood Pyruvate (mg. %)</th>
<th>Blood Lactate (mg. %)</th>
<th>Serum Potassium (mg. %)</th>
<th>Serum Sodium (mg. %)</th>
<th>Serum Magnesium (mg. %)</th>
<th>Serum Inorganic Phosphate (mg. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.63</td>
<td>60</td>
<td>152</td>
<td>325</td>
<td>223</td>
<td>3.57</td>
</tr>
<tr>
<td>30 sec. after release of cuff</td>
<td>1.26</td>
<td>31.3</td>
<td>164</td>
<td>340</td>
<td>2.50</td>
<td>3.67</td>
</tr>
<tr>
<td>2 min. after release of cuff</td>
<td>0.91</td>
<td>29.4</td>
<td>156</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6 min. after release of cuff</td>
<td>1.13</td>
<td>24.2</td>
<td>148</td>
<td>328</td>
<td>—</td>
<td>3.28</td>
</tr>
<tr>
<td>10 min. after release of cuff</td>
<td>1.01</td>
<td>16.3</td>
<td>147</td>
<td>330</td>
<td>—</td>
<td>3.28</td>
</tr>
<tr>
<td>20 min. after release of cuff</td>
<td>0.86</td>
<td>12.7</td>
<td>145</td>
<td>339</td>
<td>—</td>
<td>3.18</td>
</tr>
</tbody>
</table>

Dr. McArdle remarked that all of these results were within normal limits.

Other Investigations.—Haemoglobin was 100% (14-8 g./100 ml.); W. B. C. 4,000/c.mm. (63% polymorphonuclears, 31% lymphocytes).

The F. S. R. was 8 mm. in one hour (Westergren). The Wassermann and Kahn reactions were negative.

The serum protein-bound iodine was 34%. The total serum proteins were 7.9 g./100 ml. (albumin 4-4, globulin 3-5).

A radiograph of the chest showed a slight dorsal scoliosis, convex to the right. The lung fields and heart were normal and the thymus did not appear to be enlarged. An electrocardiogram was normal, and a basal metabolic rate was minus 11%. The urinary 17-keto-steroid excretion was 4-9 mg. in 24 hours.

Discussion and Experiments

It was clear from the information recorded that this patient fitted no clearly recognizable form of muscle disease as previously described. The non-progressive nature of the disease and the diffuse rather than selective distribution of muscular wasting and weakness made it apparent that she was not suffering from any of the common categories of muscular dystrophy. Furthermore, the pathological changes in the muscle were far less than would have been expected in a long-standing muscular dystrophy or polymyositis. She showed many characteristics reminiscent of the benign congenital myopathy or myopathic form of amyotonia congenita as described by Batten (1910) and by Aldren Turner (1940, 1949). Because of the general reduction in size of the skeletal muscles, the resemblance to Krabbe's (1946) "congenital universal muscular hypoplasia" was even more striking, since in Turner's cases the muscular wasting and weakness affected selectively the proximal muscles of the limbs and the face was not
involved. Despite these discrepancies, the results of a recent follow-up of cases of amyotonia congenita by one of us (Walton, 1956) have suggested that the disorders described by Krabbé and Turner may be the same. However, in this case there were additional unusual features: the variability of her weakness, the fatiguability, which had been a consistent feature, and the apparent response to ephedrine, suggested that there might be some defect in neuromuscular transmission akin to that seen in myasthenia gravis. It was evident that the patient was not suffering from the latter disease, in view of the diffuse muscular wasting and the failure to show a sustained response to prostigmine therapy. Rowland (1955) has recently described a number of patients who appeared to be suffering from myasthenia gravis but who showed either a very variable response to prostigmine or none at all. However, from his descriptions it seems likely that some of his cases were examples of polymyositis, a condition which may show temporary improvement with this drug (Eaton, 1954). It is clear from the clinical and pathological findings that our case was not suffering from polymyositis. An alternative possibility seemed to be that she was suffering from an unidentified disorder of muscle metabolism, although Dr. McArdle's results indicated that there was no serious defect in carbohydrate breakdown and utilization in the muscle.

In view of the apparent improvement which the patient had shown on potassium therapy it was decided to investigate the effect upon her muscular power of alterations in the serum potassium level. It was recognized that her symptoms were not like those of familial periodic paralysis, nor were they characteristic of those noted in chronic potassium deficiency (as in potassium-losing nephritis). It was also appreciated that the serum potassium level does not necessarily give a faithful indication of the intramuscular concentration of this ion. Nevertheless, since potassium is recognized to be one of the most freely diffusible ions, it was felt that if the patient's condition were due to a deficiency of intramuscular potassium, she would become significantly weaker if the serum potassium level were lowered. Another possibility seemed to be that she might have some anomaly whereby her muscles required a higher than normal concentration of potassium in order to function properly. In this case, too, a fall in extracellular potassium would increase her weakness.

It was also decided to repeat the electromyogram and to study the effects upon the muscle action potential of repetitive nerve stimulation, first under normal conditions and secondly after increasing doses of intravenous decamethonium iodide. Harvey and Masland (1941) found that in patients with myasthenia gravis, if the muscle action potential was recorded from the skin overlaying a weak muscle during repetitive supramaximal stimulation of its nerve of supply at a rate of 3 per second, the potential often showed a rapid decrease in amplitude. This was suggested as a diagnostic test, and it has been conventional to take the recording from the hypotberal eminence during stimulation of the ulnar nerve at the elbow. If this muscle group is not clinically affected, however, another must be chosen. Recently, Churchill-Davidson and Richardson (1952) have shown that in the normal individual an intravenous injection of 2 mg. of decamethonium iodide will give a significant fall in amplitude of the motor unit potential recorded from the hypotberal eminence during stimulation of the ulnar nerve at a frequency of 10 per second. Patients with myasthenia gravis, however, in whom the hypotberal muscles were not weakened by the disease, were remarkably resistant to this drug and could often take 3 mg. or more without a significant decrement in the action potential.

Clearly it also seemed important in this patient to assess, under the conditions of a controlled experiment, the effect of ephedrine, prostigmine, tensilon, and potassium upon the muscular weakness. It was decided in addition to study the effects of intravenous caffeine and of calcium, in view of the direct stimulant effect which these substances appear to have upon the muscle fibre.

Before carrying out these experiments all treatment was stopped and the patient's muscular power was assessed three times daily by one of us, at 9.30 a.m., 1 p.m., and 5 p.m., in order to see whether there was any significant variation depending upon the time of day. The power of individual muscle groups was assessed clinically and strength of grip was measured with a spring dynamometer, the value recorded being taken as the average of three maximal grips with each of the two hands. It was discovered that the latter test gave a satisfactory indication of general muscular power. Another useful test was to measure the time for which both arms could be held out horizontally in front of the body with the patient sitting in bed; the end-point was taken at the time when one hand touched the bed-clothes. Using these methods it was found that after five days in hospital, with approximately the same amount of activity carried out each day, consistent values for strength of grip and for holding out the arms were obtained from day to day. Each day there was a consistent slight decrease in these readings as the day advanced; for this reason it
was decided to carry out all experiments at approximately the same time in the mornings. All chemical estimations were carried out by one of us (N. G.) using a standard technique. The assessments of muscular power were made by J. N. W. and electrodiagnostic tests were carried out by J. A. S.

Experiment I: Lowering of Serum Potassium Level.—On May 20, 1955, the patient was starved and 5 ml. of blood was taken from the right antecubital vein at 9 a.m.; the serum potassium level, as estimated with a flame photometer, was 4.4 mEq./litre (17.2 mg./100 ml.). At 9.25 a.m. 25 units of insulin were given subcutaneously. At 10 a.m. muscular power was unaltered, but the serum potassium level was 4.1 mEq./litre (16.0 mg./100 ml.). Hence the fall in serum potassium level produced by this technique was inadequate.

On May 23, 1955, after a large breakfast, the serum potassium level was estimated at 9.10 a.m. to be 4.0 mEq./litre (15.6 mg./100 ml.). At 9.20 a.m., and again at 9.35 a.m. and 9.50 a.m. the patient was given 15 g. sodium bicarbonate in 2 oz. water. At 10.30 a.m. the patient felt somewhat tired and nauseated but there was no objective change in muscle power. At 1.15 p.m. the serum potassium level was 3.12 mEq./litre (12.2 mg./100 ml.) and 1 ml. of 1 in 1,000 adrenaline was administered subcutaneously. At 2 p.m. the serum potassium level had fallen to 3.0 mEq./litre (11.7 mg./100 ml.) but there was still no significant change in muscular power. This technique for lowering the serum potassium level will be reported in detail by one of us (N. G.) in a subsequent communication.

Experiment II: Electromyography and Effect of Intravenous Tension and Ephedrine.—On May 24, 1955, at 9.5 a.m. the patient was given 150 g. glucose orally and at 9.25 a.m. 25 units of insulin were given subcutaneously. At 10 a.m. muscular power was unaltered, but the serum potassium level was 4.1 mEq./litre (16.0 mg./100 ml.). Hence the fall in serum potassium level produced by this technique was inadequate.

At 10.30 a.m., the patient felt somewhat tired and nauseated but there was no objective change in muscle power. At 1.15 p.m. the serum potassium level was 3.12 mEq./litre (12.2 mg./100 ml.) and 1 ml. of 1 in 1,000 adrenaline was administered subcutaneously. At 2 p.m. the serum potassium level had fallen to 3.0 mEq./litre (11.7 mg./100 ml.) but there was still no significant change in muscular power. This technique for lowering the serum potassium level will be reported in detail by one of us (N. G.) in a subsequent communication.

Experiment III: Supramaximal Stimulation of Ulnar Nerve before and after Injection of Decamethonium Iodide.—On June 4, 1955, a surface electrode was applied to the left hypothenar eminence (with the indifferent electrode on the fifth finger), and at 10 a.m. recording of the action potential produced by supramaximal stimulation of the left ulnar nerve at the elbow was begun. With a stimulation frequency of 2 per second there was no significant decrease in amplitude of the motor unit potential over a 10-minute period. Stimulation was then discontinued but was restarted at 10.20 a.m. At 10.38 a.m. 1 mg. decamethonium iodide was injected over a two-minute period. Immediately the patient felt faint and dizzy (as after "tension"), her ptosis increased considerably and she developed diplopia, but the action potential of the hypothenar muscles was unchanged. At 10.44 a.m. and at 10.49 a.m. two further injections, each of 0.5 mg., were given; the patient felt subjectively weaker but there was no change in the action potential. A further injection of 0.5 mg. was given at 10.54 a.m. (total 2.5 mg.); at 10.59 the action potential showed a 13% decrease in amplitude; the patient felt weaker, was apprehensive, and refused to have further injections.

The original amplitude of the action potential was restored by 11.25 a.m. With this dose of decamethonium, the action potential of a normal subject would decrease in amplitude by more than 50%. The results of this experiment are recorded graphically in Fig. 2, using the same ordinates as those utilized by Churchill-Davidson and Richardson (1952).

Experiment IV: Therapeutic Trials.—Five substances...
were used, namely, ephedrine, calcium, potassium, caffeine, and prostigmine.

Ephedrine.—For a period of seven days from May 25, 1955, the patient was given tablets four times daily; for a part of this time the tablets were ephedrine hydrochloride (gr. ½), and for the remainder nicotinamide 25 mg. (which looked identical). This trial was designed by J. A. S. so that neither the observer testing muscular power (J. N. W.), the patient, nor the ward nurses were aware which tablet was being given at any one time, nor when the treatment was changed. At the end of this period it was clear that during the three-day period of treatment with ephedrine the patient was both subjectively and objectively stronger than when she was receiving nicotinamide.

Calcium.—On successive days the patient received an intravenous infusion of 300 ml. of fluid over a two-hour period. One of these infusions consisted of 500 mg. of calcium (as the gluconate) in normal saline while the other was saline alone. The observer concerned with the measurement of the patient’s muscular power was not aware which infusion was being administered. After each infusion the patient claimed to be considerably stronger and showed a moderate increase in power as recorded dynamometrically and by holding the arms outstretched.

Potassium.—Over a seven-day period, from June 5, 1955, the patient was given four times a day 1 oz. of an orange-flavoured preparation. For a part of this time the preparation contained 2 g., potassium citrate in each ounce and for the remainder sodium citrate. As with the trial of ephedrine neither the patient nor the observer was aware which remedy was being given at any one time nor when the change-over occurred. Throughout this period the patient’s condition remained unchanged; neither substance produced a significant change in muscular power.

Caffeine.—On June 12, 1955, the patient received three intravenous injections of comparable volume and appearance at intervals of one hour. One was caffeine sodium benzoate, 0.5 g., another ephedrine hydrochloride, gr. ½, and the other sterile saline. The injections were given by J. A. S. and neither the patient nor the clinical examiner (J. N. W.) was aware which injection was being given. It was discovered that the injection of saline had no effect, but both the ephedrine and the caffeine produced a distinct subjective and objective improvement in muscular strength of comparable degree.

Prostigmine.—On June 13, 1955, it was decided to study the effects of long-term oral prostigmine therapy, despite the fact that this treatment had proved ineffective in the past. Accordingly therapy with 15 mg. tablets of prostigmine four times daily was instituted, but was changed, at a time unknown to the patient and observer, to an inert tablet of identical appearance. This trial was continued over a five-day period. There was no doubt that both subjectively and objectively the patient was considerably stronger while on prostigmine. Indeed, she recorded higher dynamometer readings and was able to hold out her arms longer than at any time since her admission to hospital. During the next five days prostigmine therapy was alternated with pyridostigmine in equivalent dosage, but there was little difference in the effect of the two remedies, although overall improvement was maintained.

Subsequent Progress.—As a result of the findings in the experiments outlined above it was decided to give the patient combined treatment with prostigmine, one tablet of 15 mg., four times daily, and ephedrine, one tablet of gr. ½, also four times a day. For three days the improvement in the patient’s strength was sustained; she moved about the ward more easily, could lift objects of considerable weight, and climbed three flights of stairs relatively briskly. Unfortunately she then developed follicular tonsillitis with a high fever and was compelled to take to her bed. This infection resolved within a few days but left the patient depressed, tearful and weak, though no weaker than she had been on admission. She asked to be discharged, feeling that she would pick up more quickly at home; she therefore left hospital, taking both ephedrine and prostigmine, on June 23, 1955. On discharge, physical examination revealed no significant change from her state on admission.

The patient was readmitted to hospital on August 9, 1955. After returning home she had improved quickly and soon felt that her strength had returned to what it was after beginning combined prostigmine and ephedrine therapy in hospital. This improvement continued for three weeks but then she began to feel unaccountably weaker. Although she had experienced some fluctuation in her muscular strength as a result of emotional disturbances, the present deterioration was quite different, being steadily progressive. In addition, she became short of breath and could no longer lie down in bed at night, or walk more than a few paces because of dyspnoea. On examination on admission the patient was unable to walk more than a few paces with considerable effort and she was quite unable to negotiate stairs. Her resting pulse rate was 120 per minute, but the heart and chest showed no abnormality on examination. She was severely breathless, with very poor abdominal and thoracic movement and striking activity of the accessory muscles of respiration, including the sternomastoidis and scaleni. Her postis and facial weakness had not increased since her previous admission but her strength of grip was strikingly weak and she was quite unable to lift her arms from the bed. Her vital capacity could not be measured as she said she was unable to blow into the machine. An electrocardiogram was normal but for the tachycardia. Although an accurate assessment of her physical state was made difficult by emotional factors, there was no doubt that she showed persistent tachycardia and severe weakness of limb, trunk, and respiratory muscles, like that seen in severe myasthenia gravis. Intravenous tension and intramuscular prostigmine therapy produced no improvement in her condition; indeed, she insisted that these remedies made her worse. After each, some muscular fasciculation was seen but there were no abdominal symptoms. Accordingly, all therapy was
discontinued and over the next two weeks there was a gradual improvement in her respiration and in the power of the limbs; her pulse rate returned to 80 per minute. The patient was finally discharged from hospital on September 16, 1955, receiving only ephedrine gr. 1/4 four times daily. Her condition was virtually the same as when she was first admitted in May, 1955.

Conclusions

There seems to be little doubt whatever that this patient was suffering from a relatively benign, probably congenital, non-progressive myopathy, showing certain features reminiscent of myasthenia gravis. Dr. McArdle's investigations revealed no apparent defect in carbohydrate metabolism, while we were unable to produce any evidence to indicate that alterations in serum potassium affected her muscular condition. Save for the "myasthenic" features, her condition corresponds closely to the "benign congenital myopathy" or myopathic form of amyotonia congenita described by Batten (1910) and Aldren Turner (1940, 1949). However, Turner did not describe any fatiguability or apparent response to ephedrine or prostigmine therapy in his cases, nor were these features seen in the other cases of this type which were reviewed recently by one of us (Walton, 1956). On the other hand, it is evident that this patient was not suffering from true myasthenia gravis, in view of the failure to respond to tension, as well as the atypical clinical picture. Nevertheless, it must be admitted that she showed a considerable resistance to decamethonium iodide, while the sustained improvement on ephedrine therapy and the temporary response to prostigmine were suggestive of the latter disease. The apparent increase in strength following an injection of caffeine sodium benzoate was of doubtful significance.

Of great interest was the striking increase in weakness, particularly of the respiratory muscles, after prostigmine therapy had been in progress for some weeks. The associated tachycardia was suggestive of vagal inhibition but it is difficult to see how moderate dosage of prostigmine, which would be expected to give a bradycardia, could produce this effect. It seems most probable that the weakness could be attributed to this drug, despite the absence of other side-effects, and that the temporary improvement, perhaps due to its anti-cholinesterase effect, was subsequently overcome by a persistent depolarizing effect which appeared to be cumulative. It is well recognized that even patients with myasthenia gravis may become weak as a result of excessive dosage of this drug (Rowland, Korengold, Jaffe, Berg, and Shy, 1955), but the dosage administered to our patient was only 60 mg. daily which could not be expected to produce such an effect in an individual with true myasthenia. It is also difficult to understand the action of ephedrine in this case. Unlike adrenaline, this drug does not cause glycogen breakdown, hyperglycaemia, or a fall in the serum potassium level. We may ask whether its effect is unrelated to the energy metabolism of muscle and whether it may have a direct effect upon the muscle membrane or perhaps at the motor end-plate. We have no evidence which could help in deciding this problem.

The paradoxical responses which this patient showed to ephedrine, prostigmine, decamethonium, and "tensilon" suggest that in her case there may be some hitherto unrecognized defect in the muscle fibre and/or its end-plate or membrane. We can think of no better description for the type of "benign congenital myopathy with myasthenic features" while recognizing that we do not understand the essential nature of her disorder.

Although this patient, so far as we are aware, shows features which are unique, there is no doubt that other cases showing a resemblance to this clinical picture are seen from time to time. One of us (J. N. W.) in a previous communication (Walton and Nattrass, 1954) has referred to a number of cases of "myasthenic myopathy". This term is probably unsatisfactory, as it could be taken to refer to the irreversible muscular weakness and atrophy which may develop in the limb or ocular muscles of certain long-standing cases of myasthenia gravis. One of us (J. A. S.) in a recent study of a large series of cases of the latter condition has come to feel that such changes occur in a not insignificant proportion of cases and may follow a recognizable pattern, particularly in the limb muscles. However, the three cases briefly referred to by Walton and Nattrass (1954) were not of this type; rather, they were individuals with a long-standing weakness and atrophy of girdle and limb muscles who yet showed a somewhat phasic course and a definite, though sometimes temporary, response to ephedrine and/or prostigmine. Similar patients with a clinical picture like a combination of muscular dystrophy and myasthenia gravis have been reported by Laruelle and Massion-Verniory (1937), by Jezkova and Sachs (1939) and by Hosotte (1951). Hosotte's case, however, may have been one of true myasthenia gravis with eventual myopathy. In none of the cases mentioned by Walton and Nattrass did the condition begin so soon after birth as in the patient described in the present report. It is of considerable interest that one of these patients, shortly to be reported by Griffin, Nattrass, and Pask (1956), was given increasing doses of prostigmine with apparent improvement in the power of the limbs, but
with eventual respiratory paralysis, necessitating management with intermittent positive-pressure respiration. He was subsequently subjected to thymectomy with dramatic improvement.

It must be concluded that there exist a number of obscure disorders falling into the borderland of both myopathy and myasthenia gravis, of which the present case is a striking example. We have at present, however, no information to indicate the nature of the basic muscular defect in such individuals.

Summary

The case is reported of a woman who developed muscular weakness and hypotonia in the first year of life; she has shown subsequently persistent though non-progressive weakness, with moderate diffuse atrophy of the upper facial, trunk, and limb muscles. Her weakness has always become worse after exertion and she has had slight dysphagia but no diplopia.

Extensive metabolic, electrophysiological, and therapeutic experiments have revealed no defect in carbohydrate utilization or in potassium metabolism. She is more resistant to decamethionum iodide than the normal individual and shows improvement on ephedrine therapy but none following tension. Prostigmine produces definite improvement in muscular power, but if continued indefinitely in moderate dosage it appears to produce an increase in weakness, particularly of the respiratory muscles. A muscle biopsy revealed only slight, indefinite changes compatible with a myopathic disorder.

It is suggested that this condition falls into a borderland of myopathy and myasthenia and that it should be styled "benign congenital myopathy with myasthenic features". It was not possible to determine the nature of the biochemical or other defect in the muscle fibre and/or its end-plate or membrane which was responsible for this patient's condition.

We wish to thank Dr. E. A. Carmichael for permission to report this case and for his encouragement and advice. We are also grateful to Dr. J. N. Cumings, Dr. B. McArdle, Dr. W. A. Cobb, and Dr. P. A. Merton for permission to quote their findings. Fig. 1 was prepared in the Department of Photography, the National Hospital, Queen Square, Fig. 2 in the Gardiner Institute of Medicine, University of Glasgow.

References

Dr. John A. Simpson (Gardiner Institute of Medicine, University of Glasgow):

The Value of Thymectomy

Though seventeen years have passed since Blalock re-awakened interest in the role of thymectomy in myasthenia gravis, the value of the operation is still debated. Dr. E. A. Carmichael suggested that I should make an independent assessment of the National Hospital series. Sir Geoffrey Keynes invited me to extend the survey to include all patients operated on by him at New End and St. Bartholomew's Hospitals. As a control series those patients attending the National Hospital since 1934 with a diagnosis of myasthenia gravis were reviewed.

A more complete account will be published elsewhere.
The diagnosis was made by the staff of the National Hospital and by other members of the Association of British Neurologists. I examined the majority of the survivors of both series; a few patients were questioned by post. I have adopted the role of devil's advocate to avoid the chance of introducing a bias favourable to the operation. This should be borne in mind when assessing the figures to be presented as they deliberately show the operation in what I believe to be the worst light. Patients were classified at follow-up examination according to the following criteria which are based on those used by Ross (1952). The essential feature is an assessment of change of status from the pre-operative state or the state on first admission to hospital in the control cases. Hence a patient with little disability does not qualify for a high category if this state is not significantly better than at the datum point. In case of doubt a patient was assigned to the lower category. It will be seen that any bias has been deliberately applied in a sense adverse to the operation.

Categories.—A. Full working life with no restrictions. No Prostigmin. No subjective weakness (small degree of permanent objective weakness permitted).
Markedly different from pre-operative state.
B. Full life; minor symptoms not requiring Prostigmin or completely controlled by no more than 60 mg. daily.
Significantly better.
C. Full life with few restrictions. (a) demonstrable myasthenia but not requiring Prostigmin; (b) still requiring drug but at least 40% less than before and with improved response.
D. (a) Improved but on same or greater dosage. (b) Unimproved—irrespective of dosage.
(c) Worse.

Altogether 404 cases were available for study. Of the 270 female cases 11% had thymic tumours, and 13% of the 134 males. The higher age of onset and poor prognosis of these patients was confirmed and they will not be further considered here. The non-tumour cases (241 females and 116 males) showed a remarkably "normal" distribution curve for the age of onset which does not suggest that myasthenia gravis was diagnosed in a heterogeneous collection of unrelated diseases.

The operated series (182 females, 76 males) showed certain differences from the control series (59 females, 40 males). The former contained a preponderance of females at all age groups, the sex ratio varying from 4:5:1 in cases starting in the first decade to 1:1:1 in cases starting in the fifth decade. In the control series the sex ratio was even except in the 20–40 age group where females were in excess, and in the sixth decade where males outnumbered females. The two series were comparable for patients starting in the third decade and so the results of the whole comparison have been checked in this group but otherwise it has been thought advisable to compare the sexes separately. There was a statistically significant difference in the mean age at onset between the operated and not-operated series (the latter being nine years older at onset in both sexes). The standard error of the mean of the un-operated series was, however, much greater than that of the operated series. Possibly the early history of the thymectomy series has been more fully documented or the size of the sample is too small in the not-operated series. The mean survival from the first symptom was the same in the two series—females 13 and 14-7 years, males 12.5 and 12-4 years (the operated series is the first figure).

Results
The percentage of each series in each category at follow-up is shown in Table I. Two

<table>
<thead>
<tr>
<th>Category</th>
<th>F%</th>
<th>M%</th>
<th>F%</th>
<th>M%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+B</td>
<td>20.3</td>
<td>22.5</td>
<td>34.6</td>
<td>30.3</td>
</tr>
<tr>
<td>C</td>
<td>13.5</td>
<td>25.0</td>
<td>19.2</td>
<td>19.7</td>
</tr>
<tr>
<td>Data incomplete</td>
<td>13.3</td>
<td>22.5</td>
<td>7.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>28.8</td>
<td>20.0</td>
<td>7.7</td>
<td>11.8</td>
</tr>
<tr>
<td>All deaths*</td>
<td>35.6</td>
<td>22.5</td>
<td>17.0</td>
<td>21.1</td>
</tr>
</tbody>
</table>

*Including post-operative death or deaths from unrelated causes.

differences are apparent between the operated and not-operated series: (i) in each sex all survival categories are increased and the deaths from myasthenia are reduced, (ii) there is no
marked difference between the sexes in the operated series. The significance of these conclusions is tested in Tables II and III. The higher proportion of patients in the A + B category, and the decrease in deaths due to myasthenia are greater than can be explained by chance in the female sex. This level of statistical significance is not reached in the males but the trend is the same (Table II). Slightly more women than men are in category A + B after operation and fewer women than men died of myasthenia. The analysis (Table III) shows that this could have occurred by chance but it should be noted that this represents a reversal of the trend found in the un-operated series where females had a slightly poorer prognosis than males. These data may be summarized in the statement that the status at follow-up examination of the two sexes is not significantly different but that the females have more to gain from operation—a prognosis slightly poorer than the males in the not-operated series being changed to a slightly better prognosis in the operated series, the change being greater than can be explained by chance. This conclusion reconciles the apparently conflicting statements of Keynes (1949) and Schwab and Leland (1953).

The extent of the improvement due to thymectomy may seem disappointing. It must be re-emphasized that this represents the minimum estimate of the distribution of categories. There are fewer deaths due to myasthenia (and indeed fewer deaths, even including the operative risk) and the survivors show a general "shift to the left". It must not be forgotten that the method of assessment makes little allowance for the magnitude of the shift. It does not give any indication of the patient, bedridden for years, who becomes able to resume a normal life after his thymus is removed. This could be indicated only by a method of comparison of grades of severity. This was not used in the present study owing to its retrospective nature and because of the difficulty of maintaining standards over the long periods involved.

Further analysis shows that the age of onset does not differ significantly in the different categories, but Keynes' (1949) statement that the best results are obtained in patients with a short pre-operative duration of myasthenia is confirmed (though statistically significant in females only). The follow up period is comparable for all categories and there is no significant difference in their pre-operative requirements for Prostigmin.

Possible factors in the sex difference described above may be (i) thyroid abnormality was twice as common (18%) in women as in men (60% of the abnormalities noted were nontoxic); (ii) involvement of bulbar muscles at the onset was twice as common in women and initial weakness restricted to the extra-ocular muscles was more common in women; (iii) myopathic changes were twice as common in men as in women.

Electromyographic studies were presented to show that myopathic changes are common in myasthenia gravis without wasting and that the wasted muscles show changes indistinguishable from myositis. Lundervold's (1954) observation that myasthenic "fatigue" may involve derecruitment of motor units rather than decrement of individual units was confirmed. It was suggested that the evidence of electromyography and of histology as described by

---

**Table II—Significance of Difference Between Series**

<table>
<thead>
<tr>
<th>Category</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>S.E. of</td>
<td>Difference</td>
<td>S.E. of</td>
</tr>
<tr>
<td>A + B</td>
<td>+14.3*</td>
<td>6.3</td>
<td>+7.8</td>
<td>8.4</td>
</tr>
<tr>
<td>C</td>
<td>+6.7</td>
<td>5.6</td>
<td>+6.2</td>
<td>6.9</td>
</tr>
<tr>
<td>D</td>
<td>+5.7</td>
<td>5.3</td>
<td>-5.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>-21.1*</td>
<td>6.2</td>
<td>-8.2</td>
<td>7.3</td>
</tr>
<tr>
<td>All deaths</td>
<td>-18.3*</td>
<td>6.8</td>
<td>-1.4</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* % in Operated series minus % in Not-operated series. 
† Unlikely to occur by chance.

**Table III—Sex Difference in Each Series**

<table>
<thead>
<tr>
<th>Category</th>
<th>Not-operated</th>
<th></th>
<th>Operated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>S.E. of</td>
<td>Difference</td>
<td>S.E. of</td>
</tr>
<tr>
<td>A + B</td>
<td>-2.2</td>
<td>8.4</td>
<td>+4.3</td>
<td>6.3</td>
</tr>
<tr>
<td>C</td>
<td>+7.8</td>
<td>6.2</td>
<td>-1.7</td>
<td>5.8</td>
</tr>
<tr>
<td>D</td>
<td>+11.5</td>
<td>8.1</td>
<td>-0.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>+8.8</td>
<td>8.6</td>
<td>-4.1</td>
<td>8.2</td>
</tr>
<tr>
<td>All deaths</td>
<td>+13.1</td>
<td>9.0</td>
<td>-4.1</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* % Females minus % Males in each series. The sex difference is not statistically significant in either series but the trend is reversed.
Dr. Hamilton Paterson shows that the pathological changes of myasthenic muscle are not confined to the neuromuscular junction.

**Acknowledgements**

The kind permission of the Medical Committee of the National Hospital and of Sir Geoffrey Keynes to examine their patients and of the Medical Committees of St. Bartholomew's and New End Hospitals to scrutinize their records are gratefully acknowledged. Dr. E. A. Carmichael provided the facilities and gave invaluable advice throughout the investigation.

**REFERENCES**

Ross, R. T. (1952) Lancet, i, 785.
AN EVALUATION OF
THYMECTOMY IN MYASTHENIA GRAVIS

BY

JOHN A. SIMPSON

(From The Neurological Research Unit of the Medical Research Council, The National Hospital, Queen Square, London)

Few diseases have been more satisfying to the teacher of medicine than myasthenia gravis for no better meeting ground exists for clinician, physiologist and pharmacologist. Ten years ago the standard teaching was that the myasthenic response must indicate one of three possible "chemical lesions" at the neuromuscular junction: (i) insufficiency of acetylcholine, (ii) excess of cholinesterase, or (iii) a "curariform" block of transmission, presumably due to a substance carried in the blood. More recently the logical fourth possibility of an abnormality of the motor end-plates of muscle has been postulated. It seemed only a matter of time and of refinement of physiological and pharmacological techniques before the true lesion would be demonstrated. In that same period knowledge of the physiology of the neuromuscular junction has made its greatest advances, yet the solution of the problem of myasthenia evades us despite renewed and world-wide interest as evinced by two recent symposia (Acta neurol. psychiat. belg., 1955; Amer. J. Med., 1955).

Fundamental to any satisfactory theory is an explanation of the role of the thymus gland. It is not the purpose of this paper to reconsider the evidence that the thymus gland is implicated in myasthenia gravis; this has been admirably reviewed by McEachern (1943) and by Eaton, Clagett and Bastron (1953). The association of thymic pathology with myasthenia gravis has been recognized for more than fifty years and is not in question, but mere association is less important than the concept that it may be causally related to the transmission defect in muscle. It is difficult to implicate the thymus in any "chemical pathology" of myasthenia gravis with the possible exception of the neuromuscular block theory, and it is significant that the exponents of the other theories make no serious efforts to solve the dilemma. The evidence for causal relationship is of two types, (a) thymic extracts may have neuromuscular blocking properties, and (b) operative removal or radiotherapeutic destruction of the gland is followed

1Present address: Neurological Division, Department of Medicine, Edinburgh University.
by significant improvement in patients suffering from myasthenia gravis. Evidence of the first type and its antithesis has been reviewed recently by Wilson and Wilson (1955); the present paper is designed to review the evidence of the second type and to present the results of an independent survey of the largest series yet reported. A preliminary account has been presented (Simpson, 1956).

**Material and Methods**

The records of all patients diagnosed as myasthenia gravis at the National Hospital for Nervous Diseases since 1934 were scrutinized and an attempt made to trace survivors and to determine the fate of those who had died. Most of the cases seen after 1941 were subjected to thymectomy, the majority by Sir Geoffrey Keynes. The survey was extended by kind invitation of Sir Geoffrey to include his full series operated on at Saint Bartholomew's and New End Hospitals.

Criteria for inclusion of cases in the survey were:

(a) A firm diagnosis of myasthenia gravis by a consultant neurologist to one of these hospitals.

(b) A convincing case history of loss of muscle power after exercise, relieved by rest.

(c) Documented evidence of improvement with neostigmine (even if only a test injection).

(d) Absence of evidence of central neurological disease.

A total of 404 cases was available for study. Where death was not known to have occurred the patients were invited to attend the National Hospital for examination. All were traced except 12 operated and 18 not-operated patients. Doctors and hospital records were consulted for evidence of the cause of death in non-survivors. Where this was impossible the certified cause of death was ascertained. Of the 245 known survivors who could be traced, 181 were examined personally and 64 patients who were unable to attend the hospital (mainly due to residence abroad) were asked to complete a detailed questionnaire with the assistance of their family doctor.

The operated cases were classified in the following categories in which the essential feature is a comparison of the state at the time of follow-up with the state before operation with respect to (i) presence of signs and symptoms of myasthenia, (ii) exercise tolerance, (iii) drug requirements.

Category A: Full working life with no restriction. No neostigmine required. No subjective weakness. A small degree of permanent objective weakness, unrelied by neostigmine, is permitted. The present state must represent a marked improvement from the original.
Category B: Able to lead a full life with only minor myasthenic symptoms, requiring no neostigmine or controlled by not more than 4 tablets (60 mg.) daily. A significant improvement is required.

Category C: Full life with few restrictions but (a) demonstrable myasthenia not requiring neostigmine, or (b) still requiring neostigmine but at least 40 per cent less than before and with improved response.

Category D: (a) Improved, but neostigmine requirements unchanged or increased, (b) unimproved, irrespective of neostigmine dosage, (c) worse.

Post-operative death.—Death occurring within three weeks of operation.

Myasthenic death.—Death occurring at a later date where the history suggested asphyxial death or death not explicable by other causes.

Death from other causes.—Death directly attributable to significant disease where the history suggested that myasthenia was not a major factor (e.g. cerebral hemorrhage).

Patients who had not been treated by thymectomy were assessed by the same criteria with reference to the condition on first admission to hospital. The essential feature is an assessment of change of status, hence a patient with little disability (e.g. ocular myasthenia) does not qualify for a high category if this state is not significantly better than at the datum point. In case of doubt a patient was assigned to the lower category.

The data were analysed statistically with respect to sex, age at onset, duration of symptoms before the datum point, and length of follow-up to determine whether the two series ("operated" and "not-operated") could be accepted as representative samples of the population of myasthenics. Cases known to have a thymoma were considered separately. These analyses will be presented before comparing the natural history of the two series.

**PART I**

Comparison of "Operated" and "Not-operated" Series

The sex distribution and the frequency of thymic tumours in the two series is shown in Table I. It is, of course, possible that some thymic tumours

<table>
<thead>
<tr>
<th>Table I.—Distribution of Thymic Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Both</td>
</tr>
</tbody>
</table>
have not been diagnosed in the not-operated patients, but the close resemblance of the two series suggests that this is not so. Table I does, however, show a difference in the sex distribution. This is further analysed for non-tumour cases in Tables II and III and in fig. 1.

![Fig. 1.—Distribution of age at onset of myasthenia (non-tumour).](image)

**Table II.—Sex Distribution (Non-tumour Cases)**

<table>
<thead>
<tr>
<th>Age at onset (Years)</th>
<th>Operated (F: M)</th>
<th>Ratio</th>
<th>Not-operated (F: M)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>9:2</td>
<td>4.5:1</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>11-20</td>
<td>62:20</td>
<td>3.1:1</td>
<td>8:8</td>
<td>1:1</td>
</tr>
<tr>
<td>21-30</td>
<td>67:24</td>
<td>2.8:1</td>
<td>21:8</td>
<td>2.6:1</td>
</tr>
<tr>
<td>31-40</td>
<td>23:17</td>
<td>1.4:1</td>
<td>16:6</td>
<td>2.7:1</td>
</tr>
<tr>
<td>41-50</td>
<td>11:10</td>
<td>1:1</td>
<td>6:6</td>
<td>1:1</td>
</tr>
<tr>
<td>51-60</td>
<td>2:1</td>
<td>2:1</td>
<td>4:8</td>
<td>1:2</td>
</tr>
<tr>
<td>61-70</td>
<td>2:1</td>
<td>2:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71-80</td>
<td>8:2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>8:2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>182:76</td>
<td>2.4:1</td>
<td>59:40</td>
<td>1.5:1</td>
</tr>
</tbody>
</table>

Mean (years): 23.7 (Operated) 27.9 (Not-operated)
Table II and fig. 1 showing the age at onset, reveal marked differences between the two series. Inspection of the histograms of fig. 1 suggests that there is an incidence of age-at-onset which has a skew distribution about a modal value of 20 years (approx.) and that this does not differ greatly with sex, although the males have relatively more cases in the older age groups (causing a higher average age). This difference could be due to the small numbers of males, and might disappear in a larger series, but the same trend was reported by Grob and Harvey (1953). The regularity of the distribution curves in the operated series alone and in the total series suggests that they must closely represent the true population of myasthenic subjects if the age of onset is not randomly distributed. There is a preponderance of females of 4-5-1 in the first decade which steadily decreases to unity in the fifth decade (numbers are too small to attribute any significance to the possible reversal of sex incidence in later age groups). On this interpretation the "not-operated" series is gravely deficient in females under the age of 20 and contains relatively more males over 50 (hence the higher mean age for both sexes) (Table II). The series are closely similar for the age period from 16 to 35 which contains two thirds of the operated series, though only half of the not-operated series (Table III). It will be necessary to make all comparisons in this section of the case

<table>
<thead>
<tr>
<th>Age at Onset</th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>16-20</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>21-25</td>
<td>37</td>
<td>13</td>
</tr>
<tr>
<td>26-30</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>31-35</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>49</td>
</tr>
<tr>
<td>Ratio</td>
<td>2.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

material before accepting conclusions derived from comparison of the whole of both series, since the value of thymectomy has been stated to be most evident in women who develop myasthenia at an early age.

The length of survival from the onset of myasthenic symptoms (to April 1956) is shown in Table XV. It will be more convenient to discuss the breakdown into categories later. The mean survival is closely similar in the two series, indeed despite the longer time at risk in the not-operated series it is slightly lower than in the operated series, due to the long-duration survivors being matched by a higher proportion of deaths within a few years of the onset.

Associated phenomena of myasthenia gravis occurred with equal frequency in both series (non-tumour) (Table IV). "Myopathy" represents
permanently weak wasted muscles not improved by neostigmine. “Goitre” includes simple non-toxic enlargement of the thyroid and thyrotoxicosis. “Ocular myasthenia” indicates the proportion of cases in which the myasthenia remained confined to the extra-ocular muscles (including orbicularis oculi) for the duration of the survey.

**PART II**

**Results**

The classification of both series in April 1956 is shown in Table V and in percentage form in fig. 2. There are three questions to be answered by this survey: (i) Is there any significant difference in prognosis between the two groups? (ii) If there is any benefit to be gained from thymectomy, is it obtained by both sexes? (iii) Are there any other factors which affect the response to operation?

**Table IV.**—**Associated Phenomena (Non-tumour Series)**

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F M F M</td>
<td></td>
</tr>
<tr>
<td>Ocular Myasthenia</td>
<td>1-6 5-3</td>
<td>1-2 20-0</td>
</tr>
<tr>
<td>Myopathy</td>
<td>9-3 22-4</td>
<td>10-2 15-0</td>
</tr>
<tr>
<td>Goitre</td>
<td>18-0 9-0</td>
<td>8-5 2-5</td>
</tr>
</tbody>
</table>

Fig. 2.—Comparison of series (non-tumour) to show cumulative difference.
Table VI.—Comparison of Treatments for Each Sex

<table>
<thead>
<tr>
<th>Category</th>
<th>Female Difference*</th>
<th>S.E. of diff.</th>
<th>Male Difference*</th>
<th>S.E. of diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+B</td>
<td>+14.8†</td>
<td>6.3</td>
<td>±7.7</td>
<td>8.4</td>
</tr>
<tr>
<td>C</td>
<td>+6.8</td>
<td>5.6</td>
<td>±6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>D</td>
<td>+4.1</td>
<td>5.3</td>
<td>-5.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>-21.1†</td>
<td>6.2</td>
<td>-8.2</td>
<td>7.3</td>
</tr>
<tr>
<td>All deaths</td>
<td>-18.6†</td>
<td>6.8</td>
<td>-1.5</td>
<td>8.1</td>
</tr>
</tbody>
</table>

*Per cent in operated series minus per cent in not-operated series.
†Unlikely to occur by chance.

Table VII.—Sex-difference in Each Series

<table>
<thead>
<tr>
<th>Category</th>
<th>Operated Difference*</th>
<th>S.E. of diff.</th>
<th>Not-operated Difference*</th>
<th>S.E. of diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+B</td>
<td>+5.0</td>
<td>6.3</td>
<td>-2.1</td>
<td>8.4</td>
</tr>
<tr>
<td>C</td>
<td>-0.6</td>
<td>5.8</td>
<td>+7.8</td>
<td>6.2</td>
</tr>
<tr>
<td>D</td>
<td>-2.1</td>
<td>5.4</td>
<td>-11.5</td>
<td>8.1</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>-4.1</td>
<td>8.2</td>
<td>+8.8</td>
<td>8.6</td>
</tr>
<tr>
<td>All deaths</td>
<td>-4.0</td>
<td>5.4</td>
<td>+13.1</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*Per cent females minus per cent males in each series. There is no significant difference.

The first two questions may be investigated concurrently.

Comparison of results. Sex differences.—For further statistical analysis, Categories A and B will be considered together as the distinction between them is a fine one of doubtful validity. All cases in these categories are for practical purposes without any disability due to myasthenia.

In Table VI the percentages in each category are compared separately for each sex. The difference is expressed as +ve if the proportion is greater in the operated series than in the not-operated, and -ve if otherwise. If a difference in percentage exceeds twice the standard error of the difference the odds are more than 20:1 against the difference being due to chance; such a difference is accepted as statistically significant. In Table VII a similar comparison is made between male and female in each series.

This analysis answers the first two questions. The operated series contains a higher proportion of females in Category A and B than would be likely to occur by chance (at the 5 per cent level), and the death-rate from myasthenia is very significantly reduced (Table VI). Even when the operative hazard is added, the total mortality rate is significantly lower than in the not-operated series. Males show a similar trend though the difference is not statistically significant except in Category C. Table VII suggests a possible explanation. Neither series shows a significant difference between the sexes. In the not-operated series there is a higher female mortality and fewer women than men reach Categories A and B, in the operated series this trend is completely reversed. It may be that thymectomy reverses an unfavourable prognosis for women, but any
conclusions must be tentative as the analysis does not exclude the possibility of the sex difference in either series having occurred by chance. As the benefit in favour of operation for women is unlikely to be due to chance it is possible that a larger series would show that the similar trend for males is equally significant. It is also possible that differences in composition of the series may be important. The following tables are of the same type as Tables V–VII for the age group 16–35.

**Table VIII.—Results in Age-group 16–35**

<table>
<thead>
<tr>
<th>Category</th>
<th>Operated</th>
<th></th>
<th></th>
<th></th>
<th>Not-operated</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$M$</td>
<td>$F$</td>
<td>$M$</td>
<td>$F$</td>
<td>$M$</td>
<td>$F$</td>
<td>$M$</td>
</tr>
<tr>
<td>A</td>
<td>24</td>
<td>20-7</td>
<td>11</td>
<td>22-5</td>
<td>6</td>
<td>17-1</td>
<td>2</td>
<td>12-5</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>14-6</td>
<td>7</td>
<td>14-3</td>
<td>1</td>
<td>2-9</td>
<td>1</td>
<td>6-3</td>
</tr>
<tr>
<td>C</td>
<td>31</td>
<td>26-7</td>
<td>12</td>
<td>24-5</td>
<td>4</td>
<td>11-4</td>
<td>2</td>
<td>6-3</td>
</tr>
<tr>
<td>D</td>
<td>21</td>
<td>18-1</td>
<td>8</td>
<td>16-3</td>
<td>6</td>
<td>17-1</td>
<td>4</td>
<td>25-0</td>
</tr>
<tr>
<td>Data incomplete</td>
<td>4</td>
<td>3-4</td>
<td>1</td>
<td>2-0</td>
<td>7</td>
<td>20-0</td>
<td>5</td>
<td>31-2</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>9</td>
<td>7-7</td>
<td>7</td>
<td>14-3</td>
<td>10</td>
<td>28-6</td>
<td>2</td>
<td>12-5</td>
</tr>
<tr>
<td>Post-op. deaths</td>
<td>8</td>
<td>6-9</td>
<td>2</td>
<td>4-1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Deaths, other</td>
<td>2</td>
<td>1-7</td>
<td>1</td>
<td>2-0</td>
<td>1</td>
<td>2-9</td>
<td>1</td>
<td>6-3</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>49</td>
<td>35</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table IX.—Comparison of Treatments for Each Sex (Age 16–35)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference*</td>
<td>S.E. of diff.</td>
</tr>
<tr>
<td>A+B</td>
<td>+15-3</td>
<td>16-8</td>
</tr>
<tr>
<td>C</td>
<td>+15-3</td>
<td>17-7</td>
</tr>
<tr>
<td>D</td>
<td>+1-0</td>
<td>17-5</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>-20-9</td>
<td>16-8</td>
</tr>
<tr>
<td>All deaths</td>
<td>-15-2</td>
<td>16-3</td>
</tr>
</tbody>
</table>

*Per cent in operated series minus per cent in not-operated series. There is no significant difference.

**Table X.—Sex-difference in Each Series (Age 16–35)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference*</td>
<td>S.E. of diff.</td>
</tr>
<tr>
<td>A+B</td>
<td>-1-5</td>
<td>13-6</td>
</tr>
<tr>
<td>C</td>
<td>-2-2</td>
<td>14-7</td>
</tr>
<tr>
<td>D</td>
<td>-1-8</td>
<td>15-5</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>-6-6</td>
<td>15-9</td>
</tr>
<tr>
<td>All deaths</td>
<td>-4-1</td>
<td>15-3</td>
</tr>
</tbody>
</table>

*Per cent females minus per cent males in each series. There is no significant difference.

Table VIII shows no essential difference from Table V except that the male mortality rate is not apparently improved by thymectomy. This may be due to the high proportion of unoperated patients whose fate is not
known. If only the known cases are considered the deaths from myasthenia form 18 per cent of the total, which is comparable with the figure obtained in the full series. The poorer prognosis for women and its reversal by operation are again suggested by this limited series but unfortunately none of the figures are significant in the statistical sense (Tables IX and X). This is largely due to the small statistical population. The proportion of not-operated patients in the “insufficient data” category is unfortunately high, but recalculation of the figures in terms of known results only does not make enough difference to satisfy statistical criteria. Failure to satisfy these criteria shows that the results could have occurred by chance but, of course, does not indicate that they need have done so. The close resemblance to the figures calculated from cases of all ages (Table V) suggests that the trends disclosed are not chance ones and that a larger series, by lowering the sampling errors, would confirm the validity of the trends.

Factors affecting response to operation.—The third question to be answered refers to the possibility that the response to operation may be influenced by the patient’s age at the time of onset of myasthenia or at the time of operation; by the duration of illness prior to operation; or by the severity of the myasthenia.

Table XI.—Age at Onset of Myasthenia

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases</th>
<th>A-B</th>
<th>C</th>
<th>D</th>
<th>Deaths</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
</tr>
<tr>
<td>Operated</td>
<td>F 182</td>
<td>Mean 22.8</td>
<td>23.7</td>
<td>25.2</td>
<td>24.0</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>(s.e.) (1.2)</td>
<td>(1.2)</td>
<td>(1.8)</td>
<td>(2.8)</td>
<td>(0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 9.6</td>
<td>7.6</td>
<td>10.4</td>
<td>10.7</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.* 0.1-0.05</td>
<td>1.0</td>
<td>0.01-0.001</td>
<td>&gt; 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M 76</td>
<td>Mean 26.2</td>
<td>27.7</td>
<td>31.0</td>
<td>22.3</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>(s.e.) (1.8)</td>
<td>(2.1)</td>
<td>(3.4)</td>
<td>(2.9)</td>
<td>(1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 8.9</td>
<td>8.7</td>
<td>13.8</td>
<td>8.7</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.* &gt; 0.1</td>
<td>&gt; 0.1</td>
<td>0.05-0.02</td>
<td>0.01-0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not-operated</td>
<td>F 59</td>
<td>Mean 33.0</td>
<td>36.7</td>
<td>30.4</td>
<td>32.0</td>
<td>32.3</td>
</tr>
<tr>
<td></td>
<td>(s.e.) (3.4)</td>
<td>(4.0)</td>
<td>(3.2)</td>
<td>(3.1)</td>
<td>(1.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 11.9</td>
<td>11.9</td>
<td>11.4</td>
<td>12.9</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.* &gt; 0.1</td>
<td>0.05-0.02</td>
<td>&gt; 0.1</td>
<td>&gt; 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M 40</td>
<td>Mean 36.7</td>
<td>40.6</td>
<td>36.4</td>
<td>44.0</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>(s.e.) (6.7)</td>
<td>(9.1)</td>
<td>(4.1)</td>
<td>(8.7)</td>
<td>(2.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 20.2</td>
<td>15.7</td>
<td>12.9</td>
<td>24.6</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.* &gt; 0.1</td>
<td>&gt; 0.1</td>
<td>0.05-0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Probability, calculated by Student’s t test, that the mean age of the category is the same as the mean age of the whole group.
Age at onset.—It has been suggested that onset at an early age gives a better chance of good operative results in contrast with a poor prognosis without operation (Schwab and Leland, 1953; Eaton, Clagett and Bastron (1953), Table XIX). This factor is examined in Table XI for the present series. The average age of the operated series is nine years younger than the not-operated series owing to the larger representation of young people already shown in Table II but in each series the mean age of the female patients is four years younger than the male average at the onset of myasthenic symptoms. Examination of the breakdown into categories shows that of the surviving groups of not-operated patients only C-female differs significantly from the mean age of the whole series. In the operated series, group D is significantly older than the overall mean age in both sexes. There is also a trend of increasing age from A and B through C to D though the difference may not be significant. This analysis provides slender evidence for the suggestion quoted that the earlier the onset the better the response to operation. The suggested converse trend for not-operated patients is not confirmed. The age difference is clearly too small to be of practical value in selection of cases for operation. If the question asked is ‘in what respect do all other categories differ from Category D (which is that of ‘no change’) with respect to age of onset?’ the answer is that none differs significantly. The age of onset of male patients who died of myasthenia after operation was significantly younger than the mean for the series or Category D, whereas myasthenic deaths in the unoperated males occurred in patients whose average age at onset was significantly older than the group mean or Category D. The very high mean age at onset of the unoperated males who died of myasthenia has too high a standard deviation to justify further discussion. The discrepancy is not present in the 16–35 age group so it may be due to the many older cases in the full series. Because the restricted group is specially selected for age this factor has not been tabulated, but it may be stated that within that group no effect of age at onset on the final result could be demonstrated. This question is discussed more fully in Part IV.

Pre-operative duration.—The possibility that the final state is inversely proportional to the duration of myasthenia before operation is investigated in Table XII. The female Category A+B has a mean pre-operative duration which is very significantly less than the mean of all the female patients subjected to operation and significantly less than that of categories C and D. Categories C and D have a significantly longer history than the group mean. (Category C does not differ significantly from Category D.) A similar trend is disclosed by the males, though chance differences cannot be excluded. The mean pre-operative duration of those who subsequently died a myasthenic death despite operation was closely similar to the mean for the group, and in fact was not significantly different from the A+B categories. There is thus no indication that those who die of myasthenia
Table XII.—Pre-operative Duration of Myasthenia

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
</tr>
<tr>
<td>Cases</td>
<td>179</td>
<td>64</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>Mean duration</td>
<td>5:6</td>
<td>3:4</td>
<td>7:6</td>
<td>8:5</td>
</tr>
<tr>
<td>(s.e. of mean)</td>
<td>(0:44)</td>
<td>(0:5)</td>
<td>(0:91)</td>
<td>(1:2)</td>
</tr>
<tr>
<td>S.D.</td>
<td>5:9</td>
<td>4:0</td>
<td>5:9</td>
<td>7:0</td>
</tr>
<tr>
<td>s.e. of difference of D. from other cats.</td>
<td>1:2</td>
<td>1:3</td>
<td>1:5</td>
<td>—</td>
</tr>
<tr>
<td>s.e. of difference of cat. from group mean</td>
<td>—</td>
<td>0:6</td>
<td>1:0</td>
<td>1:2</td>
</tr>
<tr>
<td>P.*</td>
<td>&lt;0:001</td>
<td>0:05</td>
<td>0:05</td>
<td>0:02</td>
</tr>
</tbody>
</table>

*Probability, calculated by Student's t test, that the mean pre-operative duration of myasthenia of the category is the same as the mean of the whole Group.

Table XIII.—Age at Operation

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
</tr>
<tr>
<td>Cases</td>
<td>164</td>
<td>26-3</td>
<td>31:5</td>
<td>33-7</td>
</tr>
<tr>
<td>Mean age</td>
<td>10:9</td>
<td>1:75</td>
<td>1:9</td>
<td>2-9</td>
</tr>
<tr>
<td>(s.e. of mean)</td>
<td>(0:85)</td>
<td>(1:4)</td>
<td>(1:9)</td>
<td>(2:9)</td>
</tr>
<tr>
<td>S.D.</td>
<td>12:2</td>
<td>10:3</td>
<td>7:3</td>
<td>10:6</td>
</tr>
<tr>
<td>s.e. of difference from Category D</td>
<td>2-1</td>
<td>2:3</td>
<td>2:6</td>
<td>—</td>
</tr>
<tr>
<td>s.e. of difference from sex mean</td>
<td>—</td>
<td>1-6</td>
<td>2-0</td>
<td>2-1</td>
</tr>
<tr>
<td>P.*</td>
<td>&gt;0:02</td>
<td>&gt;0:01</td>
<td>&gt;0:05</td>
<td>&gt;0:02</td>
</tr>
</tbody>
</table>

*Probability, calculated by Student's t test, that the mean age at operation of the category is the same as the mean of the whole Group.

*Age at operation not recorded in 15 female and 3 male cases.
despite thymectomy differ in the length of history from those who survive. This point will be returned to in a later section.

**Age at operation.**—The age at the time of operation may be more important than the previous duration of illness. The analysis (Table XIII) for females shows that A+B are younger and D older than average, to a highly significant extent and that A+B are significantly younger than D at the time of operation. In the males, group D is also older than the group average though this is barely significant (at the 5 per cent level) and although A+B is not much younger than the group average the difference from D approaches the same order of significance. Those who subsequently died of myasthenia were a little younger than the group average and considerably younger than D (the "unchanged" survivors) and this difference is significant for males. Again the two sexes show the same trends.

It is not possible to say whether pre-operative duration or the age at the time of operation is the more important factor. Obviously they are interrelated. Formal analysis would require a series of cases aged less than 30 at the time of operation who had been myasthenic for, say five to ten years, for comparison with a series of older patients with myasthenia of recent onset. Individual patients of the present material could be quoted with good or bad results in both these categories so that the analysis would have no relevance in the assessment of a particular patient when operation is under consideration.

**Neostigmine requirements.**—A major difficulty in an investigation involving the comparison of large numbers of patients over a period of many years is the classification of patients according to severity. Even if it were possible for a single clinician to establish and maintain accurate standards for twenty years, it would be impossible for a later examiner to make such a decision, especially in a disease of widely varying manifestations, treated by numerous physicians during a period in which therapy was changing. The amount of neostigmine required by the patient before operation is a reasonably objective index of severity. The sexes do not differ greatly in this respect (Table XIV) and those patients who have done well after operation required little less neostigmine before operation than those who did not benefit. Those who eventually died a myasthenic

<table>
<thead>
<tr>
<th>Operated</th>
<th>F</th>
<th>10-0</th>
<th>12-6</th>
<th>19-3</th>
<th>12-4</th>
<th>14-3</th>
<th>13-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>8-0</td>
<td>12-6</td>
<td>19-2</td>
<td>10-5</td>
<td>17-5</td>
<td>13-6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Not-operated</th>
<th>F</th>
<th>5-1</th>
<th>10-0</th>
<th>7-2</th>
<th>10-0</th>
<th>13-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>4-9</td>
<td>4-5</td>
<td>8-0</td>
<td>6-8</td>
<td>9-0</td>
<td>8-7</td>
</tr>
</tbody>
</table>

*15 mg. tablets. Where parenteral injection was used the equivalent oral dose has been calculated on the basis of 0·5 mg. I.M. = 15-0 mg. oral.
death required more neostigmine than the survivors. The considerably higher requirements in Category C cannot be explained. Ross (1952) records the same trend but had too few cases in Category D to note the anomaly. It is not found in the corresponding not-operated group. The possibility must be considered that Category C is an artificial one and not truly different from Category D in which the "improvement" due to operation could be explained if many had been given more neostigmine than necessary before operation, but subsequently resumed their true requirements. No patients have been included in Category C who did not give a history suggestive of genuine improvement despite the decreased dosage of neostigmine. Apart from this discrepancy the not-operated series shows a trend similar to the operated series though the general dosage level was lower (lower dosage of neostigmine was then customary).

It is concluded that there is no clear evidence that the severity of myasthenia materially affected the final state in survivors.

Survival.—A final question must be considered before the comparative figures of Table V can be accepted. Is the follow-up period comparable for all categories? This must be known to exclude the possibility that immediate good results lapse with passage of time, or conversely, that if an unoperated patient survives long enough there is a progressive tendency to improve. Relapses do occur after operation, but in a minority of cases. The author was, on many occasions, surprised to note how closely his estimate agreed with that of Ross (1952) where the latter's note was avail-

### Table XV. — Survival from Onset

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>All known survivors</th>
<th>A - B</th>
<th>C</th>
<th>D</th>
<th>Myasth. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
<td>yrs.</td>
</tr>
<tr>
<td>Operated</td>
<td>F</td>
<td>Mean 13-0</td>
<td>10-9</td>
<td>14-2</td>
<td>15-8</td>
<td>6-7</td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td>(0-6)</td>
<td>(0-7)</td>
<td>(1-1)</td>
<td>(1-4)</td>
<td>(1-5)</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>7-1</td>
<td>5-2</td>
<td>7-7</td>
<td>7-9</td>
<td>5-8</td>
</tr>
<tr>
<td></td>
<td>P.*</td>
<td>&lt;0-01</td>
<td>&lt;0-01</td>
<td>&gt;0-1</td>
<td>&gt;0-05</td>
<td>&gt;0-001</td>
</tr>
<tr>
<td>M</td>
<td>Mean 12-5</td>
<td>12-1</td>
<td>10-9</td>
<td>15-1</td>
<td>6-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td>(1-0)</td>
<td>(0-9)</td>
<td>(1-1)</td>
<td>(3-7)</td>
<td>(2-7)</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>7-4</td>
<td>4-3</td>
<td>4-8</td>
<td>11-8</td>
<td>8-0</td>
</tr>
<tr>
<td></td>
<td>P.*</td>
<td>&lt;0-01</td>
<td>&lt;0-05</td>
<td>&gt;0-02</td>
<td>&gt;0-1</td>
<td>&gt;0-05</td>
</tr>
<tr>
<td>Not-operated</td>
<td>F</td>
<td>Mean 14-7</td>
<td>16-6</td>
<td>14-1</td>
<td>12-6</td>
<td>5-2</td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td>(1-2)</td>
<td>(2-5)</td>
<td>(2-7)</td>
<td>(2-7)</td>
<td>(1-2)</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>6-9</td>
<td>7-1</td>
<td>8-0</td>
<td>7-7</td>
<td>5-1</td>
</tr>
<tr>
<td></td>
<td>P.*</td>
<td>&lt;0-01</td>
<td>&lt;0-01</td>
<td>&gt;0-1</td>
<td>&gt;0-1</td>
<td>&gt;0-001</td>
</tr>
<tr>
<td>M</td>
<td>Mean 12-4</td>
<td>9-4</td>
<td>15-2</td>
<td>14-5</td>
<td>4-0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td>(2-1)</td>
<td>(2-3)</td>
<td>(1-0)</td>
<td>(3-8)</td>
<td>(1-2)</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>9-9</td>
<td>6-8</td>
<td>1-4</td>
<td>12-3</td>
<td>3-3</td>
</tr>
<tr>
<td></td>
<td>P.*</td>
<td>&lt;0-01</td>
<td>&gt;0-1</td>
<td>&gt;0-1</td>
<td>&lt;0-01</td>
<td>&lt;0-001</td>
</tr>
</tbody>
</table>

*Probability, calculated by Student’s t test, that the mean duration of survival from the onset is the same as the mean for all known survivors.
Table XVI.—Post-operative Survival

<table>
<thead>
<tr>
<th>Sex</th>
<th>Survivors</th>
<th>A+B</th>
<th>C</th>
<th>D</th>
<th>Myasthenic deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>7-7</td>
<td>8-2</td>
<td>6-7</td>
<td>7-3</td>
<td>2-4</td>
</tr>
<tr>
<td>M</td>
<td>7-1</td>
<td>8-1</td>
<td>6-7</td>
<td>7-3</td>
<td>1-0</td>
</tr>
</tbody>
</table>

able in the case record. (Unfortunately, this was not always present making an accurate review of the identical series impossible.)

Table XV shows the mean survival times from the onset of myasthenia in both series and the survival after operation in the operated series (to April 1956) is in Table XVI. The apparent shorter survival from onset of operated group A+B must be taken along with the figures for post-operative survival (Table XVI) which shows a longer post-operative follow-up than the other categories, thus the lower figure in Table XV merely reflects the fact that A+B tends to have a shorter pre-operative history.

There is nothing to indicate that the longer the series is followed the more cases will move from Category A+B to C or D, confirming the clinical impression. The further question regarding the possibility of improvement with time in not-operated patients is not answered unequivocally since the sexes differ and no category differs significantly from the mean for all survivors (Table XV). An interesting point emerges from Table XV. Those patients who eventually died of myasthenia gravis did so on average four to seven years after the onset of the disease whether thymectomy was carried out or not. Deaths from myasthenia gravis per se rarely occurred more than ten years after the onset of symptoms.

Conclusions.—Fewer women die of myasthenia gravis if their thymus is removed than would be expected if they were treated with neostigmine only, and a higher proportion of operated cases is very greatly improved ten or more years after the onset of the illness. This difference is unlikely to have occurred by chance. The greatest improvement tends to occur in a group with myasthenia starting rather younger than average and with a shorter pre-operative course than the less successful cases but the differences are not sufficiently marked to discount chance effects. The pre-operative severity is probably not a significant factor. Similar trends are noted in male cases. Though, considered in isolation, none of the differences noted in the operated male series are of such magnitude as to exclude the possibility that they result from chance, the fact that all trends after operation are in the same direction as in the female cases and that a direct comparison between the sexes shows no significant difference suggests that males do benefit in the same way as females. There is some evidence (Tables VI and VII) that the greater benefit obtained by thymectomy in women is due to the change from a prognosis slightly poorer than the male to one better than the male. Though the sex difference is not significant (in the statistical sense) in either series the swing is sufficiently great to be beyond chance (Table VI).
Thymomas

The number of patients in whom thymoma was known to be present was not great (29 women and 18 men); the numbers are too small to make the results statistically significant, but it is apparent from the breakdown shown in Table XVII that the mortality rate is not strikingly lowered by operation. It is very much higher in both series than in myasthenia unaccompanied by thymoma whatever form of treatment was used (Table V). Of the 9 post-operative deaths, only 2 had been shown to have thymoma by previous radiography and each of these had been operated on after a course of deep radiotherapy. In the other 7 the X-ray studies had not revealed the tumour. The deaths recorded on the line for “other causes” were a patient, described elsewhere by Chalmers and Boheimer (1954), who died of hemosiderosis without recurrence of myasthenia gravis three and a half years after operation, and another who died of acute mania complicated by bronchopneumonia. The circumstances of the other deaths were investigated as fully as possible by retrospective inquiry. In all cases the nature of the terminal illness was consistent with death due to myasthenia gravis. In at least 7 of these patients the immediate effects of operation were comparable with the non-tumour patients and indeed three of them had long periods (two and a half, six and seven years) of complete remission from myasthenia before the relapse which led to death in a short period.

Age at onset.—The mean age at onset of myasthenic symptoms was higher than in comparable non-tumour groups but the small number of cases and the wide scatter of ages make this of doubtful significance (Table XVIII). This information was not available in two male patients who have been omitted from the table.

The mean age at onset of myasthenia for all known cases of thymoma was 40·8 years for females and 39·3 years for males. The earliest onset was in a woman of 24 (died) and the oldest a woman aged 64 who at the age of 70 was in excellent health (Category A) five years after removal of

<table>
<thead>
<tr>
<th>Category</th>
<th>F No.</th>
<th>%</th>
<th>M No.</th>
<th>%</th>
<th>F No.</th>
<th>%</th>
<th>M No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>4·3</td>
<td>3</td>
<td>23·0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20·0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>17·4</td>
<td>1</td>
<td>7·7</td>
<td>1</td>
<td>16·7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>13·0</td>
<td>1</td>
<td>7·7</td>
<td>1</td>
<td>16·7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>7</td>
<td>30·4</td>
<td>5</td>
<td>38·5</td>
<td>3</td>
<td>50·0</td>
<td>4</td>
<td>80·0</td>
</tr>
<tr>
<td>Post-op. deaths</td>
<td>7</td>
<td>30·4</td>
<td>2</td>
<td>15·4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Deaths, others</td>
<td>1</td>
<td>4·3</td>
<td>1</td>
<td>7·7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 23 13 6 5
THYMECTOMY IN MYASTHENIA GRAVIS

Table XVIII.—Age at Onset of Myasthenia (Thymomas)

<table>
<thead>
<tr>
<th>Cases</th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>No.</td>
<td>23</td>
<td>12*</td>
</tr>
<tr>
<td>Mean age (s.e. of mean)</td>
<td>42.5 (6.3)</td>
<td>39.3 (2.5)</td>
</tr>
<tr>
<td>Yrs.</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

*Age at onset unknown in one male of each group.

Table XIX.—Survival from Onset of Myasthenia (Thymomas)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases survivors</th>
<th>Myasth. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>F</td>
<td>23</td>
<td>9.6</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>7.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases survivors</th>
<th>Myasth. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>9.3</td>
</tr>
<tr>
<td>M</td>
<td>5</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table XX.—Post-operative Survival—Thymomas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases survivors</th>
<th>Myasth. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>5.3</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
<td>5.5</td>
</tr>
</tbody>
</table>

A thymoma (with only post-operative radiotherapy). It does not appear as if the patient’s age when myasthenia first manifests itself is of much assistance in diagnosis or in prognosis but Keynes (1955) has remarked on the absence of cases under 20 and the low incidence under 30 (4 per cent).

Survival.—It has been thought advisable to show the survival figure of this group, though the numbers are too small for proper comparison with the non-tumour series, as there is a common tendency to give a poorer prognosis than is justified by the facts. The high mortality of this group has been discussed above, but many years of survival in a high category are possible (Table XIX). The post-operative survival is also shown for comparison with Table XVI (Table XX).

The follow-up period is of the same order as in the non-tumour cases. Furthermore Tables XIX and XX show that although myasthenic death is more likely to occur in the presence of a thymic tumour, this event tends to occur at much the same time from the onset or after operation, whether tumour is present or not. In other ways, too, the patients with a thymoma showed no obvious clinical difference from the others. The incidence of myopathic change and of goitre was the same (compare Table XXI with Table IV).
Table XXI.—Associated Phenomena (Thymomas)

<table>
<thead>
<tr>
<th></th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F M</td>
<td>F M</td>
</tr>
<tr>
<td>Ocular myasthenia*</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Myopathy*</td>
<td>8-7 23-0</td>
<td>16-7 20-0</td>
</tr>
<tr>
<td>Goître</td>
<td>21-0 8-0</td>
<td>16-7 0</td>
</tr>
</tbody>
</table>

*As defined for Table IV.

Keynes (1955) thought that the tumour cases were nearly always associated with severe symptoms which were difficult to control. The average neostigmine requirements of the thymoma group was about 25–50 per cent higher than the non-tumour series (Tables XXII and XIV) and neostigmine resistance was more common. There is a marked discrepancy in this index of severity when Category A is examined (Table XXII). In

Table XXII.—Neostigmine Requirements (Thymomas)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operated</td>
<td>F</td>
<td>30</td>
<td>23</td>
<td>13</td>
<td>10-4</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>26</td>
<td></td>
<td>1</td>
<td>17-6</td>
</tr>
<tr>
<td>Not-operated</td>
<td>F</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>40+</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>27</td>
<td></td>
<td></td>
<td>5†</td>
</tr>
</tbody>
</table>

*15 mg. tablets. Parenteral dosage equated as before.
†Not known in 2 cases.

the non-tumour series this category had a slightly lower than average pre-operative requirement for neostigmine, but in the tumour group the mean dosage was much higher than average. Too much stress should not be placed on this unexpected finding as the whole group includes only 4 cases, nevertheless, this observation once again underlines the fact that excellent recovery may follow removal of a thymoma in a severely myasthenic patient. The 3 male patients in Category A had pre-operative radiotherapy (which caused increased weakness). The female patient did not have radiotherapy until after operation. Keynes (1955) stresses the desirability of pre-operative radiation but records successful results in the absence of this precaution. Of 20 patients with thymoma treated with deep X-ray therapy followed by operation between 1948 and 1953, only 4 (20 per cent) had died at the time of his report (1953, published 1955).

Part IV

Comparison with Published Series

Comparison of one published series with another cannot be made with great accuracy. The most important reason for this is the failure to differentiate the cases associated with a thymic tumour from those in
which no tumour can be demonstrated. The numbers involved in most of the series are small and their selection varies. Sir Geoffrey Keynes has operated on almost every patient referred to him. (I have only found 3 patients who were rejected as "bad risks" so their inclusion in the not-operated series does not introduce a significantly adverse factor.) The number of patients who were not referred to a surgeon is not known, but is believed to be small. In his series of papers from 1946 to 1955 Keynes has reported the cumulative results of his experience. In the present paper (Table XXIII) his figures have been recalculated in terms of the total material instead of percentage survivors as reported. Ross (1952), reporting an independent assessment of Keynes' patients, examined "100 consecutive patients" from the latter author's series. It seems implicit that this means "100 consecutive patients who survived thymectomy." To this extent the series is not representative of the total case material now included. Keynes and Ross did not publish separate statistics for each sex, nor did Viets (1945, 1950), Harvey (1948) and Eaton and Clagett (1950), reporting the early results from Boston, Baltimore and the Mayo Clinic. Furthermore, the American reports of that period included tumour and non-tumour cases in the one analysis. Indeed some of these centres considered that the presence of a thymoma was the main indication for operation (Eaton and Clagett, 1950) and in other ways their series were more "selected" than the London series. Viets (1950), for instance, states that he had "deliberately avoided operating upon mild cases, or those fully maintained under oral medication" and excluded most patients under 40. He considered that less than half of 300 patients he had seen since 1935 were proper candidates for thymectomy. Schwab and Passouant (1952) and Schwab and Leland (1953) correlated the findings with sex and age, but still included cases with thymoma. In the last report from Boston (Schwab and Leland, 1953) 22 per cent of 78 patients subjected to thymectomy had thymomas. As tumours were present in 32 per cent of their male cases against 17 per cent of their females it is apparent that their conclusion that males received no evident benefit from the operation requires further examination. The method of selection of patients for thymectomy by Schwab and Leland (1953) "depended partly on chance, economic reasons and random persuasion in different periods by a number of different physicians, with a large number of variables. There has been, however, a trend towards selection of younger patients." A like number of patients was selected from a group of 250 myasthenia gravis patients as controls: "that matched the thymectomy patients by sex, age of onset (within five years), severity of disease and presence or absence of thymoma." The intention is most laudable, but considering the "method of selection" of cases for operation and the virtual impossibility (to the writer) of matching many cases from such a small population, the "control" must be considered dubious.
The Baltimore series, starting with the famous paper of Blalock (1941), was reported briefly by Harvey (1948) and more fully by Grob (1953) who discusses the sex difference, but unfortunately there is no report of this series in which the tumour material is separated from the non-tumour. (20 per cent of 44 operated cases had thymomas; 40 per cent of the deaths in the operated series had tumours as compared with 10 per cent of the deaths in the non-operated cases.) The criteria for selection for operation are not reported but "the average severity of disease at the time of operation was slightly greater than in the other patients with myasthenia who were hospitalized but who did not have thymectomy or irradiation." The incidence of ocular myasthenia was 4-5 per cent in the thymectomy series and 29-7 per cent in the remainder.

The failure to separate thymoma from non-tumour cases was also the cause of confusion in the first reports from the Mayo Clinic by Eaton and Clagett and their collaborators (1949, 1950). The sexes were not considered separately. It appears that "some patients were too ill or too old for major surgical procedures to be recommended." On the other hand many cases of mild myasthenia gravis were included in the control group. These authors also attempted the case-matching type of control in their later papers (1950, 1953, 1955), nevertheless in 1950 Eaton and Clagett concluded that "at present thymectomy in the treatment of myasthenia gravis is recommended by us because of the potentially malignant character of the thymomas and not because of anticipated improvement in the myasthenia gravis. Thymectomy is seldom recommended except when the following conditions prevail: (1) A thymic tumour can be demonstrated roentgenologically. (2) The condition of the patient is such that the risk of operation is not considered excessive. (3) There is no roentgenological or clinical evidence of inoperability of the tumour." When their material was redeployed to take account of presence or absence of tumour, Eaton, Clagett and Bastron (1953) expressed surprise on finding statistically significant benefit from operation in the non-tumour cases. The fullest discussion of the Mayo Clinic series including the sex factor, is made by Eaton and Clagett (1955). This is the only American paper which breaks down the material in such a way as to allow comparison with the London series. It is apparent that the earlier views were based on a surgical series in which 42 per cent of patients had thymomas against 7 per cent of non-surgical cases. The criteria for operation after this recognition were made are not reported, though it appears that the risk of operation was accepted in more severely ill patients "on the basis that thymectomy might prove to be lifesaving." In view of the very marked differences between patients selected for operation and those not so treated, Eaton and Clagett (1953, 1955) use the match-control method criticised above.

This detailed study of the constitution of the major series in the literature
is not made in any critical sense. The writer is well aware of the deficiencies and lack of planned control in the present series, but the literature has been so long bedevilled by conflicting claims it seems essential to examine the basis of these claims with care before a comparison is made. It is also necessary to examine the method of classification of results used in the different centres. This has been done in Table XXV. Where the authors define their criteria they have been quoted and this has been amplified from internal evidence in the text or tabulated data. The classifications differ widely, particularly in the large group in which improvement short of complete remission is recognized. It is not always made clear that the grading represents a change of status from some datum point such as the pre-operative period rather than a classification of physical status at the time of follow-up. Under the first system, used in this paper, a mild case of ocular myasthenia showing no spread but no significant improvement would be classed in Group D. In the alternative scheme it would be in Group B. Table XXIII shows the apparent relationship between the different reports and the further comparisons are based on this assumption. Eaton and Clagett (1955) state that their criteria for "complete remission" are stricter than those of Keynes. If a minor degree of myasthenia gravis persists these authors classify the result as "considerably improved." The definition of Group A used in the present report is restricted in the same way, but it has been considered justifiable, following Keynes (1954), to permit "myopathic" weakness (wasting, and weakness not related to effort nor reversible by neostigmine). This point is not discussed by the other authors, but since it cannot be expected that thymectomy could reverse a myopathic lesion, such a concession seems permissible. On the other hand my criteria for C and probably B are stricter. The combined A and B groups are believed to conform to the criteria of Keynes and Ross.

Table XXIV shows the relation between successive reports from London, but it should be remembered that Keynes did not operate on a small number of the present operated series which accordingly includes the "first steps" mortality of several surgical units. It is evident that the present author's assessment is similar to that made by Keynes in 1949 although the proportion not included in the improved categories is some 10 per cent higher. This is certainly attributable to the very rigid criteria adopted, for instance Group D (a) were undoubtedly improved, and might well have been in Group C in Keynes' classification, but they have been kept in the lower group because the neostigmine dosage was not decreased. This decision is certainly open to argument, but the writer's aim is to present the operative results in the least favourable light so that any result will not be open to accusation of special pleading. The discrepancy with Ross's results (which Keynes has described as "better than I have ever claimed myself") is not understood. Though it is apparent that the criteria for
Simpson, 1956

<table>
<thead>
<tr>
<th>A. Full working life with no restrictions.</th>
<th>B. Full life. Minor symptoms, not requiring neostigmine or controlled by not more than 60 mg. Significantly better</th>
</tr>
</thead>
<tbody>
<tr>
<td>No neostigmine</td>
<td>Virtually well. Minimal symptoms. Small dose of neostigmine</td>
</tr>
<tr>
<td>No subjective weakness.</td>
<td></td>
</tr>
<tr>
<td>(Small degree of permanent objective weakness permitted.) Markedly different from pre-operative state</td>
<td></td>
</tr>
</tbody>
</table>

C. Full life with few restrictions:

<table>
<thead>
<tr>
<th>(a) demonstrable myasth. but not requiring neostigmine</th>
<th>(b) Still requiring drug but at least 40 percent less than before and with improved response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. Some improvement, often considerable. Neostigmine still necessary but less than before</td>
</tr>
</tbody>
</table>

D. (a) Improved, but on same or greater dose of neostigmine

<table>
<thead>
<tr>
<th>(b) Unimproved, irrespective of dosage (even if in good health e.g. ocular myasthenia).</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. No change</td>
</tr>
</tbody>
</table>

(c) Worse

<table>
<thead>
<tr>
<th>DIED (a) Death due to myasthenia after three weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) Post-op. within three weeks</td>
</tr>
<tr>
<td>(c) Death due to other causes</td>
</tr>
</tbody>
</table>

TABLE XXIII—

<table>
<thead>
<tr>
<th>Keynes (1946, 1949, 1954)</th>
<th>Ross (1952)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Quite well. No symptoms. No neostigmine</td>
<td>A. Quite well. No symptoms or treatment</td>
</tr>
</tbody>
</table>

B. Symptoms inconstant and minor e.g. only present at menses. The patient is able to carry on with full scale of activities. Treatment minimal; significantly less (mean post-op. dose 6-8 tabs. S.D.4-8)

C. Considerable improvement. Fewer or less severe signs and symptoms, and responds to signif. smaller dose of neostigmine

D. Unchanged or worse

DIED (a) Later myasthenia

<table>
<thead>
<tr>
<th>(b) Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c) Other causes</td>
</tr>
</tbody>
</table>

DIED Later myasthenia

<table>
<thead>
<tr>
<th>Other causes</th>
</tr>
</thead>
</table>
### THYMECTOMY IN MYASTHENIA GRAVIS

#### COMPARISON OF CRITERIA

<table>
<thead>
<tr>
<th>Eaton et al. (1953)</th>
<th>Schwab and Leland (1953)</th>
<th>Grob (1953)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+4 Complete remission No symptoms. No neostigmine</td>
<td>A. Complete remission. No longer required any medication and had no symptoms</td>
<td>In a complete or nearly complete remission</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+3 Considerable improvement</th>
<th>B. Significantly and objectively improved, took less medicament and had fewer symptoms. Medical referees would all agree as to the presence of improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Subjectively well. Examination discloses (a) min. weakness due to myasthenia (b) Unequivocal sensitivity to curare</td>
<td></td>
</tr>
<tr>
<td>(2) Marked subjective improvement. Verified by increased work and activity, plus great reduction in amount of neostigmine required</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+2 Moderate improvement</th>
<th>Improved to a moderate degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) Same work and activity accomplished with signif. reduction of neostigmine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+1 No essential change</th>
<th>C. Various degrees of slight to mod. improvement which was subjective oftener than objective. Difficult to evaluate quantitatively and would not convince referees</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Greater work and activity accomplished with use of same amount of neostigmine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+0 Unchanged or worse</th>
<th>D. Unchanged or worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>(No essential change)</td>
<td>Unchanged. Worse</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>-2 Worse</th>
<th>E. All deaths</th>
</tr>
</thead>
</table>

| -4 DIED, Myasthenia gravis |

| -4 Surgical death |

| -4 Surgical death |

| -4 DIED (all cases) |
Category B are less rigid (cf. neostigmine requirements) and Ross agrees with Keynes in B. C. D. there is a difference between the proportions in Category A which can only be attributed to fortuitous selection. The apparent selection of survivors of the operation has already been pointed out. Certainly the writer was frequently able to check his own assessment with that made on the same case a few years earlier by Ross and there was rarely disagreement; and 54 per cent of the patients Ross had assessed as A made up 56 per cent of these classified as A by Keynes in 1949 (Ross, 1952). This strongly suggests that the difference is a genuine one due to examination of a consecutive series of cases which was not a true sample of the whole operated population—a well-known risk in surveys of this type. The present assessment in which no sampling is involved, should, therefore, be taken as an expression of the writer's opinion of the latest state of the whole London series when looked at in the most critical light. This can now be compared with the other major series, bearing in mind the differences in composition and in the criteria of assessment which have been presented above. The figures tabulated (Table XXV) are those most recently reported.

The closest agreement with the results of the present survey is the much smaller series of Schwab and Leland (1953). Unfortunately, 9 of the 53 females (17.0 per cent) and 8 of the 25 males (32 per cent) in the operated series had thymomas and the published data are insufficient to permit computation of the non-tumour results though it is stated that exclusion of the thymomas raised the A or B remission (A + B + C of the present classification) to 68 per cent for females, but left the rate unchanged for males (24 per cent). On the other hand, exclusion of the tumours lowered the mortality rate for males from 32 per cent to 18 per cent (though not stated it is assumed that the female mortality rate is little affected by the adjustment). These authors, then, agree closely with the London results except for the poor improvement rate in males. Their control series closely resembles the present one in all respects except for the larger number of females considered unchanged or worse. It should be remembered that the control series was selected to contain the same incidence of tumour as the operated series.

Eaton and Clagett (1953) also report results of the same order as the present ones in their operated series, but agree with Schwab and Leland (1953) in finding more females improved and more males unchanged or worse. The figures for their control series, however, are so markedly different from all the other series (which are consistent with each other) as to raise doubts about the validity of their matching technique. They report the following assessment of 142 unselected controls: A 7.7 per cent, B 9.9 per cent, C 10.6 per cent, D 49.3 per cent, Died 22.5 per cent, but 9 per cent of this series were previously reported to be cases with tumour (Eaton and Clagett, 1950). Nevertheless, the mortality rate is more consistent with that in the other not-operated series.
Table XXIV.—Progressive Assessment of London Series (Non-tumour)

<table>
<thead>
<tr>
<th>Series</th>
<th>Keynes (1946)</th>
<th>Keynes (1949)</th>
<th>Ross (1952)</th>
<th>Simpson (1956)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>A</td>
<td>9</td>
<td>27-3</td>
<td>39</td>
<td>28-4</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>33-3</td>
<td>40</td>
<td>29-2</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>24-2</td>
<td>31</td>
<td>22-6</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>15-2</td>
<td>10</td>
<td>7-3</td>
</tr>
<tr>
<td>Data incomplete</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-op. deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deaths, others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>137</td>
<td>100</td>
<td>258</td>
</tr>
</tbody>
</table>

*Per cent of survivors only.
†Both sexes combined.

Table XXV.—Comparison of Non-tumour Cases with Other Series

<table>
<thead>
<tr>
<th>Series</th>
<th>Operated</th>
<th>Not-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>London</td>
<td>Mayo</td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>22-0</td>
</tr>
<tr>
<td>B</td>
<td>13-2</td>
<td>36-7</td>
</tr>
<tr>
<td>C</td>
<td>22-1</td>
<td>20-0</td>
</tr>
<tr>
<td>D</td>
<td>17-6</td>
<td>16-7</td>
</tr>
<tr>
<td>Data incomplete</td>
<td>7-1</td>
<td>—</td>
</tr>
<tr>
<td>Myasth. deaths</td>
<td>7-7</td>
<td>6-6</td>
</tr>
<tr>
<td>Post-op. deaths</td>
<td>7-7</td>
<td>17-0</td>
</tr>
<tr>
<td>Deaths, other</td>
<td>1-6</td>
<td>—</td>
</tr>
<tr>
<td>Total cases</td>
<td>182</td>
<td>30</td>
</tr>
</tbody>
</table>

|         | London   | Mayo | Boston | Baltimore |
|         | A        | 19-7 | 17-7   | 8-0    | 22-0   | 17-5 | 13-0 | 16-0 | 17-0 |
|         | B        | 10-5 | 17-7   | 16-0   | 16-0   | 5-0  | 17-4 | 10-0 | 12-0 |
|         | C        | 23-7 | 17-7   |       | 16-0   | 7-5  | 17-4   |       |
|         | D        | 19-7 | 35-3   | 44-0   | 21-0   | 25-0 | 43-5 | 24-0 | 27-0 |
| Data incomplete | 5-3     | —    | —      | —      | 22-5  | —    | —    | —    |
| Myasth. deaths | 11-8    | 0    | 3-0    |       | 20-0  | 8-7  | 20-0 | —    |
| Post-op. deaths | 7-9     | 2-5  | 11-8   | 24-0   | 42-0  | —    | 2-5  | —    |
| Deaths, others | 1-3     | —    | —      | 1-3    | 2-5   | —    | —    | —    |
| Total cases | 76      | 17   | 25     | 19     | 40   | 23   | 25   | 41   |


Grob (1953) agrees with the present author in his grouping of male cases with or without operation except for the considerably higher mortality rate. Considering the numbers involved the female series are in reasonable agreement, but the mortality rate of the operated series is much...
higher. Unfortunately, no details are given to show how much of this represents operative risk, but the high proportion of thymomas may be responsible. 9 of the 44 operated patients (sex not distinguished) had thymomas and 6 of these "died of myasthenia gravis." Removal of these would lower the combined sex mortality from 34 per cent to 10 per cent. On the contrary, only 4 of the 41 not-operated deaths were thymomas (and 2 surviving tumours were known) giving a corrected combined-sex mortality rate of 33 per cent (37 of 112). These figures are again similar to those reported here.

To summarize, there is general agreement on the mortality rate and extent of remission in operated and not-operated series and that differences in assessment of the remainder can be attributed to differences in methods of classification or to errors of sampling in small series. All four centres agree that thymectomy results in a worthwhile improvement in prognosis for women, particularly in the reduced mortality rate, but the three American centres do not consider that males are significantly benefited by operation. On first glance at Table XXV this conclusion would appear justified, but close inspection suggests that the conclusion may be premature since the male mortality in the operated series of Eaton and Clagett (1955) is entirely an operative mortality and in Schwab and Leland's (1953) it is predominantly so.

It will further be recollected that 32 per cent of the operated males in the latter series had thymomas (double the incidence in females). Grob (1953) does not detail the distribution of the thymomas which formed 20 per cent of his entire operated series and contributed 60 per cent of the deaths. In the light of these considerations, the American position cannot be sustained until analyses of their non-tumour series are provided. Their conclusions are in line with those in Part II of this study and all four series show identical trends (in favour of operation) though no single series reaches statistical certainty. The identical trends in all series are unlikely to be fortuitous. With regard to the influence of age, Eaton and Clagett (1955) since removing thymomas from their series, now agree with Keynes and Ross (1952) and return to their opinion (of 1950), that the age at onset does not significantly affect the response to thymectomy, though earlier (Eaton, Clagett and Bastron, 1953) they had agreed with Schwab and Leland (1953) that the younger the patient at onset, the better the response to thymectomy. The latter authors, however, state that "the high proportion of thymomas in older females (in their series) probably explains their poorer result." The present study now provides further evidence that the non-tumour patients who show the poorest response are a little older than the average, although a direct comparison between the good results and the failures shows no difference sufficient to be of practical value.

An unexpected finding is that the myasthenic deaths occurring despite operation do so in a group whose characteristic age of onset, age at
operation and duration of symptoms before operation are substantially similar to those of the group deriving most benefit. From Table XV it appears that the crucial period is four to seven years from the onset, whether operation is carried out or not, and it is possible that these patients are more difficult to control by neostigmine (Table XIV). The available data do not suggest any method of selecting this unfavourable group.

The beneficial effect of shorter pre-operative duration demonstrated in this paper confirms the findings of Keynes (1946 and 1949) and Ross (1952). Schwab and Leland (1953) agree so far as females are concerned, but not for males. The high incidence of tumours again invalidates their findings. The Mayo Clinic and Baltimore groups have not commented on the influence of pre-operative duration of myasthenia. Eaton and Clagett (1950) also agree that the pre-operative duration is, on average, slightly shorter in those showing marked post-operative improvement (without distinction of sex). Nevertheless, the inference that the incidence of deaths due to myasthenia despite thymectomy would be reduced by selecting young patients with a short pre-operative history is not borne out by the present survey since the average age at onset and at operation and the average pre-operative duration in these patients is actually lower than in the unimproved (D) patients, and sometimes lower than the average of the whole group or even of Group A+B. One would expect that the response to any form of therapy would be a function of the severity of the illness at the time treatment was started, that is to say that the final category would bear some simple relation to the initial severity of myasthenia. The method of assessment used in this and in the other surveys reviewed does not permit deductions of such a nature since the factor of change of status selected for analysis takes no cognizance of the severity of the disease from which that change occurred. The necessity for this approach has been discussed already, but it is worth re-emphasizing that the bald classification of a case as Category C or even B according to the rigid definitions used does not convey how truly dramatic the change may be. Some measure of the severity of myasthenia at the datum point is given by the neostigmine requirements of the patient at that time. It was found (in agreement with Ross, 1952) that the requirements in those ultimately achieving Category A were little lower than the remainder, but certain possible fallacies (discussed in Part II) make this conclusion only tentative. None of the American series allows direct comparison. Eaton, Clagett and Bastron (1950) give criteria for classification of severity, but do not state how it correlates with the final outcome. It is, however, relevant to note that the re-analysis of their non-tumour material which forced them to acknowledge the value of thymectomy was based on cases selected as being of "average severity." Indeed a method of classification based on extent of change in state is likely to be biased against the mild
case in which any change can only be of small amount. This limitation was accepted in the present study as it was thought desirable that any bias introduced should be against thymectomy on the grounds that the onus of proof rests on the proponents of radical forms of treatment. The "devil's advocate" has nevertheless confirmed their claims that a significant saving of life results from removing the non-malignant thymus gland of patients with myasthenia gravis and that the survivors have a better chance of complete and lasting remission or of great improvement in myasthenic symptoms, particularly if operation is performed within four years of the onset of symptoms. It is perhaps not out of place to add that the extent of this improvement may be very great. It is usually permanent, though a few relapses have been noted, and it is common for improvement to be progressive for several years after operation. For the present purposes Category D has not been subdivided, but physicians considering subjecting patients to operation should know that most of the patients in this category were "unchanged," very few became worse after operation. Unfortunately, apart from duration of symptoms, this study (and parallel unpublished observations) has not shown criteria which would allow one to predict the effect of thymectomy in the individual case, but this writer is satisfied that although the chances of significant improvement, or arrest, are not overwhelmingly superior to those of non-surgical treatment, they are sufficiently great and the extent of the improvement so potentially spectacular that the operation should be seriously considered in all cases in which myasthenia is not confined to the ocular muscles. This qualification seems necessary in view of the report of Ferguson, Hutchinson, and Liversedge (1955). These workers put forward the view that the prognosis of myasthenia gravis is not unduly poor with medical management. They traced 69 of 75 cases seen over a period of twenty-two years and only 9 of these had died (2 with thymomas). Of the 60 survivors 42 were well controlled with medical treatment. They pointed out that in 27 of these (36 per cent of the total material) the myasthenia had remained confined to the ocular muscles. It is apparent that they have seen a much higher proportion of ocular myasthenia than occurs in the practice of the National Hospital (Tables IV and XXI).

Discussion

Seventeen years have passed since Blalock (1941) reawakened interest in the role of thymectomy in myasthenia gravis, yet the value of the operation is still debated. The apparent lack of agreement between the few centres with wide experience of thymectomy has caused those looking for guidance to assume that the evidence was too equivocal to carry conviction (Lancet, 1934). Keynes has pointed out on numerous occasions that the apparently poor results in some of the American reports were due to the failure to separate those patients with thymic tumours from the
Criticisms have been made of the method used in this and the earlier surveys. The deficiencies of the method are clear and are freely acknowledged. It is obvious that in a disease of protein manifestations without measurable characteristics any assessment of disability on an absolute scale would be misleading if not impossible. Nor would it be possible for even a single observer to maintain rigid standards of severity for a disease of rare occurrence. It is, however, comparatively easy by the normal means of clinical investigation for any physician to decide whether or not a patient has improved significantly from a given point in time and, if the criteria are rigidly defined and the categories are not too narrow, any experienced observer can assess the degree of improvement. Two difficulties do arise. Firstly, there must be reasonable certainty that the original diagnosis was correct. In the present study this was assumed if the diagnosis was confirmed by an experienced neurologist and a record fulfilling certain criteria was available for study. The second difficulty is more serious: Since the basis of classification is a change in clinical status it is obvious that the extent of this change may be little or great, depending on the severity of the disease at the time used as reference point. A little consideration will show that this factor cannot influence Category A and probably would not materially affect Category D, but may substantially affect the relative distribution between Categories B and C. As this effect is a random one it should not be a serious matter in a large series, but it may well explain some of the anomalies in the smaller series.

Further criticism encountered during this work has been the time-honoured "you can't reduce a complex disease in a living subject to a number." This has been answered in an admirable way by Herdan (1955) who writes:

"Some clinicians are not sympathetic to methods tending to condense the information on a patient's hospital record by disregarding certain details, in order to make the material amenable to statistical methods. They consider this inferior to working with the complete picture of the case. But such a view rests upon the illusion that our intellect is capable of integrating all the data on a patient's card or even on a number of such cards, into one clear-cut conclusion without neglecting any details. Unfortunately, our mind is not made that way. Combination of observations—and without it no conclusion can claim the slightest degree of generality—implies neglect of details. Since no two cases are completely alike they can for the purpose of combination be made alike only by dropping unnecessary detail. What statistics offer in this respect is a legitimate method free from arbitrariness, by which to replace the highly subjective method of the man who labours under the illusion that he never
neglects a detail.” No other way of assessment is open. It is not sufficient to quote a case of spectacular cure of myasthenia gravis or one in which no benefit resulted without adding the (statistical) statement that such a result may be expected to occur in a certain proportion of trials. Nor would the statement be complete without the addition of the further information of the extent to which such a result differed from that to be expected from other modes of treatment.

The last statement raises the question of “controls.” For the present purposes a series of patients treated medically by the same group of physicians was, faute de mieux, regarded as an index of the expected outcome of myasthenia gravis when treated without thymectomy, but in all other respects in the same manner. In the event, it became clear that this comparison could not be sustained since there were marked dissimilarities in the composition of the two series (“operated” and “not-operated”). This has been countered by demonstrating that the statistics of the total material are substantially the same as those of a selected sample of each group in which age and sex were comparable. It was not, however, possible to ensure that previous medical treatment was the same in both groups as the majority of the not-operated series were patients treated between 1934 and 1941 when neostigmine was customarily prescribed in lower dosage than is now practised. Two of the American series have attempted to obtain a control series of “not-operated” cases by selecting for their unoperated group a case believed to match in every respect each one in the “operated” series. The principle is good and it is probably the best method where numbers are high and operation has been advised for all cases without conscious selection. The writer does not believe that the method is at all reliable where numbers are small, operation has been selective, and the disease is so variable as to make matching in every respect a virtual impossibility. Furthermore, the “control” series is already selected by the fact of survival. It does not seem likely that if two subjects had similar clinical manifestations, but one died at one year from the onset of myasthenia, while the other survived to two years, the former would be chosen to pair a third “identical” subject operated on at nine months from the onset and still surviving at two years. Yet there would seem no reason why at the relevant time nine months the second patient should be considered a better match than the first. Without first-hand evidence no more can be said, but it may well be that considerations of this nature explain the very low mortality rate of Eaton and Clagett’s (1955) “controls” which differs so strikingly from that reported from the other centres. These authors readily concede the tentative nature of their results in view of the small size of their series. The only satisfactory method in a disease with so many variable factors (e.g. do two paralysed limbs equal one paralysed palate?) is to allocate cases randomly to one or other series and to continue each series until it is sufficiently large for all the variables
to occur without statistically significant difference and thereafter until sequential analysis shows that one series differs significantly in its natural history from the other. Unfortunately this was not done in the present instance. The physicians treating myasthenia gravis in London were sufficiently impressed by the results of the early cases which they had submitted to Keynes for thymectomy to wish to carry on in the belief that it was in the interests of their patients to do so (Proc. Roy. Soc. Med., 1946). No controlled trial was instituted at that time and it is doubtful if such a measure would now be ethically justifiable. Though not scientifically elegant the results reported here show that (in a series sufficiently large to reduce the effect of random variations) there is a great saving of life in patients subjected to thymectomy even when the hazards of operation are included, and further that a greater proportion is greatly improved at the end of a comparable period than is the case where the non-tumour thymus is not removed. This difference has been shown to be greater than can be attributed to chance in the female sex and the trend in males is the same, although the level of statistical significance is not reached. No marked sex difference could be established in either series. Slightly more women than men were in Categories A and B after operation and fewer died of myasthenia. Analysis shows that this could have occurred by chance and so does not conflict with the impression of Keynes (personal communication) that the results are equally good in both sexes. On the other hand though the sexes were again very similar in the not-operated series the trend was quite different since women had a slightly poorer prognosis than men. The change in prognosis for women when thymectomy is performed is greater than can be explained by chance. This conclusion agrees with Schwab and Leland (1953) and Eaton and Clagett (1955), thus reconciling theirs with Keynes' apparently conflicting point of view. The inclusion of tumour cases in the Boston and Baltimore series and the small size of the Mayo Clinic series have been shown to introduce fallacies which make more direct comparison impossible, but it should be remembered that two of these series agree with the writer that the benefit from thymectomy may be shown by males though statistical analysis does not exclude the possibility of chance. It would seem worth while appealing to other centres to reclassify the small numbers involved according to the criteria stated in this paper (since no others have been defined) in order that a unified body of data might be available to test those points where statistical confirmation is lacking because of shortage of cases.

**Summary**

A long term follow-up survey of 404 patients with myasthenia gravis is reported and the value of thymectomy compared with medical treatment alone. The 47 patients with thymic tumour are considered separately. The cases were classified according to definite criteria with respect to
change of status from the pre-operative period or the time of first entering hospital in the not-operated series.

In Part I the "operated" and "not-operated" series are compared with respect to sex distribution, age incidence at onset of myasthenia, length of survival from onset of symptoms, and incidence of "myopathy," "goitre," and "ocular myasthenia" (as defined).

Part II analyses the assessment of non-tumour cases. It is concluded that significantly fewer women die of myasthenia gravis if their thymus is removed than would be expected if they were treated with neostigmine only. The number of women very greatly improved ten or more years after the onset of the illness is significantly greater. Similar trends are noted in men but the benefit in favour of operation is not so clear. It is suggested that without operation women have a poorer prognosis than men but after thymectomy they have a better outlook and that the extent of this change explains the more obvious advantage of thymectomy in women.

No clinical indication of the type of cases likely to be helped by operation is noted. It is confirmed that the best results are found in a group of cases with younger mean age at onset, shorter pre-operative duration of myasthenia, and younger mean age when operation was carried out. The age differences were not sufficiently marked to be of clinical value. There is strong evidence that a good result is most probable if the myasthenia has been present for less than five years at the time of operation though some excellent results were noted where the duration was much longer. On the other hand, the pre-operative duration of those who subsequently died of myasthenia (after the post-operative period) was no different from the average. The age-at-onset and at operation was average in women who died but lower than average in men. The female myasthenic deaths were also of average age-at-onset in the unoperated group but the corresponding males were significantly older than average, due to a relative excess of older men in the unoperated group. Death from myasthenia tended to occur four to seven years from the onset in each series. During life these patients required more neostigmine than those who survived but there is no clear evidence that the severity of myasthenia materially affects the final state of survivors.

The duration of follow-up of both series was the same and did not influence the category. There was no indication that a good response was temporary (though a few relapses after early improvement were noted). The main conclusions were confirmed in part of the series omitting the extremes of age in which the case material was more homogeneous.

The effect of thymic tumour is presented in Part III. Myasthenia gravis in the presence of thymoma differed from the non-tumour group in the later age of onset (average 40 years) and the severity of symptoms which were difficult to control with neostigmine. The incidence of myopathy and
thyroid disease was the same but myasthenia never remained confined to the ocular muscles. The operative death-rate was high and combined with a subsequent mortality from myasthenia to give a poor prognosis which was little better than that obtained without operation. Nevertheless attention is drawn to the possibility of excellent response with long survival (even without radiotherapy though Keynes (1955) stresses the desirability of it). The total survival of those who died of myasthenia was closely similar to the non-tumour group.

In Part IV the other major series in the literature are analysed because of apparent discrepancies. It is submitted that the differences are due to (1) failure of other workers to report thymoma cases separately; (2) different criteria for classification; (3) methods of selection for operation; (4) selection of unoperated cases for “controls.” A tabulated comparison of series is made with the original reports adjusted to match the criteria of this paper as closely as possible. It is shown that the results of all series are then similar, allowing for the sampling errors of small series.

CONCLUSIONS

(1) There is a substantial chance of improvement after thymectomy in all cases. This is most evident, and the saving in life is greatest, when the duration is less than five years and no thymoma is present.

(2) After seven years from the onset considerable improvement after operation is less likely though it may still occur, but the risk of death from myasthenia is less whether operated or not.

(3) Improvement occurs in both sexes and the ultimate distribution of categories is the same but the extent of improvement is most significant in women as they would otherwise have a poorer prognosis than men.

(4) The prognosis for life remains poor if a thymoma is present, though pre-operative radiotherapy may be beneficial. Only one case in three survives but the improvement in myasthenia may then be as great as in non-tumour cases.

(5) The maximum improvement occurs in patients who first show symptoms at an earlier age and who have their operation younger than the average but the difference is insufficient to influence the selection of cases for operation. Death from myasthenia is more probable in cases requiring large doses of neostigmine but if they survive the ultimate category after operation is not influenced by the pre-operative severity.

ACKNOWLEDGMENTS

I am grateful to Dr. E. A. Carmichael who suggested this survey and provided the facilities. The work was carried out during the tenure of a Clinical Research Fellowship of the Medical Research Council. Sir Geoffrey Keynes kindly invited me to extend the survey to include cases operated on by him at St. Bartholomew’s and New End Hospitals, London.
I should like to thank the Medical Committees of these hospitals and of the National Hospital for permission to study their records. Dr. James Brown gave great assistance in tracing cases and the help given by almoners, family doctors, and the Registrar-General's staff is responsible for the large numbers traced. All would have been impossible without Miss Joyce Mayo.

REFERENCES

med. Ass., 117, 1529.
Chicago, 61, 467.
Proceedings of the Royal Society of Medicine (Section of Neurology) (1946) Proc. R.
Ross, R. T. (1952) Lancet, 1, 785.
MYASTHENIA GRAVIS: A NEW HYPOTHESIS*

John A. Simpson

Neurology Unit of the University of Medicine, Northern General Hospital, Edinburgh

In a part of the world which has now adopted more sophisticated methods of brain washing, it was once the custom to expose those with unorthodox ideas to a trial by ordeal which consisted of the chewing of the Calabar bean. Today I wish to present you with some unorthodox ideas so let me start by asking you to throw away the Calabar bean, or at least to keep its active constituent, physostigmine, out of sight, for I believe that this magic bean, or its synthetic competitors may have blurred our vision of the true nature of myasthenia gravis.

First let me pay tribute to Mrs Honyman-Gillespie who has made these lectures possible and the Post-Graduate Committee who invited me to contribute to this famous series. The first lecture was given by Edwin Bramwell (1938) on the contributions of the Edinburgh school to the study of the reactions of the pupil of the eye. From him I learned that the mydriatic action of the Calabar bean was discovered by Sir Thomas Fraser in 1863 when he was professor of materia medica in Edinburgh and a physician to the Royal Infirmary, and introduced to ophthalmological practice in the same year by the young Argyll Robertson in a paper read before the Medico-Chirurgical Society of Edinburgh.

Collier (1930) attributed the first British recognition of myasthenia gravis to this same Edwin Bramwell when he was starting his illustrious neurological career as a house-physician at Queen Square. Though first described by the Englishman Thomas Willis in 1672, the syndrome was unrecognized until the magnificent papers of Erb (1879) and Goldflam (1893). Indeed, Bramwell’s father, Byrom Bramwell, gives an excellent description of a case in his famous Atlas (1892a) but could not name it, and the illustration reproduced in Figure 1 is almost certainly a myasthenic child. Edwin Bramwell with Campbell reviewed the known cases in 1900 (Campbell & Bramwell, 1900) and recognition became more common from that time on. If some of the facts I shall present are unfamiliar it is humbling to record that almost all are present in that instructive review or in the interesting paper by their colleague Buzzard (1903). They have been ignored, and many later observations discarded because they are difficult to reconcile with views which have been current since attention was focussed on the phenomena of neuromuscular transmission which is presumed to be disturbed in myasthenia gravis.

I would like you to forget all that you have been taught or presumed with regard to myasthenia and look with me at the symptoms and signs present in a series of 440 cases which I have been privileged to examine through the kindness of colleagues in Edinburgh, London and Glasgow. Let us not decide what is ‘relevant’ until the whole picture is before us. I shall draw some novel conclusions from this analysis, leading to a new hypothesis. This will probably require modification in detail but has the double merit of incorporating all the clinical phenomena without exception, and of suggesting completely new lines of inquiry. In the closing part of the lecture a necessarily brief account of the pathophysiology and pharmacology of myasthenia will be given to demonstrate that the hypothesis is compatible with the known facts.

*A Honyman-Gillespie Lecture delivered on 28th April 1960.
Fig. 1. Remittent ophthalmoplegia of uncertain aetiology, attributed to syphilis. Note the bilateral ptosis and drooping of the mouth (Bramwell, 1892b). (Reproduced by kind permission of Edinburgh University Press).

Fig. 2. Distribution of age at first myasthenic symptom.

**NATURAL HISTORY**

Myasthenia gravis is twice as common in women as in men. The ratio is higher in the first three decades and reverses in the sixth decade, being more common in males in the later part of life, so that the mean age at onset of the disease is slightly less in women (26 yr.) than in men (31 yr.), but the modal age is about 20 years for both sexes (Simpson, 1958).

The smoothness of the age distribution curve (Fig. 2) does not suggest that we are dealing with a miscellany of diseases with myasthenic fatiguability as a common symptom.

The picture of generalized myasthenia is well known (Fig. 3). There is ptosis (often asymmetrical), diplopia, facial weakness with a typical vertical 'snarl' on showing the teeth. Weakness of the jaw and neck muscles cause...
Myasthenia Gravis

Fig. 3. The myasthenic 'snarl'.

the characteristic posture of the myasthenic, sitting supporting head and jaw with her hand. The limb and trunk muscles may be weak. Weakness of the tongue and laryngeal muscles causes dysarthria. Involvement of skeletal muscle of the upper pharynx and oesophagus causes dysphagia. Vocal cord and respiratory paresis cause the voice to fade and breathlessness to interfere with speech and other activities.

Those areas shaded most heavily in Figure 4 are usually first to be involved. They are also the most frequently affected altogether, and usually the most severely involved when myasthenia is generalized. Thus, though the distribution is apparently random and usually asymmetrical in the individual case, a distinct pattern emerges when a large series is analyzed. The most frequently involved are the extra-ocular muscles (including orbicularis oculi) and then the muscles of the neck, shoulder girdle and hip flexors. The proximal limb muscles are more severely affected than the distal. Extensor muscles are more involved

Fig. 4. Percentage of cases in which various muscle groups are affected at the onset (left of key) and at some time during the illness (right of key).
than flexors in the upper limbs, but flexors more than extensors in the lower limbs which are usually less severely affected than the upper. Trunk muscles, other than erector spinae, are least affected. Fortunately few patients show the generalized distribution. It is much more common for the disorder to involve a group of muscles, a single muscle, or even part of a compound muscle (e.g., extensor digitorum longus) and the initial weakness may affect any muscle. Seven patients had unilateral facial palsy which was considered to be Bell's palsy for several months until further weakness developed. Four patients had sudden severe inspiratory dyspnoea as the first symptom. Commonly the complaint is related to a muscle fatigued by a particular movement required by the patient's work. Systematic examination may reveal unsuspected weakness or fatigability of other muscles but examination must be thorough and include contraction maintained against resistance for an adequate period. If this rule is strictly followed I believe that myasthenia is less frequently confined entirely to the extra-ocular muscles than other writers have claimed. Certainly the present series gives but scanty support to the statement that myasthenia is commonly confined to these muscles. Grob (1953) states that if weakness has not spread from the extra-ocular muscles to others in 2 years it is unlikely to do so.

Weakness increases with repeated use of a muscle or with long-maintained contraction. If the patient is asked to gaze upwards the eyelids gradually droop. Eventually she blinks and immediately the ptosis lessens and may disappear. But it again reappears on refixing the gaze, and each time this happens the fatigue appears more rapidly until finally no improvement occurs. Exactly the same happens to the outstretched arms. The slow drooping followed by the cycles of sudden drop and renewed effort until fatigue is complete, looks like a hysterical manifestation to the inexperienced. Electromyography shows that the patient is taking advantage of posttetanic facilitation accumulating during a brief rest to restore full activity temporarily. A similar phenomenon in the extra-ocular muscles causes coarse nystagmus which may be monocular. The patient, if observant, will state that the object she is looking at 'suddenly slips'. The reflexes are often unusually brisk (indeed ankle clonus is sometimes observed) but may be fatigued if elicited repeatedly.

This short-term fatiguability is highly characteristic of myasthenia. It is not surprising that facilitative compensation should become less effective after the day's activities, and most patients do complain of increased weakness towards evening, but this is not so invariable as is often believed. Indeed one of the major difficulties in treatment is due to the fact that the patient is often extremely weak on first waking in the morning. This is not confined to those requiring frequent dosage who might be deprived of their drug during the night, as it occurs before treatment is started. Indeed I have had ten patients (eight of whom were males) who have noticed that they improved as the day advanced, and two actually used exercise as a means of increasing their strength. This is probably associated with the 'de-curarization' phenomenon which will be described later (Simpson & Lenman, 1959).

Permanent weakness, not responding to neostigmine, and atrophy of muscle are much commoner than generally believed (10% in this series, [Simpson, 1958]). This is the so-called 'myasthenic myopathy'. It may affect any muscle. The triceps and the facial distribution, named after Kinnier Wilson though previously described by Erb (1879) and Buzzard (1905), was only found in six cases (Fig. 5). The extra-ocular muscles quite frequently become unresponsive to neostigmine, and the triceps brachii and ilio-psoas next most commonly. Electromyographic and histological changes in these 'myopathic' muscles are indistinguishable from polymyositis.

The onset is often insidious and progress slow or rapid, but in some it has a very sudden beginning. It commonly follows a febrile disease, usually an upper respiratory infection. Only a little less common is precipitation by an emotional upset and this can be most dramatic. One patient, thrown to the ground by blast in the blitz, was unable to rise again. Another developed ptosis and diplopia the day after an accident at work, in which he was unharmed but was certain he was about to be killed. Two women had ptosis for the first
time at their wedding, and one when she found evidence of her husband’s infidelity. Very many attributed the onset to anger, quarrels, worry etc. with details too circumstantial to discount. All had myasthenia from then on, (as one woman ruefully remarked ‘girls with myasthenia shouldn’t fall in love’). A few women first had symptoms during pregnancy, and four cases were detected by abnormal response to relaxant drugs used by anaesthetists.

Later relapses were attributed to the same factors, but other causes were the pre-menstrual period, cold, warmth (especially a hot bath) and allergy to shell fish (two cases) and one case followed soon after inoculation. Sunlight often caused ptosis and blurred vision, and a few patients asserted that it also caused generalized weakness.

There can be quite surprising variation from day to day and even hour to hour. Physical exertion is certainly a factor, but I believe that the emotional state is more important. This raises the question of remissions. Remissions are generally considered so characteristic of myasthenia as to cause difficulty in evaluating therapy. It is quite true that sudden improvement can occur, but complete remission lasting for more than a month is seen in less than half of the patients, and more than one long remission is very rare. Furthermore, most of the ‘worthwhile’ remissions occur during the first 3 years, though there are notable exceptions to this rule. This is the period which sees most of the deaths from myasthenia (especially the first year of the disease) and thymectomy, to be beneficial, is best done during the first 5 years (Simpson, 1958). The impression I have is that the ‘active’ stage of the disease is limited to this period and the subsequent course depends on the extent of the damage occurring then. This is not to say that the patient is out of all danger if he survives for 5 years. On the contrary I am impressed with the ‘brittleness’ of the remissions and the delicate balance which takes place. Sudden death may occur from apparently trivial respiratory obstruction, and many examples could be quoted. Nevertheless these catastrophes are due to the precariousness of an adaptation which is sufficient for normal existence without permitting mobilization of reserve muscular power in emergency. They are not, in my opinion, usually due to sudden relapses or to a progressive disease process, though second waves of renewed activity do occur sometimes, particularly after upper respiratory infection or renewed emotional stress.

Pain is quite common in weak muscles, usually an ache which is presumably due to the extra effort required to maintain posture. This often causes headache, pain round the eyes, backache etc. Sometimes the muscles are actually tender, and pain has been noted to persist even while resting in bed. One is irresistibly reminded of polymyositis. A few patients have complained of mid-sternal pain, often on stooping, which is sometimes associated with palpitations. Other sensory symptoms are rare but cannot be ignored as they have been present in other series (Harvey, 1948). Most often there is tingling of the hands, thighs, or face, sometimes unilaterally. I have usually considered that there was a mechanical explanation, as from shoulder-girdle drop, or a misinterpretation by the patient of muscle ‘stiffness’. Deafness, often variable, sometimes occurs, from Eustachian block in pharyngeal paresis or an unusual
deafness for low frequencies which may be caused by paralysis of the tensor tympani. If the stapedius muscle is weak the patient has hyperacusis. Some authors describe objective sensory changes including a few cases of loss of taste or altered olfactory sensation which are difficult to explain (Alajouanine et al., 1957). Epilepsy was present in two of my cases and unexplained blackouts in another three. These were thought to be coincidental, but Hoefer et al. (1958) found an unusually high incidence of epilepsy associated with myasthenia. Raised protein in the cerebro-spinal fluid was present in nine cases, but the fluid was only rarely examined. There is a substantial collection of similar cases in the literature.

Tables I and II list some disorders which were present in these cases, but it should be observed that they were noted in their case records by various observers or by myself when studying the value of thymectomy and no systematic attempt was made to record the true incidence of conditions to which I then attached no significance. For this reason no attempt was made to express the figures as percentages.

Table I. Associated conditions in 440 patients with myasthenia gravis.

<table>
<thead>
<tr>
<th>Association</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma (present at onset of myasthenia)</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse lupus erythematosus</td>
<td>1</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
</tr>
<tr>
<td>‘Rheumatoid’ arthritis</td>
<td>16</td>
</tr>
<tr>
<td>Acrocyanosis</td>
<td>12</td>
</tr>
<tr>
<td>Neuritis</td>
<td>2</td>
</tr>
<tr>
<td>Allergy (precipitating myasthenia)</td>
<td>3</td>
</tr>
<tr>
<td>Sensory phenomena, other than pain</td>
<td>11</td>
</tr>
<tr>
<td>Epilepsy and recurrent blackouts</td>
<td>5</td>
</tr>
<tr>
<td>Psychosis</td>
<td>8</td>
</tr>
<tr>
<td>Cerebrospinal fluid protein raised</td>
<td>9</td>
</tr>
</tbody>
</table>

If one defines myasthenia gravis as a disorder of neuromuscular transmission it is obligatory to consider these phenomena as irrelevant coincidences, especially as the frequency of any one is too low to be statistically significant. But it may be more profitable to consider the possibility that the various phenomena are related to the muscular disorder.

Table II. Disorders of blood and reticulo-endothelial system in 440 patients with myasthenia gravis.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure red-cell aplasia</td>
<td>1</td>
</tr>
<tr>
<td>Normocytic anaemia</td>
<td>3</td>
</tr>
<tr>
<td>Microcytic anaemia</td>
<td>3</td>
</tr>
<tr>
<td>Macrocyclic anaemia*</td>
<td>4</td>
</tr>
<tr>
<td>Generalized lymphadenopathy</td>
<td>1</td>
</tr>
<tr>
<td>Splenomegaly (autopsy)</td>
<td>3</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Hyperglobulinaemia</td>
<td>4</td>
</tr>
</tbody>
</table>

* No marrow smear but clinical diagnosis of pernicious anaemia based on peripheral blood, achlorhydria and therapeutic response.

It is interesting to note that all the disorders listed in Table I and many of those in Table II were noted as manifestations of diffuse lupus erythematosus by Harvey et al. (1954). Indeed the precipitating factors and the age and sex distribution already discussed are exactly those found in that disease by these authors. It may then be significant to note that Rowland (1955) found lupus erythematosus cells in a case presenting as myasthenia gravis while Harvey et al. (1954) found myasthenic symptoms in three cases of diffuse lupus erythematosus. A list of cases is rapidly accumulating of myasthenic symptoms in other connective tissue-muscular disorders such as dermatomyositis, sarcoid and carcinomatous neuropathy. One patient in the present series had sarcoid at the onset, one had diffuse lupus erythematosus and three patients had neoplasm present or diagnosed soon after the onset of myasthenia. Can it be that myasthenia is a local manifestation of a disorder of the same type as diffuse lupus erythematosus, in which other tissues may sometimes be involved?

Myasthenic phenomena have occurred after prolonged dietary deficiency (in the Japanese ‘Kubisagari’ and in prisoners-of-war [Denny-Brown, 1947]). An outbreak in France was attributed to the chewing of tobacco infected by Clostridium perfringens (Coulonjou & Salaun, 1952). This organism
usually causes severe myositis (gas gangrene) but is related to Clostridium botulinum. Myasthenic features in anterior horn cell, peripheral nerve and muscular disorders have been discussed by Simpson and Lenman (1959).

The evidence that myasthenia gravis is a local manifestation of a disorder of variable aetiology, but often allergic, which may occasionally affect other tissues is too scanty to be dogmatic, but an interpretation of this type would satisfactorily account for all the clinical features so far discussed. Red cell aplasia (Table II) is in a slightly different category. This has been noted in patients with thymic tumour even if unaccompanied by myasthenia though more often with it than without. The first case with myasthenia was described by Wintrrope (1946) and the present one was reported in detail by Chalmers and Boheimer (1954). Some patients with thymoma have depression of other marrow elements and agammaglobulinaemia (Lambie et al., 1957).

The erythrocyte sedimentation rate is usually normal in myasthenia, and total serum proteins are within the normal range. Lowered albumin and raised γ-globulin may be demonstrated by electrophoresis (Lowenthal & van Sande, 1956), but there is some technical argument about the validity of the observation. Some of my cases have had slight elevation of γ-globulin. Blood chemistry is otherwise normal, though some authors describe abnormal glucose tolerance. I found ten patients with glycosuria. Many of these, and others with normal glucose tolerance, had a family history of diabetes.

There have been many papers devoted to the relationship between myasthenia and thyrotoxicosis. This has obscured the fact that most of the early reports stressed the presence of non-toxic nodular goitre, and the histological appearance of lymphadenoid goitre. My material has a higher incidence of thyroid disorders than usually reported (females 18%; males 10%) but most of these were either non-toxic at all times, or had a very brief spell of toxicity, lasting only a few weeks or months, but often leaving traces of exophthalmos, with thick puffy eyelids. Some patients have the ocular changes usually associated with thyrotoxicosis but without goitre or abnormal basal metabolic rate at any time. There are cases reported with myasthenia gravis and myxoedema who had never had a thyrotoxic phase.

When toxic goitre does occur in myasthenia its appearance seems quite unrelated in time. They may coincide, but either may precede the other by many years and indeed antithyroid treatment may make myasthenia worse. These facts make it unthinkable that thyroxine can cause the myasthenia, but would strongly favour a pituitary factor, since all these thyroid states, toxic or otherwise, could be associated with excess pituitary secretion. I have already commented on the fact that a family history of diabetes is not uncommon, but this is a common disorder. An observation which I believe to be new is that there is a high incidence of thyrotoxicosis in close relatives of myasthenics, even those without thyroid disorders. This relationship appears to be mainly, but not exclusively, on the maternal side. It is not, perhaps, well known that there is quite substantial evidence for a genetic factor in exophthalmic goitre. It would be reasonable to suspect that this acts by a pituitary hormone and perhaps it may express itself in other ways such as myasthenia gravis. I do not say ‘thyrotrophin’ since there is some evidence of a separate factor related to it which acts on muscles, especially the extra-ocular ones. It might be a growth hormone. Such a hypothesis would account for the reports of myasthenia gravis associated with acromegaly, diabetes, Addison’s disease, and perhaps for the effects of pregnancy, menstruation, and of the emotions acting via the hypothalamus.

Pregnancy has some sort of influence on myasthenia, but it is quite unpredictable. In general, remissions occur in the first trimester and relapses at the time of birth or during the puerperium, but some patients are worse during pregnancy and remit afterwards. Successive pregnancies may have different effects. If a hormonal influence is present it must be one of the factors involved. Schrire (1959) has recently reported low pregnandiol excretion in myasthenia, which is corrected by thymectomy. As the recovery of pregnandiol in the urine after injection of progesterone was also low it is difficult to attribute
vestigations being
by Dr K. Fotherby do not appear to confirm Schire's findings.

Interestingly enough the endocrine disorders referred to above are, like myasthenia, associated with lymphoid hyperplasia of the thymus. Comsa (1958) has confirmed that growth hormone causes enlargement of the thymus and suggests that it may be a necessary target organ through which the pituitary acts in controlling growth. Everything points towards the pituitary, but, despite a few reports of eosinophil changes or adenomas in that organ, most pathologists find it without abnormality.

If a genetic factor is involved—even if only for setting the stage for a myositis reaction—one would expect occasional evidence of familial myasthenia and this is indeed the case. I have seen three such families. One of these had the syndrome termed 'congenital myasthenia' in which there is a high incidence of ptosis and ophthalmoplegia but rare involvement of other muscles. Classical myasthenia rarely occurs in more than one person in a family, though I have examined two families with cousin and aunt involvement. On the other hand, one myasthenic girl in this series had an identical twin who is normal. Other workers have had the same experience.

These familial cases, like myasthenia occurring in young people, show no special tendency to remit.

The position is completely different in 'neonatal myasthenia', which is the name given to the myasthenia which sometimes affects the baby of a myasthenic mother. It may be serious and cause death if unrecognized as a cause of weakness, but if the child survives, with or without treatment, the weakness clears up within 6 to 7 weeks and never recurs. The suggestion of transplacental passage of a toxic substance is irresistible.

PATHOLOGY

The lymphocyte infiltrations of muscle described by Weigert (1901) were termed lymphorrhages by Buzzard (1905) to emphasize his belief that they originated from small blood vessels. He showed, and others have confirmed, that they are usually present if a search is sufficiently thorough, though they may decrease in number and size as time passes (Fig. 6). Buzzard's demonstration of similar foci in other organs, including the adrenals and pancreas, is repeatedly confirmed in the literature and in my own material but dismissed by most pathologists as 'nonspecific'. So too, the associated degenerative changes of muscle fibre described by Buzzard (1905) and recently re-investigated and classified by Russell (1953) are considered by the latter to be nonspecific.

Professor Russell described three types of muscle change. Type 1 (Fig. 7) is an acute coagulative necrosis of the muscle fibre with eosinophilic change, loss of cross striation, and inflammatory cellular reaction leading to phagocytic removal of the fragmented muscle.

Fig. 6. Photomicrograph of skeletal muscle from patient with myasthenia gravis: lymphorrhage. Haematoxylin & eosin ×210

Fig. 7. Photomicrograph of skeletal muscle from patient with myasthenia gravis: Russell's type I lesion H. & E. ×280
fibre. This process may be limited to one fibre or so widespread as to cause naked-eye changes in the muscle. Type II (Fig. 8) is the lymphorrhage, which the author considers to be secondary to solitary muscle fibre atrophy with basophilia of the cytoplasm and loss of cross striation. Type III (Fig. 9) she considered to be the least specific, being a simple focal muscle change with eosinophilia and swelling but without loss of striation or inflammatory reaction. Similar changes occurred in the myocardium, but never in true smooth muscle. It is quite true that similar lesions are seen in other conditions, notably the rheumatic disorders, connective tissue disease, chronic infections, myositis and endocrine myopathies. The lymphorrhage is found in many of these besides being the characteristic lesion of allergic reactions. Querido (1929) was impressed with a vasculitis which he found associated with lymphorrhages. 'Non-specific' they may be, but in this list one notes many of the conditions already discussed under the clinical aspects. Again one can choose to consider the muscle reactions as 'non-specific' or to look for a common factor.

Special staining techniques applied to the motor nerve endings by Coers and Desmedt (1959) have shown that the neuromuscular junctions may often be abnormal in myasthenia gravis. These authors describe two changes in the terminal arborization of motor nerves (Fig. 10). In one, the 'dystrophic' type, there is increased branching and the terminal knobs are distributed over a wider area of the muscle fibre than usual. This type is probably reactive as the related muscle fibre is usually abnormal and the same type of end-plate has been found in other neuromuscular disorders. In the other type, the 'dysplasic', there are few terminal knobs and these are arranged serially along a scanty number of terminal branches ending on a long end-plate.
region. In their limited experience the authors have not found this type in any other disease. Further work is required to confirm their results for, like the muscle fibre changes already described, they bear no direct relationship to the severity of the loss of function. Nevertheless, the evidence for structural changes in nerve and muscle is convincing and no 'chemical' theory of myasthenia is acceptable which does not take account of them.

Now what of the thymus gland? Weigert (1901) thought that the lymphorrhages of muscle were metastases from a thymic tumour but Buzzard (1905) soon disproved this. Nevertheless, the incidence of thymoma is remarkably high (15—20%) and most cases show some thymic pathology. There has been a great deal of misunderstanding about this.

Some textbooks describe 'thymic hypertrophy', and others 'failure of atrophy' such as a normal thymus is assumed to undergo. Both descriptions are wrong. Some thymus glands removed from myasthenic patients are undoubtedly larger than normal limits but the majority are not, and those with wide experience agree that normal thymic regression may occur with age (Sloan, 1943). But whether large or small, the organ shows increased content of lymphocytes (or thymocytes) in cortex and medulla and there is evidence of active formation of lymphocytes in the presence of 'germinal centres' (Fig. 11). This finding is very characteristic (Castleman & Norris, 1949). The epithelial cells of the thymus are not proliferated unless there is a tumour but in thymomas they may be arranged in cords or tubes, giving the appearance of a secretory tissue. Even then the surrounding non-tumour gland shows the typical 'germinal centres' (Fig. 12). It is interesting then to note the occasional presence (usually in young patients) of hyperplasia of lymph nodes and of lymphoid tissue in the spleen. One patient in the present series died of lymphosarcoma (Table II). Disorders of blood formation and of the plasma proteins have already been referred to. Each of these conditions is rare, but the total picture suggests that the thymus
is participating in an activity of reticuloendothelial type rather than a glandular one in the conventional sense and certainly no evidence of glandular activity can be seen.

Myasthenia has occurred after a thymoma has been removed. A small thymoma associated with severe myasthenia may continue to grow while the myasthenia remits. Operative removal of a thymoma does not usually affect the course of the disease but occasionally does so. Removal of the non-tumour gland, on the other hand, definitely improves the prognosis and saves may lives, but in general only if it is removed within the first 5 to 7 years of the myasthenia and even then the results are unpredictable for the individual and improvement may be immediate or delayed (Simpson, 1958). I have seen patients improve immediately and others not till months later, but improvement may continue for 3 years or more. Why should this be? Why too should improvement be most marked in young women, to the extent of changing their prognosis from one which is poorer than men to one which is better? (Simpson, 1958). Men may improve too, although the statistics are equivocal and their gain is less obvious.

A HYPOTHESIS

These facts do not suggest that the thymus is the source of a toxic substance or an endocrine gland with some necessary function. Rather it may act as an accessory organ in some mechanism, perhaps as a storage organ or possibly (as suggested by Comsa [1958]) in another context) as a target gland of pituitary growth hormone which might atrophy in later life. The thymic-inhibitory effect of the thyroid and adrenal described by that author could be manifestations of the well-known pituitary feedback inhibition. Unfortunately, we know too little about the thymus to do more than speculate. Certainly the organ does not impress one as an endocrine gland, but more as a reticulo-endothelial organ. Metcalf of Sir F. Macfarlane Burnet’s laboratory in Melbourne has recently isolated a factor secreted by the epithelial cells of thymus (in tissue culture) which appears in the blood plasma as a ‘lymphocyte stimulating factor’ (Metcalf, 1956). Though the main source of globulin antibodies is believed to be the plasma cells, the lymphocytes do seem to have some role in immunity reactions.

Fig. 13. Suggested role of pituitary and thymus in controlling growth and differentiation. Differentiation of blood cells and production of protein antibodies may be a surviving remnant of these functions in the adult. In myasthenia the thymus may produce an antibody against normal muscle, especially after upper respiratory infection.

It is interesting to speculate on the possibility that a genetically-determined pituitary hormone acting on muscles might do so via a thymic secretion which releases an antibody carried by lymphocytes (Fig. 13) or alternatively that the thymus reacts ‘allergically’ to the breakdown products of muscle cells damaged by abnormal pituitary secretion or by a delayed-type allergy caused by infection.

During the last 5 years Dr John Anderson of Glasgow and Dr Roland Alexander of Edinburgh have collaborated with me in a search for muscle antibodies in myasthenia gravis, but without success, perhaps because the substance sought is intracellular. We were encouraged to search for better methods on reading the recent report of Nastuk et al. (1959). Searching for a neuromuscular blocking substance, these authors found that blood from myasthenic patients (and a few control
subjects in lesser degree) caused lysis of frog muscle cells. Following up this surprising observation they found that the serum complement activity was within the normal range in most myasthenic patients, but was above or far below the normal range in a few cases and this tended to correlate with remissions and exacerbations respectively.

Last year Smithers (1959), studying the rôle of the thymus and lymphocytes in disease, concluded that they were implicated in auto-immunity and that the thymus changes of myasthenia gravis were strongly suggestive of an auto-immune process. Perhaps, too, the occasional benefit to myasthenics from corticotrophin or cortisone is further evidence of this. The muscle protein may be rendered antigenically 'foreign' by the same type of association with upper respiratory infection as is believed to occur in the rheumatic disorders and in acute nephritis. The postulated pituitary factor might predispose to this by disturbing cell membrane integrity and indeed if protein which is normally confined within the muscle cell were allowed to escape, the auto-immune process would be initiated. It is possible that we have here an example of the influence of the endocrine glands upon immune and allergic responses to bacterial infection demonstrated by Long (1955). Perhaps the place of the thymus in growth is as a regulator of cell-differentiation, a function which becomes unnecessary in the adult except for the formation of blood cells and plasma proteins. This action would include the recognition of 'foreign' proteins, and the reactions appropriate to them, that is the function of the reticulo-endothelial system. One can only speculate where knowledge is incomplete, but I do so without apology because the purpose of this lecture is to suggest new ideas for research on myasthenia gravis.

**PATHO-PHYSIOLOGY**

I have taken you a long way from the current ideas on myasthenia, but not far from those of the earlier workers who knew nothing about neuromuscular transmission or auto-immunity, and little about endocrinology. For my unorthodox ideas I must undergo the ordeal of the Calabar bean. How does the notion of an endocrine or auto-immune disorder combine with the patho-physiology and pharmacology of the disease?

The resemblance of myasthenia to curare poisoning had occurred to Oppenheim (1887) and to Jolly (1895) but it was Mary Walker's (1934) demonstration of the dramatic relief afforded by physostigmine coinciding with Dale and Feldberg's (1934) confirmation of the rôle of acetylcholine in neuromuscular transmission which turned all thinking towards transmission block. The effect of physostigmine and a variety of related synthetic compounds with anti-cholinesterase properties, suggested that a defect of acetylcholine transmission from nerve ending to muscle end-plate was present and would account for the weakness. Jolly (1895) had shown that the fatiguability could be reproduced by faradic stimulation of a motor nerve while the 'fatigued' muscle would still respond fully to locally applied galvanism. Modern electromyography shows that the loss of power is accompanied by a decrement of the evoked action potential of the muscle (Fig. 14). This seemed explicable by three hypotheses:

1. diminished acetylcholine synthesis or release;
2. increased acetylcholine destruction by cholinesterase;
3. competition for receptors by a curare-like substance.

Before discussing these I must describe the finding of the opposite effect—an incremental response to tetanic stimulation—which is not uncommon in myasthenia (Fig. 14) and in polymyositis (Simpson & Lenman, 1959).

Both effects may occur in the same patient. One is reminded of the decurarizing effect of tetanic stimulation when the dose of curare is low. The phenomenon is most simply explained if neuromuscular block in myasthenia is competitive in type. Post-tetanic facilitation, which is characteristic of true myasthenia gravis though not always present, is also strongly suggestive of a curare-type block.

It may be that one or more of the other mechanisms is responsible for the 'symptomatic myasthenias' found in motor neurone disease, polyneuritis, and polymyositis (Simpson & Lenman, 1959).

Perhaps more than one abnormality is present in myasthenia gravis, for instance the histological findings might suggest that both nerve and muscle are abnormal. Normal
Myasthenia Gravis

Fig. 14. Electromyograms from patients with myasthenia gravis: (a) Ulnar nerve at wrist stimulated with supramaximal electric shocks repeated 4, 8 and 50 times per second. Action potential recorded from abductor digiti minimi muscle by surface electrodes shows decrement ('fatigue') with fast tetanization only. Note post-tetanic facilitation at arrows.

(b) The classical response from abductor digiti minimi but the triceps shows a temporary incremental response.

Physiology does not help one to predict the effects which would then occur, but it is desirable to attempt an explanation of the findings in disease in terms of the known normal mechanism. Pharmacological studies have recently added a new body of evidence which has first to be considered.

It has been recognized for some time that the myasthenic patient can be paralysed by an unusually small dose of d-tubocurarine or quinine, but Churchill-Davison and Richardson (1952) showed that myasthenic muscle was extraordinarily resistant to the depolarizing blocking drug decamethonium (C10). A small dose might facilitate, or sometimes a depolarizing type of block might change to a competitive type with response to neostigmine (a 'mixed block'). Grob et al. (1956) showed a similar alteration of the response to choline—one of the breakdown products of acetylcholine. Zaimis et al. (1952) believe that these facts indicate an alteration in the motor end-plate, which is one possible interpretation. Arguments about whether the myasthenic muscle is hypersensitive or hyposensitive to intra-arterially injected acetylcholine have
been shown to be due to usage of massive test doses (Engbaek, 1951). With suitable dosage there is no doubt that the myasthenic muscle has a raised threshold. This, too, makes the acetylcholine deficiency theory unacceptable. The pharmacological results, often seemingly conflicting, are difficult to summarize, but can be harmonized by the following hypothesis (Fig. 15).

It is proposed that the effective condition for depolarization of the end-plate membrane may be the density of ionic charges attached to the postulated receptor substance. Thus an applied substance will be effective only if (1) its ionic charge is adequate (Riker, 1953) and (2) there is an adequate charge per unit of receptor area. If this limiting charge density is not reached no depolarization occurs and the attached charges merely block receptors.

If an adequate charge density appears (probably with certain time factors) the membrane permeability is altered and a 'local response' causes depolarization. If this reaches a 'critical level' it becomes self-completing, an action potential propagates and the muscle twitches. If the charge density rises too high, or the stimulating chemical is resistant to hydrolysis by cholinesterase, depolarization persists causing neuromuscular block of a type which is made worse by anticholinesterase compounds or by the addition of acetylcholine ('depolarization block'). As the concentration of depolarizing substance falls, charge is lost from the end-plate which repolarizes. Inexcitability of the membrane due to 'desensitization' prevents further stimulation (Axelsson & Thes...
left, 1958), but if the ionic charge remains within the 'critical zone' sufficiently long it may facilitate neuromuscular transmission for a brief spell (Grob et al., 1956) until the charge-density falls too low and it enters the zone of 'competitive block' where it will tend to inhibit response to indirect stimulation or to application of further depolarizing chemicals until the 'sticky molecules' (Zaimis et al., 1952) become slowly detached. The two latter phases would be more prominent with large doses and with compounds resistant to hydrolysis. This hypothesis would explain all the phenomena described.

If now, ionic charge-density (note not necessarily the total charge) is decreased, the 'critical level' is raised. The competitive zone is passed too quickly at first to cause clinical signs, stimulation occurs at a higher dose level, 'depolarization block' only occurs with the bigger doses and is soon followed by a prolonged 'competitive block' (the 'mixed response' of Zaimis). Smaller doses merely stimulate and drop rapidly through the competitive zone (C10 resistance etc.). Time factors will differ with different drugs and animal species, but all the reported phenomena can be fitted into the scheme. What light does it throw on the alternative hypotheses?

1. Diminished acetylcholine production is unlikely unless the charge is dispersed spatially. Coers and Desmedt's (1959) histological findings would be compatible with this.

2. Increased cholinesterase would decrease the rate of rise, lower the crest and steepen the falling phase of the curve. The observed results do not suggest that this occurs, and of course increased cholinesterase has never been demonstrated (in muscle or in circulation).

The remaining theories (i) alteration of the end-plate's properties so that it requires a greater charge density and (ii) occupation of some receptor sites by molecules with a negligible charge, would both fit the observations. The first is the 'end-plate abnormality' theory, the second the 'curare-substance' theory. The pharmacological results are compatible with each, or with dispersed acetylcholine receptors. Further studies of this type are unlikely to settle the matter if the physics of the situation are those postulated. Selection between the alternatives must depend on other factors. It may be that more than one factor is present, but when the decurarization effect, the post-tetanic phenomena, the natural history of the disease, and above all the probability of placental transmission of the disorder are taken into account the only satisfactory single theory is the 'competitive-blocking' substance (Table III). This substance must have the unusual property of persistence in the myasthenic baby for several weeks.

Despite claims to the contrary—especially Strümpf's (1954) recent work—there is no satisfactory evidence that serum from myasthenic patients will cause block of normal neuromuscular junctions. Cross transfusion to normal subjects has no effect. Wilson and his colleagues in Liverpool have tried for years to isolate such a substance from the thymus (Wilson et al., 1953). Their early

Table III. The ability of theories of causation of myasthenia gravis to explain the known phenomena of the disease.

<table>
<thead>
<tr>
<th>Theory</th>
<th>'Indirect' tetanus depression (fatigued)</th>
<th>Augmenting Carolus (curare-ized)</th>
<th>Post-tetanic facilitation (Ch. C10)</th>
<th>Pharmacology</th>
<th>Remissions</th>
<th>Placental transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Cholinesterase excess</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>?</td>
<td>0</td>
</tr>
<tr>
<td>End-plate change</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Competitive block</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

433
results with extracts of glands removed from myasthenic patients were most promising but have been criticized as being attributable to potassium in the extract. They have recently isolated a series of substances from the thymus of horse foetuses but they seem to block by depolarization (Nowell et al., 1959). In view of the warning I have given of the different effects occurring with varying dose levels of quaternary onium compounds it would be unwise to dismiss these extracts from further consideration, but they do not seem to have the desired properties. (For instance a sudden relapse should cause spontaneous twitching or a depolarization block and be aggravated by anticholinesterase compounds).

Where, then, are we to look for a blocking substance which must be of competitive type, transmissible through the placenta, with persistence in the child for a few weeks only, but not transmissible to another adult? If one looks at the mechanism of attachment of acetylcholine to receptor protein (Fig. 16) one is immediately reminded of the Ehrlich theory of antibody action. Let us suppose that antibody was developed against the 'receptor substance' of the end-plate protein. Would not this substance have exactly the properties described? All that is required of it is that it should be detachable and should only occasionally lead to lysis of the muscle cell. That is, it should have the properties of an incomplete antibody (odd that the alternative name of 'blocking antibody' should have been coined by the haematologists!). Perhaps a 'complete' antibody, or one modelled to the rest of the muscle membrane gives rise to 'polymyositis'.

Fig. 16. Two molecules of acetylcholine and one of antibody have similar configuration, based on end-plate receptor 'templates'. They will compete for receptor sites.

SUMMARY AND CONCLUSIONS
In summary then, my suggestion is that myasthenia is an 'auto-immune' response of muscle in which an antibody to end-plate protein may be formed. This would have the properties of an acetylcholine-competitive-blocking substance, specific to the individual, and occasionally to the foetus of a myasthenic mother. Nerve endings, muscle fibres, and on occasion the central nervous system might sometimes be involved, in close analogy with diffuse lupus erythematosus and dermatomyositis. Myasthenia gravis is, therefore, a restricted form of myositis. It may be the result of an auto-immune response to an infection, usually of the upper respiratory tract, or the reticuloendothelial system, specifically the thymus, may react to muscle end-plate protein as if it were 'foreign' in disorders of the thymus. The latter is probably under the influence of the pituitary gland, probably by a growth hormone, and this may be a necessary condition for the allergic response to infection. A familial incidence of thyrotoxicosis or diabetes is reported for the first time.

These conclusions have been reached from a detailed analysis of 440 cases and of the literature. No other theory of myasthenia gravis attempts to explain all the phenomena, clinical and experimental. The present attempt is speculative, but I hope it is justified by the many new lines of research immediately suggested by it. Let me close by quoting Huggings Jackson (Taylor, 1958):

'The use of hypotheses if the method of science. To suppose we can make discoveries by the Baconian method is a delusion. A hypothesis or supposition is not a conclusion; it is only a starting point for methodical observation and experiment, the endeavour being not only to prove it, but to disprove it.'

ACKNOWLEDGEMENTS I am most grateful to the medical committee of the National Hospital, Queen Square, London; Sir Geoffrey Keynes; and colleagues in Glasgow and Edinburgh for their generous permission to examine their patients; to Dr A. J. Maloney and Professor W. Blackwood for pathological reports; to Dr J. Anderson (Glasgow), Dr R. Alexander (Edinburgh) for antibody studies; Dr S. L. Tomsett and Dr K. Fotherby for biochemistry and the clinical photography departments of Glasgow and Edinburgh Universities for the illustrations.

Experimental studies have been supported by grants from the Secretary of State for Scotland on the recommendation of the Advisory Committee on Medical Research, and from the Muscular Dystrophy Group.

REFERENCES


BRAMWELL, E. (1938). Upon the pupil reactions—with special reference to contributions by alumni of the Edinburgh school. Edinburgh post-graduate lectures in medicine. 1, 1, Oliver and Boyd

BUZZARD, E. (1905). The clinical history and post-mortem examination of five cases of myasthenia gravis. Brain, 28, 428


COLLIER, J. (1930). Oculomotor palsy resulting from infective and toxic processes. Trans. ophthal. Soc. U.K., 50, 244

COMA, J. (1958). Influence of the thymus upon the reaction of the rat to anterior pituitary growth hormone. Nature (Lond.), 182, 728


DENNY-BROWN, D. (1947). Neurological conditions resulting from prolonged and severe dietary restriction. Medicine (Baltimore), 26, 41


Myasthenia Gravis
RUSSELL, D. S. (1953). Histological changes in the
striped muscles in myasthenia gravis. J. Path. Bact.,
65, 279

SCHRIRE, I. (1959). Progesterone metabolism in
myasthenia gravis. Quart. J. Med., 28, 59

SIMPSON, J. A. (1958). An evaluation of thymec-
tomy in myasthenia gravis. Brain, 81, 112

of frequency of stimulation in neuromuscular
disease. Electroenceph. clin. Neurophysiol., 11, 604

SLOAN, H. E. (1943). The thymus in myasthenia
gravis. With observations on the normal anatomy
and histology of the thymus. Surgery, 13, 154

gland in relation to some general concepts of ne-
plasia. J. Fac. Radiol. (Lond.), 10, 3

STROPPLER, A. (1954). Experimentelle Unter-
suchungen zur Pathogenese der Myasthenie. Z. ges.
exper. Med., 125, 244

TAYLOR, J. (1958). Selected writings of John Hugh-
Books, Inc

WALKER, M. B. (1934). Treatment of myasthenia
gravis with physostigmine. Lancet, 1, 1200

WEIGERT, C. (1901). Pathologisch-anatomischer
Beitrag zur Erbach'sen Krankheit (Myasthenia
Gravis). Neurol. Zbl., 20, 597

2nd ed. p. 572. Philadelphia: Lea & Febiger

ZAIMITIS, E. J. CHURCHILL-DAVISON,
H. C. RICHARDSON, A. R. (1952). Motor end-
plate differences as a determining factor in the mode
of action of neuro-muscular blocking substances.
Nature (Lond.), 170, 617

Some effects of extracts of thymus glands removed
from patients with myasthenia gravis. Lancet, 2,
368

436
THE DIFFERENTIAL DIAGNOSIS AND MANAGEMENT OF CRISIS IN MYASTHENIA GRAVIS
WITH NOTES ON THE USE OF OXIMES IN THE TREATMENT OF CHOLINERGIC CRISSES
Based on communications to the British Pharmacological Society, July 1961
and to the Scottish Society of Physicians, October 1961.

John A. Simpson
Neurological Unit, Northern General Hospital, Edinburgh

The outlook for the sufferer from myasthenia gravis was transformed by Mary
Walker's demonstration of the miraculous, if temporary, restoration of power
caused by an injection of physostigmine (Walker, 1934) and soon after that
by its synthetic homologue neostigmine (Walker, 1935). There is still no
agreement about the basic defect in myasthenia, but general acceptance that
the therapeutic effect of neostigmine and related drugs is due to inhibition
of cholinesterase at neuromuscular junctions, thus potentiating and prolonging
the action of acetylcholine released at the motor nerve terminals. Direct
stimulation of receptor sites of motor endplates and pre-junctional effects
probably exist, but the validity of the anticholinesterase hypothesis of drug
action is not seriously challenged.

It is believed that molecules of acetylcholine released at motor nerve
terminals unite with a receptor substance on the endplate of striated muscle
and this in some way alters the permeability of the surface membrane of the
endplate, permitting a sudden transfer of sodium and potassium ions across
the membrane. When this electrolyte transfer is of a critical value, it causes
a propagated action potential to pass along the muscle fibre and the electro-
chemical change appears to trigger the contractile mechanism of the muscle
fibre. When the electrolyte shift occurs, the ratio of ions on each side of
the cell membrane is altered, destroying (or reversing) the metabolically
maintained imbalance which causes the resting cell to be electrically polar-
ized. The cell membrane is said to be 'depolarizes' at that site and this
depolarization is the essential precursor to muscle contraction. Some restor-
ation of polarization (not necessarily complete) is essential before another
action potential can be propagated and a twitch of the fibre result. Thus the
efficiency of muscle as a machine is increased if acetylcholine is destroyed
immediately it has performed its detonating action, so allowing repolarization
of the muscle cell in readiness for the next packet of acetylcholine trans-
mitted across the neuromuscular junction. If the amount of acetylcholine
available at the endplate is inadequate it may be rendered more effective if
its destruction is delayed by inhibiting cholinesterase, and this would pertain whether the insufficiency is due to failure of production or release of acetylcholine, a raised threshold of the endplate, or a competition for receptor sites (as by curare). This was the rationale for the use of neostigmine in myasthenia gravis. It was not widely appreciated until recently (although pointed out by Kennedy and Wolf in 1938) that the transmitter must be destroyed at a reasonable rate to allow repolarization of endplates for the next action potential otherwise they can not respond to further increments of acetylcholine. This is termed depolarization block and the weakness resulting, when it occurs in man, is "cholinergic crisis".

Clearly the clinical effect of anticholinesterase substances will depend on three factors 1) dose, 2) rate of destruction or excretion of the substance, 3) the state of the particular synapse with respect to the adequacy of its acetylcholine / receptor ratio. The last factor suggests that muscles unaffected by myasthenic weakness will be liable to depolarization block with a dose which is in the therapeutic range for affected muscles. The possibility of causing paralysis by overdosage with anticholinesterase drugs in the treatment of myasthenia gravis has been recognized for some time though mainly with respect to the long-acting organic phosphate drugs such as OMPA and TEPP. Many authors considered that the risk was remote with neostigmine, less than the risk of underdosage. Rowland et al (1955) proved that cholinergic block could be induced in myasthenic patients by intravenous administration of neostigmine. I am convinced that it is a real hazard of treatment which is commonly overlooked, and that so-called "neostigmine resistance" often indicates depolarization block. The fact that different effects may be seen in different muscles of the same patient has not, so far as I am aware, been emphasized.

The purpose of this paper is to define the differential diagnosis of myasthenic and cholinergic crises and to draw attention to the possibility that they may coexist. The management of crises of weakness occurring in myasthenic patients will be discussed in the light of personal experience.

**Signs of overdosage with anticholinesterase drugs**

It is well known that most patients with myasthenia gravis can take large doses of anticholinesterase drugs without experiencing muscarinic side effects. Though not invariably true, colic, diarrhoea, sweating and hypersalivation usually indicate overdosage. For this reason Schwab (1954) advises against the routine administration of atropine to myasthenic patients since it may prevent early recognition of toxicity.

In my personal experience, fasciculation of muscles has been an earlier sign but recognition of this has not always enabled me to avoid cholinergic crisis. The reason for this requires some consideration as it has not been discussed
in earlier papers on the subject. In Case 1 (described) fasciculation always occurred in muscles which were unaffected by myasthenic weakness, usually in the lower limbs in other cases. This might be predicted. No harm results if the dose is not further increased to the level of cholinergic block and indeed some patients were known to have had fasciculation for years. Difficulty was experienced when further deterioration of the myasthenic muscles took place because the increased dose of pyridostigmine or neostigmine required was sufficient to cause cholinergic block of the non-myasthenic muscles.

(Case 2 described). Undoubtedly fasciculation is a valuable sign that the danger level of dosage has been approached, but it may be absent or so transient as to be overlooked (Osserman, Kaplan and Besson, 1953; Rowland et al, 1954). Apparent absence of fasciculation should never be used to discard a diagnosis of cholinergic crisis.

In the circumstance of increasing weakness, three possibilities exist:--
1) there is an increasing myasthenic state, 2) the myasthenic muscles are becoming resistant to neostigmine, 3) cholinergic block is developing. If myasthenia is becoming more severe, an increased dose of anticholinesterase is required but this will not be effective in the presence of "drug resistance" and positively dangerous in cholinergic crises. I am not convinced of the reality of the neostigmine-resistance so often described in the past. It is true that individual muscles become atrophied and permanently fail to respond to the drug, notably the extraocular muscles and the triceps brachii (Simpson, 1958, 1960) but this is a local phenomenon and other muscles continue to respond fully. I believe that most cases reported as showing no improvement or even deterioration with progressively larger doses of neostigmine were in reality suffering from neostigmine-poisoning. Examples are Cases 2-5 (described). In these cases the errors made were quite obvious in retrospect but the picture was not so clear at the time and it is the purpose of this paper to show why the warning signs were ignored or missed.

Fasciculation had been present in Case 5 for a long time without affecting the wellbeing of the patient. The knowledge that this was a response of "healthy" muscle, and the long spell of reasonable health encouraged me to discount such fasciculation as an indicator of the state of the muscles which were affected by myasthenic weakness but were a constant reminder that any clinical deterioration might be due to either cholinergic block or to increased neostigmine requirements by the damaged muscles. Most physicians who have treated myasthenic crisis must have been impressed by the inadequacy of successive increments of
dosage with neostigmine. Eaton (1947) described the use of a daily dose of 1000mg neostigmine, and Rowland et al (1954) mention a case requiring 2500mg daily. Rowland et al (1956) describe patients who failed to respond to doses as high as 18.0mg of neostigmine injected intravenously during one hour. This phenomenon is usually attributed to refractoriness. Accepting that atrophic or "myopathic" muscles fail to respond to anticholinesterases, there is no good evidence that general refractoriness can occur within the space of a few days. Viets (1944) considers that "refractoriness" is "merely an acknowledgment of inadequate dosage". In my own experience, cholinergic block is at least as common as a cause of apparent refractoriness to neostigmine. In this situation one requires a simple means of testing the status of individual muscles.

Edrophonium test for cholinergic state

Osserman and Kaplan (1952) introduced edrophonium chloride (Tensilon) as a test for myasthenia gravis. It resembles neostigmine in its anticholinesterase effect but probably has a greater direct action on the endplate and it differs in its rate of action. Response to intravenous injection may be observed in about half a minute, and most of the action is complete (at the clinical level) in five minutes. Confirmation of relief of myasthenic weakness is obtained rapidly while the action on non-myasthenics (fasciculation, especially of the eyelids, and sometimes colic or sweating) is mercifully brief compared to that of an injection of neostigmine. I have sometimes found no response in patients subsequently shown to have true myasthenia but have never found significant improvement (by objective criteria) in other conditions. Osserman, Kaplan and Besson (1953) suggested that edrophonium might be used to test the cholinergic status of treated myasthenics. If temporary improvement occurs after an injection, the need for a bigger dose of anticholinesterase drug is indicated. If temporary deterioration occurs there must be presumed to be cholinergic block. If no change is observed, these authors wisely advise interpreting this as evidence of incipient cholinergic block. The theoretical basis of this test -- a sort of 'titration' -- is sound, but I have found difficulties in practice.

In Cases 3 and 4 the weak muscles improved after edrophonium so the dose of pyridostigmine was increased. Despite this, the patients' breathing and swallowing deteriorated. A possible explanation is that the respiratory muscles had not been affected by the myasthenia and so were overdosed into cholinergic block while the ocular muscles were still myasthenic. An important lesson to be learned from these cases is that when the edrophonium test is used to assess the state of a patient who is deteriorating under treatment, the index of response should be a respiratory excursion, preferably using some instrument
capable of recording inspiratory volume.

Case 5 (described) died soon after an edrophonium test. There had been good reason to believe that neostigmine requirements were increasing despite persistent fasciculation of the legs. In fact, respiration was improved by edrophonium but it provoked intense salivation which the patient could not swallow sufficiently rapidly. She panicked and would not obey instructions to lean forward. Death was very sudden despite immediate artificial respiration. The possibility of ventricular fibrillation or cardiac arrest can not be excluded. There is considerable uncertainty about the actual state of this case but it is included to underline the fact that edrophonium may be dangerous when given to a patient who is already taking a large dose of neostigmine, and the "transient" muscarinic effects may be fatal even when there is awareness of the danger and resuscitation measures are at hand. Neostigmine may potentiate vagal reflexes affecting the heart and lungs (Furman and Geiger, 1952) and there is little doubt that edrophonium would do likewise.

Many authors have stated that the possibility of giving too much neostigmine to a patient in crisis is remote and that there is a greater likelihood of giving inadequate amounts when a patient is critically ill but not in a respirator (Eaton, 1947; Harvey, 1954; Viets, 1946). On reading the brief descriptions in this text, the reader may conclude that the diagnosis was easy. Unfortunately, this was not so. Although suggestive signs or symptoms have been recorded chronologically in the case reports, they were sometimes elicited retrospectively by close questioning of junior nursing staff who had known of increased sweating, diarrhoea, muscle twitching, increased restlessness etc. without appreciating their significance. Usually the observer had understood that these symptoms were to be expected in any patient taking neostigmine and indeed junior medical staff sometimes reassured them. There is some truth in this belief, but the new appearance of such symptoms or signs should always be interpreted as evidence of impending cholinergic crisis.

It is in just such circumstances that the edrophonium test might help. Unfortunately this has been less valuable than anticipated. The possible dangers of edrophonium in a cholinergic crisis have been discussed above. These might be avoided by giving atropine sulphate o.1mg intravenously before injecting edrophonium. A more serious difficulty (illustrated) is that the edrophonium might cause temporary increase in muscle power when the patient is actually on the verge of cholinergic block. In explanation of this anomaly it may be
suggested that some neuromuscular junctions (those severely affected by the disease) could be in a sub-optimal state with a dose of anticholinesterase which was causing depolarization block of the more normal endplates. Thus the ocular and shoulder girdle muscles might improve with an injection of edrophonium while the respiratory muscles (which are often less severely affected) may be in danger of cholinergic block. The brief action of edrophonium is then a disadvantage because it does not permit fatigue-testing of a range of muscle groups yet there is sufficient persistence to make it inadvisable to repeat the injection more than once in an hour (Osserman, Kaplan, and Besson, 1953). If the test is being used to decide therapy in a deteriorating case, I advise that the vital capacity be measured with a respirometer (or a Benedict-Roth B.M.R. machine) before and after an injection of edrophonium. An objective assessment of this type is desirable. A statement from the patient that he "feels stronger" or "breathes more freely" is an unsound basis for treatment. Case 6 illustrates this point. Because of its unique value it is reported in greater detail by Simpson (1964).

Case 6 was a male aged 60+ who had myasthenia gravis associated with thymoma for 11 years. The thymus had been treated elsewhere with radiotherapy. During the year before he came under my care he had been getting weaker despite an increasing dose of neostigmine. On admission he was taking 46 tablets (690mg) neostigmine daily. Reduction to 40 tablets confirmed that he needed a bigger dose. Increments were given by intramuscular injection. On the ninth day finger twitching was noted, the pupils were small, but edrophonium still improved eye movements. Muscarinic signs became prominent on the following day. Edrophonium now caused no objective improvement but the patient claimed to feel stronger. Nonetheless, cholinergic crisis was diagnosed so atropine was given and 500mg of pyridine-2-aldoxime methiodide (P.A.M.) injected intravenously. Some restoration of power occurred, confirming the diagnosis, but this preparation, claimed to be an antidote to anticholinesterase poisoning, caused severe hypotension and acute haemolysis with anuria. Since neostigmine and its analogues are excreted by the kidneys there was then great difficulty in establishing adequate treatment so that despite drastic reduction in dosage the patient had three cholinergic crises in the next six weeks. His survival for this period was due entirely to positive pressure ventilation.

This unusual case gave opportunity to study the signs and treatment of
cholinergic crisis under controlled conditions. From it we learned that the edrophonium test was a reliable indicator of the need for more neostigmine only when a measurable increase of muscle followed its administration. If improvement in strength was equivocal, even though the patient claimed to be stronger, there was invariably a recurrence of muscarinic signs and cholinergic block soon followed, lasting for an hour or more. We concluded that the test was reliable if absence of measurable response was always interpreted as a warning that cholinergic block was impending. Respiration could not be tested in this case as the patient was being ventilated passively through a tracheostomy, but it was noted that at times of cholinergic block the respirator had to work against increased resistance though bronchial toilet was adequate and radiography showed no pulmonary collapse. This suggested the presence of severe brochoconstriction and it may be that some of the respiratory failure occurring in the other cases was due to this.

It was confirmed in this case that muscular fasciculation occurred in the 'normal' muscles at a dose level which was perfectly safe; indeed as cholinergic block occurred the fasciculation naturally became less prominent. The muscarinic signs were also misleading. Sweating and pallor resulted from other causes such as pulmonary infection and hypoxia. Diarrhoea or rectal incontinence was fairly closely related to the cholinergic state but was also precipitated by oral administration of potassium salts. Bradycardia was never observed, indeed tachycardia was the rule. Only two signs were consistently related to cholinergic crisis, hypotension and miosis. Drop in blood pressure was not gross, only apparent from retrospective study of the charts except for the first attack which, perhaps erroneously, was attributed to the injection of P.A.M. Small pupils very consistently accompanied the cholinergic state and usually preceded muscular weakness by several hours. A pupil diameter of 2mm in a dimly lit room usually indicated impending crisis and the diameter decreased to 1mm or less when weakness became severe.

At this point it will be advantageous to summarise my present evaluation of diagnostic signs in determining whether increasing weakness is due to myasthenic or cholinergic crisis.

Muscular strength: If deterioration follows an emotional upset or in the course of a pyrexial illness and is first noticed more than two hours after the last dose of anticholinesterase it is very likely to be myasthenic. If it occurs in the course of treatment in which there has been gradual increase in dosage because of inadequate response it may be either myasthenic or cholinergic, and the latter is highly probable if the daily dose exceeds 20 tablets of
neostigmine or its equivalent, or if weakness develops within one hour of taking tablets. If the patient has previously been well controlled at a stable dosage level and there is no obvious precipitating factor for myasthenic relapse, remission may be occurring so that the previous therapeutic dose is now too much. Case 7 (described) is an example of this. This situation is not uncommon in the 2nd - 4th day after thymectomy and reduction of dosage may be necessary for a few days at that time.

**Fasciculation** This is very unlikely to be present in myasthenic crisis but it may be seen in the adequately controlled patient and disappear as cholinergic block ensues. Absence of fasciculation does not controvert a diagnosis of cholinergic crisis.

**Muscarnic effects** These are extremely rare in myasthenic crisis even if the dose of neostigmine is very high. Their presence in association with deterioration of muscle power should be taken as prima facie evidence for a cholinergic state. Diaphoresis, nausea, vomiting, and abdominal pain may be associated with other illness which is responsible for precipitating myasthenic relapse, but sweating with a cold pale skin, rapid pulse, and drop in blood pressure strongly suggests overdosage. Rectal incontinence, as distinct from diarrhoea, is also a significant sign. Lachrymation and hypersalivation are useful signs of overdosage though the patient may drool his normal saliva simply because he cannot swallow. If the saliva is thick and glairy it is more suggestive of myasthenic crisis. The size of the pupil is a good guide provided there is a previous record of its size in comparable lighting and no opiate or similar pupilloconstricting drug has been given (important after thymectomy). If the pupil diameter is known to have decreased to 2mm great care is essential in raising the dose of anticholinesterase any further. If it decreases to 1mm and becomes unresponsive cholinergic crisis is imminent.

**Edrophonium test** In principle, the concept of measuring the discrepancy between the acetylcholine released at nerve terminals by willed contraction and an optimal level is good and the edrophonium test is undoubtedly a major advance in the management of myasthenia gravis. With increasing experience, Osserman et al (1955) have modified their original technique and now recommend the following procedure:

1. **Timing** When used to adjust dosage in the average myasthenic the test is best applied at the end of the second hour after neostigmine has been administered. A positive response at that time indicates that neostigmine dosage is insufficient. In 'adequate' treatment no response will be obtained; in overdosage there will be temporary deterioration. A false impression may be
gained if the test is carried out at the end of the first hour when the action of neostigmine is maximal as a patient may fail to improve further at that time even though the general level of dosage is sub-optimal. Conversely, three hours after neostigmine is too late. Even a null response at that time may indicate overdosage. There is no information about the timing of the test in cases treated with pyridostigmine but the two-hour test is probably reliable for this drug also, provided that absence of objectively determined improvement is always interpreted as indicating optimal or overdosage. At times of crisis the test may be used at any time but should not be repeated for at least an hour.

b) Method For the test, the usual dose is 10mg edrophonium (1ml). The first 2mg (0.2ml) should be injected intravenously and the needle kept in the vein. If the patient is hypersensitive to the drug, or in a cholinergic state, this may cause fasciculation, blepharospasm, dizziness, faintness, and slight muscarinic symptoms. In this event the test should be discontinued and atropine injected if necessary. If no ill effects occur in 30 sec the remaining 8mg is injected. If the above symptoms occur in the next 15 minutes it is certain that no myasthenic weakness exists, but this type of response is by no means invariable in normal subjects. During the period 15-5 minutes various groups of muscles should be tested for fatiguability. If this is found it may be concluded that these muscles require a larger dose of anticholinesterase drugs. It is my contention that it is not justifiable to extrapolate this conclusion to every muscle and in particular to those unaffected by myasthenia. On the contrary, the latter muscles may be dangerously overdosed. Unfortunately the effective duration of action of edrophonium is often too short to allow testing of more than one group of muscles, hence the importance of testing respiration and swallowing.

Rowland et al (1955) observed the effect of neostigmine infusions in myasthenic patients and confirmed that an excess would cause significant weakness. They made the important observation (which is confirmed by my experience in the patients described) that any preceding improvement in power was usually incomplete. That is to say that there is no stage of full muscular power at the transition between myasthenic and cholinergic weakness. They further concluded that muscles which had not been clinically weak previously were usually affected simultaneously with 'myasthenic' muscles although "paradoxical" observations were made in one case. This is obviously
a point that urgently requires clarification since the validity of the edrophonium test in differentiating myasthenic from cholinergic crisis depends on it.

c) Dose The method just described is that recommended by Osserman and colleagues. It is a very useful test if there is unequivocal improvement or decrease in muscle strength. Unfortunately it is often when its help is most required that the result is equivocal. In my experience doubling the test dose did not give extra information. This procedure is not recommended for routine use and may be dangerous for use in a patient who is not having assisted respiration.

Jolly-Harvey-Masland test The most objective way to test the neuromuscular status would be to tetanize a peripheral nerve and to observe the resulting muscular contraction or its action potential for evidence of myasthenic 'fatiguability'. With cholinergic block the first twitch and its action potential should be greater than subsequent responses but the latter should remain at a subnormal plateau without showing the decrementing response typical of the myasthenic state. I found this procedure helpful in Cases 3-6. Unfortunately no permanent electromyographic record was obtained. Again, the main caveat concerns the extrapolation of findings from one nerve-muscle complex to another. This problem will be studied further as occasion arises.

Treatment of myasthenic crisis

In mild cases of myasthenia gravis respiratory distress is very rarely due to weakness of inspiration. Sudden respiratory distress in such patients is more likely to be due to inhalation of food or a foreign body, so prompt postural drainage with manually assisted cough may be lifesaving if suction apparatus is not at hand, Weakness of inspiration is usually preceded by progressive weakness of limb and bulbar muscles and an increasing dosage of neostigmine. For this reason it is unwise to temporise if the patient has shallow fast respiration or is restless. Cyanosis is rare. Neostigmine 2mg should be injected intramuscularly at once and a respirator obtained. (Intravenous injection is not necessary and may be dangerous.) It is possible to use a tank respirator, but in emergency it is safer to use positive-pressure ventilation through an endotracheal tube or tracheostomy since inspiratory weakness is usually associated with weakness of swallowing. Bronchial secretions should be aspirated as soon as possible. Neostigmine should then be given through a nasogastric tube. If intubation is not possible it may be given intramuscularly. As a starting point the equivalent of the previous oral dose may be calculated on a basis of 1.5mg i.m. for every 15mg given orally according to Viets (1950); my own impression is that a truer equivalence would be 1mg i.m. equals 15mg oral.
This dose may be repeated hourly for 2-3 hours. If an edrophonium test shows that treatment is still inadequate the dose may be increased steadily. This is less likely to give rise to cumulative effects than is shortening the intervals between injections although this may also become necessary. Provided an unequivocal response to edrophonium is obtained it is safe to give atropine in addition, but its effect in thickening of bronchial secretions is undesirable and so it should be avoided if possible. If progressive increments of neostigmine do not help, especially if evidence of incipient cholinergic block appears, it is sometimes effective to withdraw all medication, maintaining respiration artificially. When neostigmine is resumed a few days later it is often effective at much lower dosage. The general principles of management of a respirator patient apply and need not be detailed here.

**Treatment of cholinergic crisis** If increasing weakness due to overdosage of anticholinesterases is recognized before respiratory failure takes place it is sufficient to omit the next dose and to give atropine sulphate 2mg i.v. The edrophonium test should be repeated every two hours and no more anticholinesterase given until there is definite improvement with edrophonium. If there is respiratory failure the patient should be respirated through a cuffed endotracheal tube. This must be replaced by a tracheostomy tube in 24 hours if further artificial respiration is necessary. This is a much more dangerous state than the myasthenic crisis because of the haemodynamic changes present and the possibility of bronchospasm so intubation must not be delayed. There will be no worthwhile recovery an hour later and the patient may lose his chance of survival.

Atropine should be continued in big doses (2mg i.v.) every hour until muscarinic signs abate or signs of atropine toxicity appear. (This should be considered if restlessness or confusion develop in the well oxygenated patient.) Atropine has little if any effect on the neuromuscular junction of skeletal muscles. There are two possible antidotes to cholinergic poisoning:—

1) to displace some of the acetylcholine from endplate receptors with curare. This method was tried in Case 3. Temporary restoration of power was achieved but we had no means of determining the necessary dose. Nevertheless this form of treatment might be exploited further.

With later plastics it has been possible to keep an endotracheal tube in place for a week or more. Tracheostomy is usually avoided.
2) to reactivate acetylcholinesterase at the endplates. This may be achieved by the use of oximes such as pyridine 2-aldoxime methiodide (PAM) or its methane sulphonate (P₂S) which reactivate the enzyme when it is inhibited by an organic phosphate drug. Grob and Johns (196) claimed that oximes were also effective in the treatment of poisoning by quaternary ammonium anticholinesterases such as neostigmine. Both of these substances were tried in several crises in Case 6 (Simpson, 1964) under the unique controlled conditions described above. It was confirmed that some recovery of power and reduction of muscarinic effects took place but there was a latent period of $\frac{1}{2}$-1 hour before any clinical effect could be seen, the restitution of power was not impressive, and the effect lasted for about one hour. Even repeated doses did not make a material contribution to the handling of cholinergic crises in this case. Certainly it would be unwise to postpone artificial respiration because of an expected response to either of these "antidotes". Positive pressure respiration with normal management routines and injections of atropine must be continued until the patient is clearly myasthenic again., and re-established on a satisfactory dosage of anticholinesterase.
Case 6 (MN5095) Gradual onset of cholinergic weakness with increasing fasciculation, miosis, and sweating (Stage 1). Equivocal results of edrophonium testing based on subjective responses. The neostigmine injected i.m. is charted on an equivalence of 1mg to 10mg by mouth. Unsatisfactory response to P.A.M. 500mg i.v. Hypotension soon after the injection of P.A.M. Haemolysis and oliguria three days later but less fasciculation and improved power. Stages 2 & 3 show renewed onset of cholinergic crisis, delayed response to P.A.M. and icterus three days later. Neostigmine was resumed on Day 24 and pyridostigmine on Day 34. The latter was associated with gradual return of cholinergic signs. Sudden crisis on day 40 was treated with P2S. Stage 4 was a period of relatively smooth control on a small dose of pyridostigmine (360mg/day). Sweating and fasciculation persisted, especially when potassium was added, but the pupils were not contracted. Gradual deterioration (Stage 5) started at Day 58 despite absence of muscarinic signs. Oliguria continued. New features were aregenerative anaemia, pneumonitis, oesophagitis, and spells of dyspnoea. Terminal deterioration with signs of hypoxia resembled the muscarinic signs of the cholinergic state.
Stage 2. Poor response of second cholinergic crisis to repeated injections of P.A.M. Temporary increase of slight hypotension.

Stage 3. Response to $P_2S$ occurred one hour after injection, but pupils were relatively unaffected.

Stage 5. Terminal pseudo-cholinergic state not improved by injection of $P_2S$ and atropine.
EATON L.M. 1947 Care of the patient who has myasthenia gravis. Medical Clinics of North America, July, 907.


OSSERMAN K.E., KAPLAN L.I. and BESSON G. 1953 Studies on myasthenia gravis. Edrophonium chloride (Tensilon) test as a new approach to management. Journal of Mt Sinai Hospital, 20, 165.


VIETS H.R. 1944 Myasthenia gravis treated with large doses of neostigmine methylsulfate intramuscularly and intravenously, and with neostigmine bromide orally. American Journal of Medical Science 208, 701.


WALKER M.B. 1934 Treatment of myasthenia gravis with physostigmine. Lancet, 1, 1200.

WALKER H.R. 1935 Case showing the effect of prostigmine on myasthenia gravis. Proceedings of the Royal Society of Medicine, 28, 759.
Myasthenia Gravis and Autoimmunity

Sir,—The paper by Sir Macfarlane Burnet (September 29, p. 807) and his recent Jephcott lecture are of great interest to me in the authoritative support they provide for my suggestion that myasthenia gravis is a disease of autoimmunity with the thymus as the source of lymphocytes antagonistic to muscle. I have been working on this idea for eight years, and in April, 1960, described the clinical evidence for it in a Honyman-Gillespie lecture which was later published. At that time there was little positive evidence of the physiological role of the thymus gland, and it is gratifying to note that my speculation is being so rapidly substantiated.

My experimental evidence is still not satisfactory. I have found antinuclear factors in the serum of some myasthenic patients but in a much smaller proportion of a larger series than recently reported by R. G. White and A. H. E. Marshall.

My hypothesis went further than Burnet's in postulating a pituitary control of the thymus. It will be interesting to see if this speculation is also confirmed by his laboratory. Sir Macfarlane rightly points out that the pharmacological nature of the effect on the muscle is still unknown. My paper suggests that an antibody to the protein of the end-plate of a muscle fibre would act as a competitive-blocking substance which would be specific to the individual or, in occasional cases, to the foetus of a myasthenic mother.—I am, etc.,

Neurological Unit,
Northern General Hospital,
Edinburgh 5.

John A. Simpson.

References

Carcinomatous Myasthenia

Sir.—Your leader (October 20, p. 1039) draws attention to an important cause of "symptomatic" myasthenia. Relative sparing of ocular and bulbar muscles, loss of tendon jerks, and incomplete response to neostigmine should raise suspicion about the diagnosis as suggested by your leader-writer, but unfortunately the electromyographic changes described are not so specific as suggested. I have previously reported cases of acquired myopathy of other types ("polymyositis") in which progressive increase in the heights of the muscle action potentials evoked by repetitive stimulation of a peripheral nerve was a very striking feature. These cases have now been followed up for more than five years without evidence of neoplasm. It is also not sufficiently well known that a similar facilitation phenomenon may be found in true myasthenia gravis. I have sometimes found this response in some muscles while others show the classical Jolly fatigability. There appears to be a correlation with the different responses to decamethonium found in myasthenia gravis. Even the frequency of stimulation (which must be supramaximal) affects the response in myasthenia gravis. One occasionally finds a typical myasthenic response with stimulation rates from 3 to 20 per second, yet faster stimulation produces the "facilitation" response. (This is not the place to discuss the validity of the terminology.) Drechsler in Prague has made similar observations and no doubt they are known to other experienced electromyographers. This brief note is written to draw the attention of others to the non-specificity of the electromyographic features described in your annotation.—I am, etc.,

J. A. SIMPSON.

Neurological Unit,
Northern General Hospital,
Edinburgh 5.

REFERENCES

4 Drechsler, B., personal communication, 1962.
Myasthenia Gravis—1

JOHN A. SIMPSON, M.B., Ch.B., F.R.C.P.E., F.R.E.P.S.G., M.R.C.P., Senior Lecturer in Neurology, Edinburgh University; Physician in Charge, Neurological Unit, Northern General Hospital, Edinburgh

Myasthenia Gravis is a disorder of skeletal muscle with many unusual features. Its most outstanding characteristic is that weakness increases progressively when the affected muscles are contracted and disappears after a short rest. Thus a patient who looks perfectly strong and healthy in the consulting room may describe severe weakness when at his work. The apparent inconsistency is the main reason why this disease is often missed by those who are not familiar with it. It is not a rare disease though many doctors state that they never see a case.

Clinical Features

Symptoms tend to be intermittent at first and are often precipitated by an emotional upset. This, too, prejudices the doctor towards a diagnosis of 'hysteria'. An infection of the upper respiratory tract may precipitate the first or later attacks, but once the disease has started the symptoms are brought on by physical effort or by emotional upset. Even then the symptoms may subside for periods of months or years or there may even be spontaneous cure.

Nevertheless, the tendency is for more and more symptoms to appear during the first two or three years and, if untreated, death may occur during this period. Death is less likely if the patient manages to survive for five years but a risk of choking remains for many years if the muscles of swallowing and respiration are affected.

Myasthenia gravis is commoner in women. It occurs at any age, but usually appears for the first time in the early 20s. In the older age group it is rather commoner in men.

Signs and Symptoms

Any skeletal muscle may be involved (the smooth muscle of the gut, etc., is not) and the symptoms will vary accordingly. The most commonly affected muscles are those of the eyes and eyelids so that the patient complains of double vision when tired, and her eyelids tend to droop and may close completely. After a short rest they can be reopened until they again become tired. Other symptoms may be difficulty in chewing or swallowing, loss of the voice after speaking for a time, difficulty in keeping the jaw closed or holding the head up, weakness of the limbs, especially about the shoulders and in fact loss of strength of any muscles including, in severe cases, the respiratory muscles. Symptoms tend to be worse towards evening but for some patients the hour immediately after waking is the worst of the day.

There is no pain, though weak muscles may ache, and no loss of sensation. Reflexes are present but may fatigue if elicited repeatedly.

Diagnosis

If myasthenia is suspected (and the facial appearance of a severe case is easily recognizable—Fig. 1) the diagnosis is easily confirmed by asking the patient to make a sustained effort with the affected muscles. For instance she may be asked to keep her gaze held on an object held above eye level, to count up to 100, to swallow barium, or hold her hands outstretched for a minute, when the symptoms suggest fatigability of eyelids, speech, swallowing, or shoulder girdle respectively. In each case the attempt will be terminated by increasing weakness.

Final confirmation is given by the dramatic improvement after use of the appropriate drug. The most rapid result is given by Tensilon (edrophonium). A prelimi-
Nursing Times, January 11, 1963

37

Fig. 2. Left, before, and right, one minute after intravenous injection of edrophonium.

Nursing Times, January 11, 1963

As the effect wears off in a few minutes it is not suitable for treatment. Neostigmine (Prostigmin) on the other hand takes longer to act (half to one hour after 1 mg. injected subcutaneously) but its effect continues for three hours or more, making it more suitable for treatment. It is also used as a test though the response is not so rapid.

Nature of the Disease

The two drugs just mentioned are from the group known as anticholinesterase drugs which prevent the action of cholinesterase, an enzyme present in muscle just beneath the motor end-plate. The stimulus for a muscle to contract passes from the spinal cord along the motor nerve to its terminal. There it releases acetylcholine, a chemical which crosses the gap (the neuromuscular junction) to the muscle end-plate (Fig. 3). Acetylcholine stimulates the muscle to contract but if it then remains in contact with the receptor substance of the end-plate, no further muscle twitch can occur. Cholinesterase normally destroys the acetylcholine as soon as it has done its duty, releasing the receptors for the next packet of transmitter chemical.

The symptoms of myasthenia gravis are probably due to deficiency of this mechanism. Various possibilities have been suggested and all have their supporters among research workers (Fig. 3).

(i) The nerve fibre may produce too little acetylcholine.
(ii) Cholinesterase may destroy acetylcholine too quickly.
(iii) There may be a substance resembling curare which will compete with the acetylcholine for the available receptors ('neuromuscular block').
(iv) The receptors may be abnormal and so fail to respond in the normal way.

I favour the third theory because occasionally a myasthenic mother has a baby who has myasthenia gravis for the first few weeks of its life then recovers fully. This strongly suggests that some substance has been passed from mother to child. I have suggested that the blocking substance may be an antibody, a concept which has started a lot of new research on this baffling disease.
Whichever theory is correct, the acetylcholine produced by the nerve would be less effective than it should be. For this reason it is rational to use a drug which will depress the action of cholinesterase and so allow the acetylcholine to act more strongly and for a longer time. It may have occurred to you that if this is overdone, the muscle will be made unresponsive for the time until acetylcholine is gradually destroyed. In fact this is a serious danger which to some extent limits the use of anticholinesterase drugs. The resulting paralysis is called a cholinergic crisis, and it is important for the nurse to learn its signs.

Pathology

The idea of a 'neuromuscular block' has fascinated physiologists so much that they have ignored the long-known facts that microscopic changes can be seen in the muscles. A few of the muscle fibres may be degenerated, but the most characteristic lesion is little collections of lymphocytes between the muscle fibres (thromborrhages). Recently it has also been shown that the nerve terminals are deformed. These changes could result from an auto-immune reaction in the muscle (Simpson, 1960).

The other characteristic lesion which indicates that myasthenia gravis is more than a simple chemical disturbance at the neuromuscular junction is the common finding that the thymus gland has failed to atrophy and shows the microscopic changes indicating active formation of lymphocytes (cells which carry antibodies). About 16 per cent. of cases also show neoplastic change in the gland; a thymoma, as the tumour is called, can usually be seen in an X-ray film of the chest (Fig. 4). It may be a benign or a malignant tumour. In the remaining cases although the gland is abnormal it does not show on the radiograph.

Myasthenia gravis has a peculiar link with disorders of the thyroid gland. The patient may have a goitre, simple or toxic, but this may occur years earlier or later than the myasthenia so it cannot be a cause. I have found an increased incidence of thyroid disease in relatives of myasthenics so it may be that they have a common hereditary background, possibly involving the pituitary gland (Simpson, 1960).

NEXT WEEK: Myasthenia Gravis—2
Myasthenia Gravis—2

JOHN A. SIMPSON, M.B., Ch.B., F.R.G.P.E., F.R.F.P.S.G., M.R.C.P., Senior Lecturer in Neurology, Edinburgh University; Physician in Charge, Neurological Unit, Northern General Hospital, Edinburgh

The name myasthenia gravis was given to the disease when no treatment was known. With the discovery of the effect of physostigmine and later of neostigmine (a synthetic anticholinesterase) by Mary Walker in 1935 the mortality was reduced from about 80 per cent. in the first year of the illness to approximately 15 per cent. Even so 20 to 30 per cent. of medically treated patients die of myasthenia within seven years.

In view of the constant abnormalities of the thymus gland, surgical removal (thymectomy) has been advocated for many years and practised in a few centres. Despite the considerable experience acquired by a few surgeons there was no agreement regarding the benefit or otherwise of thymectomy because American surgeons did not appreciate that there was a great difference in prognosis between cases with a thymoma and those without.

Sir Geoffrey Keynes of London, the most experienced surgeon in this field, has always maintained that patients with a thymoma tend to do badly whether operated on or not, but if there is no thymoma the outlook is greatly improved by surgery. His claims were fully confirmed by my independent examination of cases operated on by Keynes and other surgeons (Simpson, 1958). If there was no thymoma the mortality due to myasthenia was halved in women, especially if they had myasthenia for less than seven years. Furthermore, the survivors were found to have a much better chance of recovering completely or partially. Men also tend to improve after thymectomy but the benefit is not so striking. On the other hand a thymoma is a tumour and it is advisable to remove it for this reason though there is less chance of the myasthenia improving.

Response to Operation

The response to operation is very strange. Some patients respond immediately, others in a few weeks or months and the remainder notice little difference at the time. Nevertheless when they look back from the vantage point of two or three years, most patients are certain that ‘the tide turned’ when the thymoma was removed. It may be possible sooner or later to discontinue medical treatment, but often it has to go on with a lower dosage until recovery takes place. Thymectomy has not abolished the need for drugs; both have a role to play.

Largely, thanks to modern drugs, most myasthenic patients can now look forward to a normal life. The disease can be controlled, and even cure is possible if the thymus is removed in time.

Anticholinesterase Drugs

Edrophonium (Tensilon) is a short-acting drug used intravenously as a test and not for maintenance treatment.

Neostigmine (Prostigmin) may be given by injection to the severely ill patient who is unable to swallow (1–3 mg. subcutaneously) but is usually given by mouth. One to four 15 mg. tablets may be required every two to four hours in a severe case. An average dose is 10 tablets in a day. Mild cases may only require one tablet before meals (usually given half to one hour before so that the maximum effect is present while the patient is eating).

Pyridostigmine (Mestinon) is a 60 mg. tablet which has a strength approximately equal to the neostigmine tablet. It gives less boost to strength but has a smoother and slightly longer effect (like the difference between lente insulin and soluble insulin). It is preferred by most patients, and is especially useful to reduce the frequency of dosage during the night as it may act for six hours or more.

Ambenonium (Mysuran) is another anticholinesterase with a long action. It is less frequently used in this country because, like other long-acting drugs which will not be described here, the disadvantage of prolonged action is a tendency for the effect to be cumulative and cholinergic crisis may occur before the medical staff is aware of the danger.

Cholinergic Crisis

Anticholinesterase drugs also act on smooth muscle and glands and so may cause gastric upsets, diarrhoea, salivation, sweating, pallor, and bradycardia. For some unknown reason the myasthenic patient can tolerate large amounts of anticholinesterase drugs before these symptoms appear and as they are not in themselves dangerous it is common to suppress these side-effects by
the regular use of atropine. This has the disadvantage of removing early warning of impending danger. If the cholinesterase of skeletal muscles is too strongly inhibited the muscles first fasciculate and then become paralysed. Too often the increasing weakness is taken to mean that myasthenia is increasing and still more anticholinesterase may be prescribed. Death from respiratory paralysis is certain if this is not realized. For this reason the nurse should always report the presence of any of the above symptoms. Danger is very close if any of the following are present:

(i) increasing weakness one hour after the last dose;
(ii) fasciculation of muscles;
(iii) headache, confusion, restlessness;
(iv) cold sweating with pallor;
(v) constricted pupils.

I find that the pupils are often the best guide but if in doubt, report at once, because death can take place suddenly. Naturally, severe weakness may indicate that myasthenia has become worse — myasthenic crisis — and a bigger dose is required. The differential diagnosis can be extremely difficult even for the most experienced. The Tensilon test can be valuable here. If the crisis is myasthenic, Tensilon will cause brief improvement and give valuable evidence that the patient requires more anticholinesterase medication. If the crisis is cholinergic, Tensilon will not help. It may even make him worse, but this will only last for a few minutes. It is wise to be ready with equipment for mouth-to-mouth breathing or some other efficient form of artificial respiration before the test is carried out. If respiration is seriously impaired before the test it is wise to pass an endotracheal tube before carrying out the Tensilon test.

If myasthenic crisis is confirmed, an injection of 1–2.5 mg. neostigmine will relieve the situation in 15–30 minutes. The additional speed of intravenous medication must be matched against the greatly increased risk to the heart, and neostigmine should always be accompanied by atropine if given by this route. If cholinergic crisis is diagnosed, no further anticholinesterase drug is given. Atropine, gr. 1/6, should be injected intravenously and a further gr. 1/6 should be given intramuscularly every hour until sweating stops and the pupils become larger than 3 mm. The dose can then be reduced, but no more anticholinesterase should be given until Tensilon causes unequivocal improvement.

Always have atropine available when you have a myasthenic patient in the ward.

Artificial Respiration

Most deaths in myasthenia gravis are due to asphyxia. Either the respiratory muscles become paralysed or a foreign body becomes impacted in the glottis, trachea or a bronchus and the patient cannot cough with sufficient force to clear the obstruction. Early suction or bronchoscopy or the passage of an endotracheal tube will be life-saving. Tracheostomy may be necessary and should never be delayed until the chance of saving life is gone. Remember that myasthenia is reversible and the most severely paralysed patient may have complete remission. Most patients now lead a satisfactory life and many can look forward to recovery. For this reason it is not a kindness to delay intubation.

If artificial respiration is required for more than a few hours tracheostomy is essential but in the first instance an endotracheal tube can be connected to a positive pressure respirator or even to an anaesthetic machine with an oxygen supply. For this purpose it is necessary to use a cuffed tube so that air passed through it will go into the lungs and not escape back into the pharynx. The cuff helps in another way by sealing off the trachea and preventing saliva, etc., from entering the lungs. This is especially important in cholinergic crisis when saliva may be very severe.

Other Measures

For obvious reasons this article has concentrated on the treatment of emergencies in myasthenia gravis, but one must keep a sense of proportion. Most cases do not need these desperate measures. Indeed myasthenia may be quite a mild condition, especially if it remains confined to the eyelids and extracocular muscles. In this event the outlook is so good that there is no need to take the slight risk of operation. Even so, care must be taken to avoid circumstances which will make the illness severe.

1. Excessive fatigue, emotional upset, infections, and menstruation may cause relapse.
2. The patient should not eat grapes because of risk of inhaling seeds (fruit must be eaten with care).
3. Sedatives (morphine in particular) must be used with care to avoid depression of respiration; and special care must be taken if the patient requires surgery. Relaxant drugs such as curare have abnormal action.
4. No enema should ever be given to a myasthenic patient. For some unknown reason an enema may cause sudden collapse.

Fortunately with reasonable care and regular use of anticholinesterase drugs (as a diabetic cares for himself with insulin), most myasthenic patients can now look forward to a normal life. Unlike the diabetic, the myasthenic may even hope for a remission during the first four years, and if her thymus is removed in time she may even be cured.

REFERENCES

Immunological disturbances in myasthenia gravis with a report of Hashimoto’s disease developing after thymectomy

JOHN A. SIMPSON

From the Department of Medicine, Edinburgh University, and the Neurological Unit, Northern General Hospital, Edinburgh

During the years 1953-55 I was privileged to examine a large number of patients who had been treated for myasthenia gravis at the National Hospital and other centres in London. The survey was made in order to evaluate the efficacy of thymectomy in the treatment of that disease. In reviewing the results it was remarked that myasthenia gravis was a favourite subject for the demonstration of the physiological mechanisms in disease yet, despite a period of unprecedented advances in knowledge of the physiology of the neuromuscular junction, the nature of the myasthenic response remained unknown. None of the theories current at that time attempted to account for the facts of thymic pathology and of clinical response to thymectomy. The role of surgery was indeed still debated, but the review showed that there was a statistically significant benefit to a myasthenic patient provided that the thymus was removed within five years of the onset of symptoms (Simpson, 1958). This temporal limitation is difficult to account for if the thymus is producing a ‘curare-like’ substance as ordinarily understood.

It was decided to approach the problem without bias by recording as fully as possible the medical history of each patient interviewed. No clinical fact was rejected on grounds of apparent irrelevance. When the assembled data on 440 cases were reviewed (the London series supplemented by the first 33 of a personal series in Glasgow and Edinburgh) some surprising observations were made. First it was found that a few cases had evidence of temporary disturbance of the central or peripheral nervous system and supporting cases were found in the literature. Furthermore, certain non-muscular disease appeared to be related closely to myasthenia apart from disorders of the thyroid gland. Nevertheless, certain disorders of the blood, reticulo-endothelial system, and joints were noted. At that time a working hypothesis was considered, namely, that myasthenia gravis was only the most prominent feature of a disorder which could occasionally involve other organs. The age distribution, the high incidence in females, and the natural history of the disease were suggestive of a pathological process resembling systemic lupus erythematosus. Furthermore it was known that myasthenia gravis may occasionally complicate systemic lupus erythematosus (Harvey, Shulman, Tumulty, Conley, and Schoenrich, 1954; and the author’s series).

In Glasgow in 1955, Dr. J. R. Anderson kindly helped me to attempt to produce myasthenia in mice by inoculation of homologous muscle with Freund’s adjuvant, but we had no success. (This could not be mentioned by Simpson at the time (1960) as the Honman-Gillespie lecturer is forbidden to discuss animal experiments.) Nevertheless the concept seemed to account for more details of the disease than any hitherto available though it involved the assumption that the thymus did not function as an endocrine gland in the usual sense but as a reticulo-endothelial organ which was important in immunity reactions, probably the first suggestion of this role. During the next three years the concept of autoimmunity as a mechanism in disease was gaining ground. My conviction that it was a factor in myasthenia gravis was increased when in 1959 Smithers remarked that the histological changes in the thymus were suggestive of an autoimmune process and Nastuk, Strauss, and Osserman (1959), while searching for a neuromuscular blocking substance, found that blood from myasthenic patients sometimes caused lysis of frog muscle cells. A lesser degree of cytolysis was caused by blood from some normal subjects. They then examined the activity of serum complement. In most myasthenic patients it was within the normal range but in a few cases it was either high or far below normal and this tended to correlate with remissions and exacerbations respectively (Nastuk, Plescia, and Osserman, 1960).
Though Smithers (1959) did not suggest how the muscle disease could be caused and the American team did not consider a role for the thymus, their independent evidence encouraged me to formulate a hypothesis which was described in a Honyman-Gillespie lecture in April 1960. It was suggested that the thymus might stimulate the production of lymphocytes carrying antibody against the endplate protein of muscle, and possibly also against muscle fibres, nerve endings, and on occasion the central nervous system and other organs such as the bone marrow and the joints, in close analogy with systemic lupus erythematosus. It was shown that an antibody could act as a neuromuscular blocking substance of 'competitive' type which would account for the myasthenic phenomenon. (The American cytolytic theory does not explain the reversible nature of the weakness.) Furthermore, an antibody against muscle could account for the facts that a baby born to a myasthenic mother may have temporary myasthenia, yet transfusion of blood from a myasthenic patient into another adult does not cause myasthenic signs. Coincident with the publication of this lecture (Simpson, 1960), Strauss, Seegal, Hsu, Burkholder, Nastuk, and Osserman (1960) demonstrated the presence of a muscle-binding globulin in myasthenic serum and this has been confirmed by Beutner, Witebsky, Ricken, and Adler (1962) and Feltkamp, van der Geld, and Oosterhuis (1963b). These authors describe four different types of reaction of globulin to muscle based on immunofluorescence techniques: (1) fluorescence of the sarcolemma, which also occurs with control sera; (2) fluorescence of the A bands of muscle fibres; (3) similar involvement of only about half of the muscle fibres ('zebra' type); and (4) nuclear fluorescence. Only types (2) and (3) seem to be specific for myasthenia gravis. There is not at present any direct evidence for an antibody confined to the endplate zone such as I had postulated, nor to the nerve terminals which was suggested as an alternative. A further search should be made for these as the present evidence suggests that the antibodies against the muscle fibres only occur in significant quantity in the presence of a thymic tumour (side infra). The nuclear fluorescence is due to the presence of antinuclear factors in the serum (Feltkamp, van der Geld, Kruyff, and Oosterhuis, 1963a).

The response to the autoimmune hypothesis was immediate. Marshall and White (1961) showed that direct injection of bacterial antigen into the guinea-pig thymus produced a histological reaction which was essentially the same as that seen in myasthenia gravis. Their studies suggested that a blood-thymus barrier exists which would preclude any cellular reaction of the normal thymus to circulating antigen and so facilitate the development of immunologically competent cells active even against an individual's own tissues. By this time experimental biologists were coming to the conclusion that the thymus played a very important role in the development of immunological response (Miller, 1961, 1963; Burnet, 1962a, b). It seems very likely that immunological mechanisms are disturbed in myasthenia gravis. It remains to be demonstrated whether they are causative or not.

One question which immediately arises is whether the immunological response is organ and species-specific. The cytolytic effect described by Nastuk et al. (1959) on frog muscle, and the globulin-binding on human and rat skeletal muscle (Strauss et al., 1960; Feltkamp et al., 1963a, b) demonstrate that the antibodies are not species-specific. Strauss et al. (1960) found that the globulin which binds to skeletal muscle does not do so with cardiac or uterine muscle and they also had negative results against thymus tissue obtained from two myasthenic patients. The reaction was shown to cause fixation of complement. Their positive results were obtained from the pooled sera of 10 myasthenic patients, of whom six had a thymoma. Many others investigated showed no muscle-binding globulin in their serum. Beutner et al. (1962) confirmed their results with sera from two of 10 myasthenic patients. Both sera, which were obtained from the only cases with a thymoma, also showed fluorescence with heart muscle by the direct and indirect fluorescent antibody techniques, but the complement-fixing and staining technique using heart tissue was negative. The presence of at least two antibodies is suggested, one specific to skeletal muscle and the other reacting with skeletal and cardiac muscle. van der Geld and Oosterhuis (1963), using an antiglobulin consumption test with or without the aid of immuneelectrophoresis, found that 40% of sera containing antibodies reacting with skeletal muscle also reacted with thymus. They did not encounter sera with antibodies acting exclusively against thymus. Cross-absorption experiments suggested that there were two distinct circulating antibodies, one of which reacted with skeletal muscle or thymus, the other with skeletal muscle alone. No reaction was found with heart, liver, pancreas, or kidney tissues. The incidence of anti-muscle antibodies was consistently higher in cases with thymoma (van der Geld, Feltkamp, Loghem, Oosterhuis, and Biemond, 1963) and this may account for our failure to find convincing evidence of anti-muscle antibodies since there were no patients with thymoma in the small series investigated. It would be interesting to examine the correlation between muscle antibodies of the Strauss type and the atrophic muscular weakness found in
some myasthenics which does not respond to anticholinesterase drugs. This 'myasthenic myopathy was not a feature of the cases I examined for muscle antibodies. It is more common in cases associated with a thymoma though not confined to them (Simpson, 1958).

Burnet (1962b) classified myasthenia gravis in his group 1 (organ-specific) type of autoimmune disease while acknowledging that a possible relationship with rheumatoid arthritis might necessitate revision of this provisional classification. This putative relationship was based on the present author's paper (see Burnet, 1962a). In the original report (Simpson, 1960) the disorder was listed as 'rheumatoid' arthritis to indicate that it resembled true rheumatoid arthritis but might differ from it. This was done because the arthropathy affected the small joints (Fig. 1) but was sometimes transitory. Van der Geld et al. (1963) have recently reported that the rheumatoid factor was present in the serum of five of 111 (4-5%) cases of myasthenia gravis (Tables I and II). Six patients in their series had rheumatoid arthritis (sic), but it seems from a letter by Feltkamp et al. (1963a) that only one of these patients had the rheumatoid factor in the blood so it would appear that the other positive findings were

![Image](http://example.com/figure1.png)

**FIG. 1.** Arthropathy of metacarpo-phalangeal joints in a case of myasthenia gravis.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISORDERS ASSOCIATED WITH MYASTHENIA GRAVIS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>Scotland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid (all types)</td>
<td>72</td>
<td>15</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes and glycosuria</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>'Rheumatoid' arthritis</td>
<td>12</td>
<td>6</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
<td>1</td>
<td>1(2)</td>
<td></td>
</tr>
<tr>
<td>Raynaud's syndrome</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Epilepsy and 'blackouts'</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Psychosis</td>
<td>9</td>
<td>1</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Patients</td>
<td>407</td>
<td>84</td>
<td>322</td>
<td>90</td>
</tr>
</tbody>
</table>

1 The series reported by Simpson (1960) includes all the London cases and the first 13 from Scotland.
2 Includes one case of Hashimoto's disease and two of lymphadenoid thyroid found at necropsy.
3 Considered to be caused by medication.

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIBODIES DETECTED IN SERA FROM PATIENTS WITH MYASTHENIA GRAVIS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Present series</th>
<th>Positive</th>
<th>Rheumatoid Factor</th>
<th>Muscle</th>
<th>Thyroid</th>
<th>Stomach</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>8</td>
<td>9</td>
<td>39</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>White and Marshall (1962)</td>
<td>Positive</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van der Geld et al. (1963)</td>
<td>Positive</td>
<td>11</td>
<td></td>
<td>38</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>111</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 One case of carcinomatous myasthenia excluded.
2 Rheumatoid factor—S.S.C.T. in present series, latex test in others.
made on myasthenic patients without clinical evidence of arthritis. Arthritis was present in three of 15 cases of myasthenia gravis reported by White and Marshall (1962), and two of these had a positive latex test for rheumatoid factor and hyperglobulinaemia at the time of examination or previously. One of these was later diagnosed as systemic lupus erythematosus. Antinuclear factor was demonstrated in six of the 15 cases and another two gave positive nuclear fluorescence with undiluted serum. Thyroid tissue was used to detect the presence of antinuclear factor in the serum. The positive reactors included

### TABLE III

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age</th>
<th>Thymectomy before Tests</th>
<th>Thyroid</th>
<th>Gastric</th>
<th>Liver</th>
<th>Muscle</th>
<th>Anti-nuclear Factor</th>
<th>S.S.C.T.</th>
<th>Associated Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>294</td>
<td>F</td>
<td>73</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>Arthritis</td>
</tr>
<tr>
<td>3054</td>
<td>F</td>
<td>49</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>3233</td>
<td>F</td>
<td>48</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>3609</td>
<td>F</td>
<td>24</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>3912</td>
<td>F</td>
<td>32</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4301</td>
<td>M</td>
<td>55</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4536</td>
<td>F</td>
<td>22</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4585</td>
<td>F</td>
<td>22</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4586</td>
<td>F</td>
<td>13</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4601</td>
<td>F</td>
<td>19</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4611</td>
<td>F</td>
<td>53</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4649</td>
<td>F</td>
<td>48</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4656</td>
<td>F</td>
<td>30</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4934</td>
<td>F</td>
<td>32</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5315</td>
<td>F</td>
<td>27</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5401</td>
<td>F</td>
<td>39</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5409</td>
<td>M</td>
<td>39</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5494</td>
<td>F</td>
<td>78</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5503</td>
<td>F</td>
<td>72</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5625</td>
<td>F</td>
<td>21</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5652</td>
<td>M</td>
<td>28</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5683</td>
<td>F</td>
<td>38</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>5706</td>
<td>F</td>
<td>23</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6209</td>
<td>M</td>
<td>39</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6433</td>
<td>F</td>
<td>14</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6576</td>
<td>M</td>
<td>30</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6481</td>
<td>F</td>
<td>41</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6503</td>
<td>M</td>
<td>34</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6522</td>
<td>F</td>
<td>57</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6780</td>
<td>M</td>
<td>65</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6792</td>
<td>F</td>
<td>22</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6807</td>
<td>M</td>
<td>39</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6811</td>
<td>M</td>
<td>54</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6933</td>
<td>F</td>
<td>17</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>6998</td>
<td>F</td>
<td>52</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7145</td>
<td>M</td>
<td>31</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7390</td>
<td>M</td>
<td>36</td>
<td>—</td>
<td>25,000</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>7476</td>
<td>F</td>
<td>71</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7543</td>
<td>F</td>
<td>40</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7550</td>
<td>M</td>
<td>52</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7557</td>
<td>F</td>
<td>31</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7720</td>
<td>F</td>
<td>50</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7730</td>
<td>F</td>
<td>50</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7781</td>
<td>F</td>
<td>40</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>7855</td>
<td>F</td>
<td>17</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>RL 1</td>
<td>M</td>
<td>67</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>RL 2</td>
<td>M</td>
<td>66</td>
<td>—</td>
<td>25</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>—</td>
<td>16</td>
<td>—</td>
</tr>
</tbody>
</table>

Positive: 2

Total: 48
all but one of the arthritic cases, one case of cutaneous lupus erythematosus, and three cases without evidence of joint involvement. Strauss (1962) found no evidence of antinuclear factor against skeletal muscle-cell nuclei but Beutner et al. (1962) and Felkamp et al. (1963a and b) reported its presence. The incidence of positive results against muscle nuclei is not recorded but, using human leucocytes as antigen, Felkamp et al. (1963a) found antinuclear factor in 15 of 111 cases (13.5%). Antinuclear factor was not detected in any of 70 normal controls (type unspecified). In a personal series of 40 cases of myasthenia gravis the antinuclear factor against human leucocytes was detected in eight. No L.E. cells were detected. (The series is additional to the 440 cases described by Simpson (1960) in which one patient had L.E. cells in her blood.) The tests were performed on human leucocytes according to the technique of Alexander, Brenner, and Duthie (1960) who found 4% positives in 580 controls (healthy young blood donors of both sexes). The original series (Simpson, 1960) contained one case of pure red-cell aplasia. Holborow, Asherson, Johnson, Barnes, and Carmichael (1963) report a positive antinuclear factor test in a case of this disorder associated with a thymoma. There were four cases of pernicious anaemia in the earlier report and this has now increased to nine (Table I). Red-cell aplasia has been associated with myasthenia gravis and thymomas by several authors but no correlation has been described between myasthenia gravis and pernicious anaemia though it has been mentioned in one report (Rowland, Hoefer, Aranow, and Merritt, 1956). My own cases are described in another paper (in preparation). It has recently been suggested that pernicious anaemia is associated with an immunological disorder with production of organ-specific antibody to intrinsic factor and to the parietal cells of the stomach (Schwartz, 1960; Irvine, Davies, Delamore, and Williams, Wynn 1962; Taylor, Roitt, Doniach, Couchman, and Shapland, 1962). In the present series of myasthenic patients, antibody active against human stomach was detected in three of 35 cases of which one had pernicious anaemia. With the method used positive results are obtained in 8% of middle-aged female blood donors. 

FIG. 2a. Section of thyroid from case MN 7390 showing lymphadenoid goitre which was unsuspected during life. × 30
FIG. 2b. Lymphadenoid goitre from a case of thymoma with myasthenia gravis. × 30
(Irvine, 1964). The other patients with pernicious anaemia listed in the full series (Table I) are no longer available for immunological studies. It has also been reported recently that pernicious anaemia may be associated with rheumatoid arthritis (Partridge and Duthie, 1963), with autoimmune thyroiditis (Irvine et al., 1962; Taylor et al., 1962; Markson and Moore, 1962), with thyrotoxicosis and systemic lupus erythematosus (Doniach, Roitt, and Taylor, 1963), and with diabetes mellitus (Arakakis, Bock, Williams, and Witts, 1963; Beckett and Matthews, 1962). There is thus an overlap between several diseases associated with abnormal immunological reactions and to these myasthenia gravis should now be added.

The relationship between thyrotoxicosis and myasthenia gravis has been known for many years but Simpson (1960) showed that the relationship was indirect and could be genetic. Many of the cases previously described have shown diffuse lymphoid changes in the thyroid. Case MN 5503 (Table III) was found to have thyroiditis of Hashimoto type at necropsy. This was unsuspected during life until thyroid antibodies were found in her blood. One case of myasthenia with thymoma had Hashimoto’s struma (not included in the Table) (Fig. 2). A clinical diagnosis of Hashimoto’s disease was made in the following case (MN 7390). A point of special interest is that Hashimoto’s disease developed five to seven years after thymectomy.

J. McD (male) was found to have active pulmonary tuberculosis and at the same time he developed piosis, diplopia, dysphagia, and weakness of the arms in 1943 when aged 19. Myasthenia gravis was diagnosed and treated with neostigmine until 1955 when thymectomy was carried out by Mr. Paterson Brown in Edinburgh Royal Infirmary. Neostigmine requirements were reduced from 25 to 10 to 15 tablets daily.

From 1960 he complained at intervals of abdominal pain, sweating, and tiredness, which were alleviated by regulation of the dosage of neostigmine and pyridostigmine. During one such episode he was admitted to Inverness Royal Infirmary where it was noted that he had a diffuse goitre and pigmentation of the face (Fig. 3). He was complaining of tiredness and dyspnoea which were not improved by regulation of dosage and were not apparently due to reactivation of tuberculosis. He was overweight and showed no clinical evidence of thyrotoxicosis.

A radioiodine uptake test gave a T.P.I. of 1-6 and the protein-bound iodine was 1-4 mg. per 100 ml. Serum cholesterol concentration was 200 mg. per 100 ml. Antibody titres against thyroid tissue were T.C.H. 1/2,500,000; C.F.T. 1/256; precipitin test positive within 24 hours. The zinc sulphate turbidity was 14-4 units and thymol turbidity 8-2 units. A diagnosis of Hashimoto’s disease was made and treatment started with 1-thyroxine (0-1 mg. daily) in February 1963. He was admitted to the Neurological Unit in July 1963. The E.S.R. was 70 to 85 mm. in the first hour. No L.E. cells were found in his blood and antinuclear factor was not detected (Dr. W. R. M. Alexander). Antibody titres against thyroid were estimated by Dr. W. J. Irvine as T.C.H. 1/25,000, C.F.T. 1/16. No antibodies active against gastric or liver tissues were found. Serum electrophoresis showed increased gamma globulin. Thymol turbidity was 5 units, and the cephalin-cholesterol flocculation test was positive. The pulmonary tuberculosis was considered to be inactive. No mediastinal tumour could be detected by radiology. Myasthenia was well controlled by pyridostigmine 60 mg. t.d.s. and neostigmine 15 mg. t.d.s. (alternating) and ephedrine 1/4 t.d.s. Persisting tiredness was considered to be caused by Hashimoto’s disease.

Thyroid antibodies have been present in 10 of 38 cases examined in this series by the method described by Irvine (1964). Six of the positive reactors have had confirmed or suspected thyrotoxicosis at some time in the past and another four cases with a similar history have not had a significant level of antithyroid substances in their serum. The others shown in Table III have not been examined for thyroid antibodies. Van der Geld et al. (1963) found antithyroid antibodies in 36 of 111 cases of myasthenia gravis.

No detailed studies have yet been made on the relationship with diabetes mellitus. This may be coincidental but is worth further study since there
also appears to be a familial relationship between the two conditions (Simpson, 1960). Other diseases believed to be autoimmune in type have occasionally been associated with myasthenia gravis. Raynaud's syndrome present in more than 2% of myasthenic patients (Simpson, 1960) may be caused by auto-agglutinins in the blood (Forbes, 1947). Ulcerative colitis and hepatic cirrhosis were associated with systemic lupus erythematosus which followed thymectomy for myasthenia gravis in a patient recently reported by Alarcón-Segovia, Galbraith, Maldonado, and Howard (1963). I have seen two myasthenic patients with acute hepatitis and two with acute haemolytic anaemia. In each instance it was considered that the liver or blood disorder was due to the toxic effect of a drug such as chlorpromazine or P.A.M. (Simpson, 1961) but cases recorded in other series are listed in Table I. One patient (MN 5409), who had had myasthenia gravis for 17 years, developed Addison's disease which caused his death. At necropsy it was shown that both adrenal glands were caseous owing to tuberculous infection. No abnormal antibodies were found in his serum.

DISCUSSION

The evidence gathered together in this paper supports the hypothesis that myasthenia gravis is associated with disturbed antigen-antibody reactions but demonstrates that there may be several organ-specific antibodies present and that there is a clinical and serological overlap with many other autoimmune disorders. Antibodies against muscle fibres are rare except in the presence of a thymic tumour and may not account for the defect of neuromuscular transmission. Some of the permanent weakness found in severe myasthenia, notably in association with a thymoma, may be due to this type of reaction. The possibility of a more localized end-plate reaction remains unconfirmed. The multiplicity of antibodies makes it unlikely that each organ is 'leaking' antigen which then 'instructs' the reticuloendothelial system to form appropriate antibody. It is more probable that the antibody-producing mechanism is producing proteins of which some react with various tissues. There can now be little doubt that the thymus plays a key role in the regulation of antibody production at least in foetal life but the persistence of myasthenia after thymectomy or the development after thymectomy of myasthenia gravis (Green and Booth, 1958), thyrotoxicosis (personal experience), ulcerative colitis, and systemic lupus erythematosus (Alarcón-Segovia et al., 1963) make it certain that the thymus is not necessary for the continuation of the disease or for the subsequent development of autoimmune disorders. The present paper records a case of Hashimoto's disease which probably occurred at least five years after thymectomy.

The observation that thymectomy is most beneficial if performed within five to seven years of the onset of myasthenia gravis might imply that it is an essential element at first but that other parts of the reticuloendothelial system may then continue to produce autoantibodies without its presence (Simpson, 1958, 1960). Nothing is known of the nature of the possible changes of immunological tolerance. Burnet (1962a) discusses various hypotheses involving failure of the homeostatic control of the thymus and White and Marshall (1962) suggest a breakdown of a blood-thymus barrier. Since the first part of the writer's hypothesis has proved so acceptable it might not be inappropriate to draw attention to the other part. A study of the personal and family histories of 440 patients with myasthenia gravis suggested that the altered immunological tolerance might be due to a genetic factor acting on the thymus through the pituitary gland. This may provide the intrinsic (non-immunological) stimulus to the thymus or the homeostatic mechanism required by Burnet's theory. Burnet (1962a) proposes that the thymus is primarily concerned with the maintenance of the chemical integrity of the body, including the mechanism of specific protein synthesis. He suggests that the development of the immunological competence of the body is part of the process of morphological and functional differentiation of the embryo and young animal as previously postulated by Simpson (1960). Weiss (1950) and Burwell (1963) have developed alternative theories of control by the reticuloendothelial system of cellular growth and differentiation. If my further suggestion that this mechanism is controlled by the pituitary gland is as fruitful as the rest of the hypothesis it would open up a new field of experimental biology.

SUMMARY

The author's hypothesis of an autoimmune mechanism in myasthenia gravis is summarized and further evidence reviewed. A high incidence of thyroid disorders, pernicious anaemia, and a rheumatoid type of arthritis is reported. Many cases had anti-nuclear factor and others had antibodies in their serum specific against thyroid or gastric tissue. No significant antimuscular activity was found in a small sample and it is suggested that this disagreement with other workers is due to absence of thymoma in the cases examined. The occurrence of Hashimoto's disease in a myasthenic patient is recorded. This is the first report of the association and also of the occurrence of Hashimoto's disease in a patient without
a thymus. A hormonal homoeostatic mechanism for the thymus is suggested.

I am grateful to the Medical Committee of the National Hospital, Queen Square, London, for permission to investigate the London cases, to Dr. W. Lancaster of Inverness Royal Infirmary for referring the patient with Hashimoto’s disease, and to Drs. W. R. M. Alexander, J. R. Anderson, and W. J. Irvine for the immunological studies. Dr. A. F. J. Maloney kindly provided the histological studies.

ADDENDUM

Since this paper was written Daly and Jackson (1964) have reported a case of myasthenia gravis associated with Hashimoto’s disease. An additional case in the author’s series has been found at necropsy. As no antibody studies were carried out this case is not included in Tables I to III.

REFERENCES


187. SIMPSON J.A., Glasgow, Great Britain - Autoimmunity in myasthenia gravis

SIMPSON (1960) presented evidence that myasthenia gravis may be a multisystem disease and drew attention to resemblances to Systemic Lupus Erythematosus with respect to age, sex, course, precipitating factors, and pregnancy. It was suggested that the abnormal thymus was the source of circulating or cell-borne antibodies active against the junctional region of muscle and sometimes against other organs. Transplacental passage explains neonatal myasthenia but antigenic difference accounts for failure of cross-transfusion and animal experiments. STRAUSS et al. (1960) and later workers demonstrated muscle binding complement-fixing antibody in many myasthenic sera. Titre is highest in cases with thymoma. Antimuscle titre is highest in cases with thymoma. Removal of a hyperplastic thymus is beneficial for myasthenia of less than 5 - 7 years duration (SIMPSON 1958). It is suggested that immunologically competent cells may be produced by other lymphoid structures without thymic control after that period. A significant incidence of hypergammaglobulinaemia and raised protein in cerebro-spinal fluid is reported.


SIMPSON, J.A. (1958) Brain, 81, 112.


VENTERS, J. (1955) Immunology, in press.
Simpson (1960) presented evidence that myasthenia gravis may be a multisystem disease and drew attention to resemblances with Systemic Lupus Erythematosus (S.L.E.) in respect of age, sex, course, precipitating factors, and pregnancy. The pathology of muscle, thymus and other organs was in favour of an immunological disorder. It was suggested that the abnormal thymus was the source of circulating or cell-born antibodies active against the junctional region of muscle and sometimes against other organs. Transplacental passage explains neonatal myasthenia but antigenic difference accounts for failure of cross-transfusion and animal experiments. Removal of a hyperplastic thymus is beneficial for myasthenia of less than 5-7 years duration (Simpson, 1958). After that period it is possible that other lymphoid structures produce immunologically competent cells without thymic control.

Strauss et al (1960) demonstrated by fluorescence that many myasthenic sera contain complement-fixing antibody which binds to the 'A bands' of skeletal muscle fibres. Titre is highest in cases with a thymoma. Vettes (1965) considers that similar fluorescence seen with many normal sera is situated on the 'I band'.

The immunological hypothesis is supported by later demonstration of associated Hashimoto's disease, pernicious anaemia (Simpson, 1964), ulcerative colitis and S.L.E. (Alarcon-Segovia et al, 1963). Anti-nuclear factor (A.N.F.) and antibodies against thyroid and gastric parietal cells are more common in sera from myasthenics (Simpson, 1964). There is some uncertainty about the presence of a raised level of rheumatoid factor (Van der Geld et al, 1963).

Serum gammaglobulin was lowered in 5 and raised in 1 case reported by Thévenard and Béna (1955). Lowenthal and van Sande (1956) urged measurement of absolute rather than percentage levels. They reported hypergammaglobulinaemia and lowered albumin in 12 of 16 cases. Other reports are reviewed by Costerhuis et al (1964). These authors found relative hypergammaglobulinaemia in 3 of 15 patients. Two of these had a thymoma.

Total plasma globulin was estimated in 41 cases of the present series, 37 with electrophoretic separation. Absolute hypergammaglobulinaemia was found in 11 cases. Five others showed increased γ or β globulin. In the 4 cases with a thymoma the electrophoretic changes were slight unless other complications existed. On the other hand, absolute hypergammaglobulinaemia was found to be closely associated with Hashimoto's disease. Abnormal cephalin-cholesterol flocculation and thymol turbidity tests were found in 4 cases including 4 with normal levels of gammaglobulin. These abnormalities were associated with 'rheumatoid' arthritis, lymphadenopathy, Hashimoto's disease or a previous history of goitre, hepatitis, megablastic and haemolytic anaemia (single case with thymoma), or a positive A.N.F. test. No complicating factor was noted in 2 cases.

Ten patients had C.S.F. protein levels exceeding 50 mg/100 ml. One had minor seizures and one had polymyositis. The others had no evident neurological disease but 6 had some of the non-neurological complications previously listed by Simpson (1960, 1964). The protein abnormalities support the author's hypothesis of a breakdown of immunological tolerance in myasthenia gravis. Increased or altered globulins occur in the serum or C.S.F. in myasthenia complicated by clinical disorders of immunological type. A thymoma need not be present.
Myasthenia gravis has always been a puzzling disease, but for our generation it seemed that the solution was not far off in view of the rapid advance of knowledge about transmission at the neuromuscular junction. Since the successful use of anticholinesterase drugs in 1934 it has seemed probable that the disorder would be explained by a biochemical disturbance at the junction which caused transmission failure during sustained or repeated use of the junction. Certainly I was taught that myasthenia was brought on by exercise and relieved by rest.

In some cases the onset of weakness is insidious but in many it is quite rapid and the same is true of relapses in the remittant form of the disease. When the circumstances of the rapid onsets are investigated it is surprising to find that physical exertion is a minor factor. The most common precipitating factor is infection (usually of the upper respiratory tract) and emotional causes are almost as common.

In some of these cases the relationship with emotional disturbance was very striking.

Three patients whom I examined at the National Hospital, Queen Square, London, attributed the first onset of symptoms, or a relapse, to "shock" during the bombardment of London. One of these cases, a young woman aged , was thrown to the ground by blast while she was running to an air raid shelter. She was unable to rise again. A diagnosis of hysterical palsy was made at the hospital to which she was taken, but later events caused the diagnosis to be changed to
myasthenia gravis.

Five patients developed their first symptoms of myasthenia soon after a near relative had died suddenly, and in three women the symptoms appeared to be precipitated by anxiety about the illness of a child or husband.

Two young women first became aware of ptosis while they were being married, but another developed the same symptoms soon after she found a letter from another woman in her husband's pocket. Yet another attributed the onset to the worry of divorce proceedings. Two women developed ptosis at a party and many other patients with established myasthenia have this symptom every time they enter a crowded room.

One of the most striking cases of emotional precipitation was a crane driver aged . The crane was overturned by accident and he was thrown into a position where he was certain he was going to be crushed to death by the falling crane. In the event, he was completely unhurt. Another three patients dated the onset of myasthenic symptoms from a sudden fright. In all of these cases the onset of symptoms was clearly associated in the patient's mind with the emotional disturbance, whether the latter was unpleasant or, as in the weddings, presumably pleasurable. The same is true of many of the later relapses. If the patient is questioned closely it is rare to find that relapse is attributable to physical stress but common to find an emotional cause. An excellent example under my care at present is a young married woman who has a remission when her elderly and rather "difficult" father-in-law goes to live with other relatives but relapses when he comes back to her house. But emotional stress may have the opposite effect. One patient
had a long remission when she was blown up by a "doodle bomb" during the war. Another patient with severe myasthenia dropped a hot water bottle when her hands became too weak to hold it. She suffered a severe burn and was admitted to a hospital where it was not known that she required a large dose of neostigmine. Nevertheless she had no myasthenic symptoms during the next two weeks until she was recovering from the burn when she suddenly remembered about the muscular weakness. A third patient had a complete remission when her husband was injured by falling from a roof and she had to go to his assistance. Perhaps the most striking example of remission associated with emotional disturbance is a patient who was under my care in Edinburgh. He was a 29 year old man with a previous history of exophthalmic goitre. In March 1960 he had the first of a series of epileptic fits. At the same time there was intellectual deterioration sufficient to cause loss of employment as an electrician. One year later when I saw him a clinical and E.E.G. diagnosis of left temporal-lobe epilepsy was made. In May 1962 the attacks became associated with brief "choking". A few days later he complained of pain in his back, difficulty in controlling his head, and severe weakness of his legs, especially in the evening. This was followed in another week by upper limb weakness, ptosis of the right eyelid, double vision and loss of voice when speaking. Examination at that time showed typical very severe generalised myasthenia gravis.

Despite increasing dosage of neostigmine he became extremely weak. Thymectomy did not benefit him and for the following year he required almost constant passive respiration. Fifteen months after the operation,
when the prospect of recovery seemed remote, a patient from his home town was admitted to the neighbouring bed. He remarked that he had recently met the myasthenic patient's wife at a dance. This infuriated him and he pulled out his tracheostomy and jejunostomy tubes. He was very agitated and paranoid for some time, but from that day he began to improve rapidly. A response to neostigmine reappeared, the muscles became more bulky, subcutaneous fat reappeared and his mental state rapidly returned to normal. In one month he was able to spend weekends at home and in two months was discharged from hospital.

Myasthenic patients tend to be nervous and excitable, the personality resembling that associated with thyrotoxicosis. Severe anxiety states are not uncommon and earlier writers have remarked on the frequency of depression (Buzzard, 1905; Boothby, 1935; Collins, 1939). Kennedy and Moersch (1937) noted depression in 8% of cases. It is not surprising that fear of choking should cause panic or hysterical reactions, and depression with fear of dying during sleep is fully understandable. This did not seem to be an adequate explanation of the psychotic behaviour exhibited by twelve of my five hundred patients. One patient whom I saw in 1958 had had mild myasthenia gravis for six years. It was well controlled on 3-4 tablets of neostigmine daily and he considered that the weakness was trivial compared to his worries. He was referred to me from the Royal Edinburgh Hospital because it was desired to give E.C.T. for the depression and there was some concern about the use of muscle relaxant drugs. The treatment was given without incident. The psychiatric illness was depressive with prominent paranoid symptoms in ten cases, and there
was one case each of acute mania and of schizophrenia.

It is possible that these psychiatric abnormalities are coincidental but, as I have pointed out before (Simpson, 1960, 1965) there is a comparable incidence in two other large series of myasthenic patients in the literature (Osserman, 1958; Storm-Mathisen, 1961). A new series recently reported by Oosterhuis & Wilde (1964) reports acute confusional states with hallucinations occurring in myasthenics. I have seen this but have always considered that it was due to respiratory insufficiency. These authors draw attention to the apparent return of full muscular power during the period of disorientation. The patient described above who made the remarkable recovery when hope had been virtually abandoned, was able to pull out two firmly anchored rubber tubes though he was unable to grip firmly on the previous day. Oosterhuis and Wilde (1964) considered that four of their 150 cases of myasthenia gravis had psychotic episodes (two endogenous depressions, one schizophrenia) and 89 showed neurotic traits. Most of these were overmeticulous and abnormally clean, some demonstrated phobic phenomena, vague anxiety and obsessions. In general they were pessimistic in outlook. They tended to be depressed or had clearly hysterical features. They examined their cases further with the Amsterdam Personality Questionnaire and found an increased tendency towards introversion.

It is difficult to assess the significance of the psychiatric illness, but I would agree with Oosterhuis and Wilde (1964) that it is usually present before the onset of muscular symptoms and so cannot reasonably be attributed to the latter. We have become so used to
statistical tests of significance that we forget that data my still be meaningful even though not significant in the statistical sense. In 1960 I drew attention to the fact that all the major series of myasthenia in the world literature, including my own, showed an apparent association between the muscular disease and disorders of other organs. Psychosis and epilepsy were included in the neurological correlates. Since I drew attention to this (Simpson, 1960, 1965) other workers have reappraised their clinical material and confirm that their own cases are similar. It is, of course, possible that the relationship is coincidental, but then the same coincidences have been happening in different parts of the world. Alternatively the relationship is meaningful. I have postulated that there is a break-down of immunological tolerance to muscle in myasthenia gravis and that this may sometimes cause "autoimmune" disorders of other organs (Simpson, 1960). The evidence in favour of this concept is now rather strong (Simpson, 1964).

The acceptance of this concept has not included what I believe to be an important link in the argument. It has been recognized for many years that there is a close link between myasthenia gravis and thyrotoxicosis. Indeed until the autoimmune theory/myasthenia gravis gained prominence the disease was usually considered to be a disorder of endocrinological type. There is, in fact, some evidence for hormonal disorder. I feel that the link between the emotional factors I have been describing and the disorder of the thymus gland which causes myasthenia gravis is likely to be hormonal, probably acting through the hypothalamus and pituitary gland. I have argued (Simpson, 1960) that there is a genetically
determined state of the hypothalamic-pituitary axis which may lead to either thyroid disease or myasthenia or both.

The endocrinological approach has also been suggested by the well known effects of menstruation and pregnancy on the myasthenic woman. Many but not all of them get worse before a period and improve when it starts. But the relationship with pregnancy is much more puzzling. Some patients have a relapse when they become pregnant whereas others have a remission. In general there is a tendency for relapse to occur in the first trimester, improvement in the second and third trimesters and then relapse again after the child is born. It has not proved possible to correlate these changes (and especially the opposite effects in different patients) with the hormonal state. I have pointed out (Simpson, 1964) that the changes closely parallel the emotional responses of many normal women to their pregnancy, labour and puerperium. A wanted pregnancy sometimes seems to be associated with remission, and so is a desired termination of pregnancy. On the other hand an illegitimate pregnancy or an accidental miscarriage may precipitate a relapse. Two interesting cases should be recorded.

One woman suddenly improved her myasthenic status. She was later convinced that this coincided with the date of conception which she was able to define accurately as it occurred during her husband's return on short leave during the war. It was doubtless a joyful occasion. At any rate the myasthenic remission occurred before she missed a period and she relapsed again just before the first post-partum period three months after the baby was born.
Mrs. I.M., on the other hand, relapsed two years after successful thymectomy at the time she realised she was pregnant. Pregnancy was terminated at the fifth month and myasthenic symptoms disappeared on the same day. I find it difficult to believe that these sudden changes could be due to a hormonal influence which is too subtle to detect by present techniques and suggest that more attention should be paid to the emotional state of the patient.

To summarize: I have suggested that psychotic disorders may occasionally be part of the myasthenic syndrome - in analogy with Systemic Lupus Erythematosus which I believe to be a related disease. More commonly, there is a neurotic background to myasthenia gravis which may determine its onset, relapse or remission. It is very important to remember this before making a retrospective diagnosis of hysterical palsy. Many of my patients have been diagnosed as hysterics for long periods. Consider the situation. A young healthy-looking girl enters the consulting room with a tale of double vision, loss of voice, or paralysis of limbs. The doctor elicits a history of emotional upset, often an unhappy love affair. Superficial neurological examination is negative as she has been resting in the waiting room. A diagnosis of hysteria is self evident. If she had complained of progressive "fatigue" or of weakness increasing towards the evening the true diagnosis might have been made, but in fact surprisingly few myasthenics describe their symptoms in this way until specifically questioned about the effect of exercise. If the patient uses the word "fatigue" rather than loss of power in describing his symptoms it is much more likely that the diagnosis
is psychoneurosis. A neurologist sees many cases presented as "myasthenia", the majority of which do not have myasthenia gravis. I would agree with Schwab and Perlo (1965) that one in three of these patients have some form of psychogenic fatigue associated with psychoneurosis or hysteria. (The remainder have organic disease of the muscles or nervous system though not myasthenia gravis). A therapeutic test with edrophonium or neostigmine may be misleading in the hands of inexperienced workers. It is essential to have objective evidence of improved muscular performance at the appropriate time after the injection. A professed feeling of improvement by the patient should never be accepted as evidence (Simpson, 1965). The diagnosis is not easy and I have no doubt that some neurotic patients are masquerading as myasthenics. This is, however, less serious than the opposite mistake since untreated myasthenia gravis represents a constant danger to life. Meyer (1965) describes sudden, cataleptic-like intensification of myasthenic weakness caused by anger or by terror, and aggravation of symptoms over a period of 1-2 days brought about by a mixture of anxiety and depression. Chafetz (1965) considered that certain personality types responded more poorly to medical treatment of myasthenia gravis but Meyer (1965) was unable to associate the disease with any particular personality pattern. There is therefore not only a diagnostic difficulty but a therapeutic one. Adequate psychotherapy, whether formal or otherwise, may prevent relapses and encourage recovery though, as Meyer (1965) points out, in some instances where obvious aggravation of symptoms from emotional stress is present, a psychiatrist may be unable to be of help. I must close
with a warning of the possible danger of using tranquillising drugs to contest the psychiatric disorder in the myasthenic. Some myasthenic patients have their weakness increased by taking chlorpromazine, (McQuillen, Gross & Johns, 1963). I have one patient who responded in this way to chlorpromazine and also to chlordiazepoxide.

I have pointed out how many putative cases of myasthenia gravis are found to be suffering from a neurotic syndrome. Nevertheless, in view of what I have just said, you will realise that it is always wise to consider the possibility of myasthenia gravis in the hysterical patient who has muscular weakness, especially if this involves speech, respiration, swallowing, or double vision. A neurologist will always be glad to see them.
Many people have doubted whether myasthenia gravis is a disease entity. Undoubtedly the symptom 'myasthenia' is not peculiar to one disease. It is characterized by the development of an abnormal amount of weakness in voluntary muscles following repetitive contraction or prolonged tension, with a marked tendency to recovery of motor power after a period of inactivity or lessened muscular tension. The decrementing response to maximum innervation is sometimes described as 'fatigue'. The term will serve for clinical description so long as it is clearly understood that it has practically nothing in common with physiological fatigue. It may be present in muscles affected by polymyositis, systemic lupus erythematosus, dermatomyositis, or one type of carcinomatous myopathy, often only at the onset of the illness. It is occasionally found in disorders of the lower motor neurone (Simpson, 1960a, Simpson and Lenman, 1959). Some authorities consider that a therapeutic response to anticholinesterase drugs is also necessary for the definition of myasthenia gravis. Some response may be found in these 'symptomatic myasthenias' but it is rarely dramatic and the response often fails after a few weeks of treatment. It is, therefore, true that the myasthenic response is a symptom found in different diseases. Nevertheless I agree with those who consider that myasthenia gravis is a clearly recognizable disease in which the response of the symptom to anticholinesterase drugs is more dramatic than in the other types, and which has an individual natural history and pathology, though the theme I will develop will show that the first group of symptomatic myasthenias, the acquired myopathies, is closely related to true myasthenia gravis.

**NATURAL HISTORY**

No race is immune to myasthenia gravis. Estimates of prevalence range from 1 in 50,000 to 1 in 10,000 of the population. It affects both sexes, women twice as frequently as men. In young people the female preponderance is as high as 4.5 to 1 but myasthenia starting in the latter half of life is commoner in males. This difference in distribution makes the mean age of onset a little lower for women (26 years) than for men (31 years) but the
modal age of onset is about 20 years for each sex (Simpson, 1958 and 1960b).

The onset may be insidious or sudden. Common precipitating factors are (i) emotional upset, (ii) a febrile disease, usually an upper respiratory infection, (iii) pregnancy or the puerperium, (iv) severe physical stress. Despite accepted teaching the latter is the least common and the more myasthenia I see (and I have now personally examined more than 400 cases) the more I am impressed with the importance of psychological factors. Perhaps this accounts for the fact that most patients are first diagnosed as hysterics.
The characteristic course is one of relapses and remissions, but the long-term remissions are not so common as many believe. Fewer than half of the cases have a remission of a month or more and long remissions rarely occur more than once and then usually within the first 5 years of the disease. Further relapses are influenced by the factors described above but additional causes are menstruation, extremes of heat or cold, inoculation or vaccination and, occasionally, allergy. Most of the deaths directly attributable to myasthenia gravis occur during the first 5 years, particularly during the first year, with a second danger period from 4 to 7 years after the onset (Simpson, 1958). After 10 years death from myasthenia per se rarely occurs though the patient may be constantly at risk of asphyxiation from inhaled foreign bodies because of the diminished expiratory reserve. Some cases undoubtedly continue to deteriorate for many years but in the average case the 'active' stage of the disease whether for remission or severe relapse is limited to a period of 4 to 7 years and the subsequent course depends on the extent of damage occurring during that period. It is during this time that thymectomy must be carried out if the operation is to be beneficial (Simpson, 1958 and 1960b). Later cases are not helped by surgery and in the final stages of 'burned out' myasthenia the response to drugs may disappear. At that stage the clinical picture closely resembles polymyositis and has been termed 'myasthenic myopathy'.

The course may be benign and the affection may remain limited to a few muscles. Grob (1953) and Ferguson et al (1955) state that 20 to 30 per cent of cases show only extra-ocular muscle involvement. If there is no further spread in 2 years the prognosis is good. In my experience this restriction has been rare except in males and this might account for the fact that I have found a worse prognosis in females, contrary to the usual opinion (Simpson, 1958). The course is greatly influenced by the nature of the associated thymic pathology. A thymic tumour is found in 10 to 20 per cent of cases. It is more common in males and according to Keynes (1955) is rarely found in patients under 30 years of age. The illness is often particularly severe in these cases and difficult to control by drugs or surgery, but this is not invariable.

CLINICAL PICTURE

In addition to the long-term fluctuations just described, there is a very characteristic variability in muscular strength from day to day or even from hour to hour. Short term weakness is often due to physical exertion when it tends to 'fatigue' steadily with repeated or continuous use of the
Biochemical Aspects of Neurological Disorders

affected muscle. The failure of contraction may, however, be sudden and the observer unfamiliar with this fact may be misled into a diagnosis of hysterical paresis, particularly as emotional upset is just as important in causing short-term weakness as in precipitating relapses in the general course. Power is restored by a short rest or by administration of an anticholinesterase drug but the maximum power obtainable is often suboptimal. This is an important point which requires emphasis as an overdosed patient may proceed from myasthenic to cholinergic weakness without an intervening stage of full muscular power. This is probably due to the presence, even in early cases, of unresponsive muscle fibres which are presumably affected by the necrotic changes described below. In later stages 'myopathic' changes are not uncommon especially in the extraocular muscles (Keynes, 1954), the triceps brachii, and the tongue where it causes the triple grooving described by Buzzard (1905) and Wilson (1954).

In 'burned-out' cases the permanent weakness may be difficult to distinguish from polymyositis or from exophthalmic ophthalmoplegia.

Any muscle may be affected by myasthenia gravis but especially the extra-ocular, bulbar, neck and shoulder girdle muscles. The order of incidence of involvement is virtually the same as the sequential order in which muscles are involved in most cases (Fig. 2) but there is great individual variation. A diagnosis of myasthenia gravis need not be rejected because of sparing of the extra-ocular or bulbar muscles though this is more common in the 'symptomatic myasthenias'. In the latter the tendon reflexes are reduced pari passu with muscle strength and in carcinomatous myasthenia they are often absent. In true myasthenia gravis, on the contrary, they are usually brisk and even clonus is not uncommon, though the reflex jerk may decrease progressively if elicited repetitively.

An account of the symptoms resulting from myasthenia of different muscles is unnecessary in this chapter for I have given an account of the more important manifestations elsewhere (Simpson, 1964). The symptoms of progressive 'fatigue', in the sense used above, are so characteristic that the diagnosis is readily made if only it is considered. Confirmation is obtained by testing the ability of each muscle to maintain contraction for a reasonable time. For instance the arms can normally be outstretched for more than a minute and no ptosis will normally result on looking upwards for the same period of time. The brief contraction against resistance which is adequate for the detection of most forms of muscle weakness is just not good enough. It is my personal belief that if this rule is followed the cases of 'pure extra-ocular myasthenia' are few indeed.
**Diagnostic Tests**

Fatigue tests appropriate to the various muscle groups will readily suggest themselves and it may be possible to record a progressive decrease of muscle power with a dynamometer or ergograph (Fig. 3). Performance tests are conveniently combined with pharmacological tests. These may be (i) therapeutic, or (ii) provocative. The most useful therapeutic test is the response to an intravenous injection of edrophonium chloride ("Tensilon"). This has the great advantage of rapidity since clinical improvement occurs in half to one minute after an injection of 10 mg of the
drug and as the main effect passes in 5 minutes the muscarinic action in non-myasthenic subjects is not too unpleasant. Subjective assessment of improvement by the patient is unreliable and no diagnosis should be based on it. It is essential that the doctor be convinced that there is an unequivocal measurable improvement in some performance test. Since the muscles chosen for observation may be affected by 'myopathic' changes the short duration of action may be a disadvantage and so it is sometimes preferable to test the response to an intramuscular or oral dose of neostigmine which gives more time for multiple or repeated tests of muscle strength.

Provocative tests are less useful as interpretation may be difficult and the weakness induced may be dangerous. All anticholinesterase medication

should be withdrawn for 24 hours and facilities for resuscitation prepared before carrying out a provocative test. Injection of d-tubocurarine in 2-5 per cent of the dose required to paralyse a normal subject, or the oral administration of quinine may precipitate dangerous weakness. In the case of quinine this cannot be counteracted by the use of neostigmine. For these reasons I have deliberately avoided giving any detail of the test procedures. Those who wish to use them should consult original sources or the detailed account given by Simpson (1964). One provocative test is of sufficient theoretical interest to require further consideration. Churchill-Davidson and Richardson (1952) have shown that the action of depolarizing neuromuscular blocking drugs such as decamethonium is different from the normal, and not simply an increased sensitivity as with curare and

\[\text{Fig. 3. Ergographic record of repeated hand grips, at intervals after taking Pyridostigmine by mouth.}\]
Myasthenia Gravis

59

quinine, since they tolerate an unusually large dose. Tolerance is particularly marked in clinically unaffected muscles, whereas the muscles showing myasthenic weakness are first to be affected but the type of block differs from normal. Depolarization block, if it occurs at all, is brief and soon changes to a longer competitive (curare-like) type of block. This 'dual response' is characteristic of myasthenia gravis. The nature of the block is shown by the fact that competitive block is reversed by injection of neostigmine (which would make depolarization block more profound).

The nature of the response to drugs is best studied by the use of electromyography to monitor the muscle response, and the subjective factor is removed by stimulating the motor nerve by brief supramaximal electric shocks (Harvey and Masland, 1941). In the absence of facilities, the original Jolly (1895) test using the mechanical response to faradization will serve. The response can only be interpreted if the stimulus is supramaximal, to make sure that any inadequacy of response is not due to failure to stimulate every motor nerve fibre. A positive response is obtained when successive muscle action-potentials or twitches decrement rapidly, even if stimulated more slowly than 30/sec. The initial decrement may be temporarily counteracted by facilitated release of acetylcholine caused by tetanic stimulation. This 'myasthenic reaction' may not be seen until the trains of stimuli have been applied several times or the nerve-muscle complex has been 'fatigued' by prolonged voluntary contraction. In some cases a slowly repeated stimulus causes a myasthenic response but a fast tetanization evokes a progressively increasing muscular response. This incrementing type of reaction may be the only abnormality seen and Simpson (1960b) suggests that it may be most evident in muscles showing resistance to decamethonium. It is seen in its most dramatic form in carcinomatous myasthenia (Eaton and Lambert, 1957; Wise and Mac Dermot, 1962), but also occurs in polymyositis (Simpson, 1960a; Simpson and Lenman, 1959).

Conventional needle electromyography records a progressive simplification of the pattern as voluntary 'fatigue' occurs. The electrical configuration of the motor units may be normal but there is commonly an increased incidence of 'myopathic' units and I have seen spontaneous positive sharp potentials such as are found in myositis. The units may be seen to decrease in amplitude during sustained contraction but often cease firing quite suddenly (Simpson, 1956). I mention these observations to draw your attention to two facts. Myasthenia gravis is associated with a functional defect of neuromuscular transmission (since, as shown by Jolly,
the 'fatigued' muscle will still respond to direct stimulation) but there is also evidence of some other lesion or lesions, either in the motor nerve or in the muscle fibre. I now want to turn my attention to an examination of some other facts which are difficult to reconcile with the current view that myasthenia gravis is a disorder of function of the neuromuscular junction.

**CLINICAL EVIDENCE OF DISSEMINATED DISEASE**

When medical opinion is unanimous about the nature of a disease any observation which conflicts with the concept is rejected as irrelevant or mistaken. This has been the experience of myasthenia gravis. In 1953–55 I had the opportunity to examine a large series of myasthenic patients to assess the value of thymectomy. I decided to record every detail mentioned by patients (or their hospital records) without reference to any particular concept. No special questions were put to the first 400 (London) cases so the recorded details represent the minimum occurrence of certain phenomena and should not be used to estimate true incidence. Many complaints were found which are clearly described in the first comprehensive review by Campbell and Bramwell (1900) but which have been ignored since then. Pain is quite common in weak muscles and may be the presenting symptom. It is usually an ache which is presumably due to the extra effort required to maintain posture. This often causes headache, pain round the eyes, backache, etc. Sometimes the muscles are actually tender, and pain has been noted to persist even while resting in bed. A few patients complain of mid-ternal pain. It tends to occur while stooping and may be associated with palpitation (Russell, 1953; Simpson, 1960b). A sensation of 'stiffness' is not uncommon and there may be paraesthesiae of hands, thighs or face (Harvey, 1948; Simpson, 1960b). There is usually a mechanical explanation such as traction of a nerve by a drooping shoulder girdle, but some sensory phenomena are difficult to account for in this way. Transitory anaesthesia of the face has been recorded. Symonds (1922) described one case with temporary anaesthesia of the pharynx and another with temporary loss of the sense of taste. I have seen a patient with the same symptom and Alajouanine et al (1957) have recorded anosmia in myasthenia gravis.

Other neurological disorders occasionally present are psychotic disturbances and epilepsy (Table 1). Neither of these is common but it is surprising that each of these and other conditions appear in the lists of
associated disorders in each of three large series (Harvey, 1948; Osserman, 1958; Simpson, 1960b). Storm-Mathisen (1961) also noted psychosis and Hocfcr, Aranow and Rowland (1958) report eight patients with epilepsy in 180 cases of myasthenia gravis. Increased content of protein in the C.S.F., reported by de Haene and Roussel (1955) was present in nine of the London case records in my series. The true incidence is unknown since lumbar puncture was rarely performed. Unquestionably these neurological abnormalities are rare, but it must be considered whether their occasional presence must be explained by postulating coincident unrelated disease. In view of some of the other associated conditions, Simpson

Table I
Disorders other than reticulo-endothelial, associated with myasthenia gravis

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disorders (all types)</td>
<td>86</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes/glycosuria</td>
<td>16</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>'Rheumatoid' arthritis</td>
<td>19</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma (non-thymic)</td>
<td>19</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Acute nephritis</td>
<td>2</td>
<td>1</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Acrocyanosis</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy and recurrent blackouts</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Psychosis</td>
<td>10</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>C.S.F. protein raised</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td><strong>490</strong></td>
<td><strong>325</strong></td>
<td><strong>90</strong></td>
</tr>
</tbody>
</table>

(1960b) suggested that myasthenia gravis can no longer be considered to be a disorder limited to the neuromuscular junction. These other conditions will now be described.

Endocrine Disorders
A relationship between myasthenia gravis and disorders of the thyroid gland has been recognized for many years. Millikan and Haines (1933) found that the incidence of hyperthyroidism before, during, or after detectable myasthenia gravis is about 5 per cent. In my experience about 9 per cent of males and 18 per cent of females with myasthenia gravis
show signs of a thyroid disorder at some time during their life but this need not be thyrotoxicosis (Simpson, 1958). Non-toxic goitre and, more rarely, primary myxoedema may be found and indeed most of the early reports stress the presence of non-toxic nodular goitre, and the histological appearance of lymphadenoid goitre. Many cases have thyrotoxic symptoms for a brief spell of weeks or months which then subside but which may leave traces of exophthalmos with thick puffy eyelids (Simpson, 1960b). Thyrotoxic symptoms may be subsiding when myasthenia gravis appears and vice versa. In a limited period of observation this may lead to the conclusion that the two disorders have a 'see-saw' relationship to each other (McEachern and Parnell, 1948). It must be concluded that the thyroid disorder does not cause myasthenia gravis. Simpson (1960b) observed a high incidence of thyroid disease in relatives of myasthenic patients (even those who did not have clinical thyroid disease) and suggested that both conditions might be linked by a common genetic factor. The possibility of auto-immune thyroiditis was considered in view of the author’s concept of myasthenia but was considered less likely than genetic linkage. Lymphadenoid changes are common in the goitres associated with myasthenia gravis. The single case of myxoedema reported by Simpson (1960b) had L.E. cells in her blood, and one case of unquestionable Hashimoto thyroiditis associated with myasthenia gravis has been reported recently (Simpson, 1963). Doniach and Roitt (1962) suggest that there is a close relationship between familial thyrotoxicosis and auto-immunizing thyroiditis including Hashimoto’s disease.

Deficient glucose tolerance was present in 2 per cent of 440 cases of myasthenia reported by Simpson (1960b). Many of these had symptomatic diabetes mellitus which sometimes coincided in onset with a severe myasthenic relapse. Some of these patients and other myasthenics without obvious diabetes had diabetic relatives. Diabetes mellitus is a common disease and the relationship could be coincidental, but Simpson (1960b) postulated that diabetes, thyroid disorders, and myasthenia gravis might be diseases related by common genetic abnormality of the hypothalamic-pituitary axis. Such a mechanism could account for the precipitating role of emotional disturbance as well as the effects of menstruation and pregnancy. These effects are inconstant, but weakness is often greater in the pre-menstrual period. The effect of pregnancy is equally variable and may differ from one pregnancy to the next (Harvey, 1948). The most common event is for exacerbation of weakness to occur during the first trimester and then for remission to take place until the baby is born when rapid relapse may again be found (Fraser and Turner, 1953).
Fig. 4. Identical twins. Only the twin on the right of the picture has myasthenia gravis.
(By courtesy of Dr. E. A. Carmichael)
Fig. 5. Photomicrographs of thymus from patient with myasthenia gravis: (a) thymoma, with tubular arrangement of epithelial-type cells; (b) germinal centres in thymic tissue surrounding a thymoma. Germinal centres are found in non-tumorous thymus glands but the glandular appearance is only seen in thymomas (Simpson, 1966b). (By courtesy of the Editor of the Scottish Medical Journal.)
Myasthenia Gravis

GENETIC FACTORS
This is a convenient point to mention that the baby may show evidence of myasthenia at birth in about 1 in 7 live births to myasthenic mothers (Osserman, 1958). Later children born to the same mother are rarely affected in this way. The neonatal myasthenia (which must be distinguished from infantile myasthenia) is only temporary, and if the child survives, recovery is complete in 1 to 12 weeks. This strongly supports the concept that it is due to transplacental passage of some toxic substance and not to hereditary transmission. The occurrence of myasthenia gravis in related patients is rare but does occur. Only one of a pair of identical twins may be affected (Fig. 4). Perhaps this might be accounted for by a genetic abnormality of variable expression (Simpson, 1960b).

Table 2
Disorders of blood and reticulo-endothelial system in 490 patients with myasthenia gravis

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure red cell aplasia</td>
<td>1</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>9</td>
</tr>
<tr>
<td>Normocytic anaemia</td>
<td>3</td>
</tr>
<tr>
<td>Microcytic anaemia</td>
<td>3</td>
</tr>
<tr>
<td>Haemolytic anaemia (toxic ?)</td>
<td>2</td>
</tr>
<tr>
<td>Generalized lymphadenopathy</td>
<td>1</td>
</tr>
<tr>
<td>Splenomegaly (autopsy)</td>
<td>3</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Hyperglobulinaemia</td>
<td>4</td>
</tr>
</tbody>
</table>

RARER ASSOCIATED DISORDERS
The possible association of other disorders with myasthenia gravis has been ignored for the twin reasons of (i) irrelevancy in a disease believed to be confined to the neuromuscular junctions, and (ii) the current thraldom to the 'statistically significant' with the false assumption that any lesser degree of association must be accidental. One exception to this rejection has been pure red cell aplasia which has been associated with thymic tumours, with or without myasthenia gravis (Green, 1958). The single case in the London series was reported by Chalmers and Boheimer (1954). I have drawn attention to a more common association between myasthenia gravis and pernicious anaemia (Simpson, 1960b, 1963) (Table 2). The importance of this will be discussed below.

Myasthenia in childhood may be associated with enlargement of the
spleen and lymph glands. One patient previously reported developed lymphosarcoma (Simpson, 1960b) and two further cases are known to the author (Lesny, 1962; Whitty, 1963—personal communications). Hyperglobulinaemia is an occasional finding (Lowenthal and van Sande, 1956; Simpson, 1960a). These features suggest that there may be a related disorder of the reticulo-endothelial system.

The other disorders which were recorded in my 1953-55 survey are individually rare with the exception of an arthropathy resembling rheumatoid arthritis or ankylosing spondylitis (two cases) but comparison with other reported series shows that the relationship is unlikely to be fortuitous (Table 1) (Osserman, 1958; Simpson, 1960b, 1963; Storm-Mathisen, 1961).

THE AUTO-IMMUNE HYPOTHESIS

The age and sex incidence, natural history, and the relationship to other diseases suggested to the author that myasthenia gravis may be one expression of a disorder resembling systemic lupus erythematosus (Simpson, 1960b). Such a hypothesis would bring myasthenia gravis close to the 'symptomatic myasthenia' of dermatomyositis, systemic lupus erythematosus, and possibly of carcinomatosis. This hypothesis offered, for the first time, a rational explanation for the morbid anatomy of the disease, and incidentally was the first publication to state that the thymus was probably an organ of the reticulo-endothelial system rather than an endocrine gland (as ordinarily understood) and that it played a significant role in auto-immune diseases. In 1955-56 attempts to produce myasthenia in mice injected with homologous muscle and Freund's adjuvant proved unsuccessful (Simpson and Anderson, unpublished) but I was encouraged by the report of Nastuk, Strauss and Osserman (1959). These authors were searching for a neuromuscular blocking agent in the blood of myasthenic patients. Like all previous workers they were unsuccessful, but they observed that serum from myasthenics caused cytolysis of frog muscle cells. The further work of this group, who independently reached a similar conclusion, will be described below after a brief presentation of the morbid anatomy of myasthenia gravis.

PATHOLOGY

Lymphocytic infiltrations of skeletal muscle were described by Weigert in 1901. Buzzard (1905) named them lymphorrhages and also described
Myasthenia Gravis

de generative change of muscle fibres. These were classified into three types by Russell (1953), who considered that they may be non-specific on account of similar appearances occurring in the muscles in certain rheumatic type diseases and endocrine myopathies. Simpson (1960b) suggested that this could be due to a similar auto-immune mechanism in each of these diseases.

By special supravital staining techniques abnormalities of the motor nerve terminals can be demonstrated (Bickerstaff and Woolf, 1960; Coers and Desmedt, 1959; MacDermot, 1960). The most characteristic change is probably reduction in the number of terminal knobs which are arranged serially in a remarkably elongated end-plate region. Minute nerve fibres proliferate from these knobs suggesting a regenerative process. Though the specificity of these changes is not yet established it is obvious that there is no validity in the frequent statements in recent years that myasthenia gravis is a functional disorder without morbid anatomy.

The most difficult fact to account for by all previous theories of myasthenia is the consistent disorder of the thymus. The incidence of thymic tumours (malignant or benign) is remarkably high (10-20 per cent) and there are histological abnormalities in the thymus of most if not all the others. There has been a great deal of misunderstanding about this. Some textbooks describe 'thymic hypertrophy' and others 'failure of atrophy' such as a normal thymus is assumed to undergo. Both descriptions are wrong. Few of the glands removed from myasthenic patients are larger than normal and the change in size with age is within normal limits (Sloan, 1943). The characteristic change is not the size but the presence of 'germinal centres' in the cortex and medulla (Castleman and Norris, 1949). The epithelial cells of the thymus are not proliferated unless there is a tumour but in thymomas they may be arranged in cords or tubes. This is the only time that the thymus has the appearance of a secretory tissue and even then the surrounding lymphoid tissue shows the germinal centres which are typical of the disease (Fig. 5). The thymus has all the appearances of an active lymph organ associated with immunological reactions (Simpson, 1960b; Smithers, 1959).

IMMUNOLOGICAL REACTIONS

The clinical and pathological features of myasthenia gravis led the author to publish his hypothesis that myasthenia gravis was associated with an auto-immune mechanism involving the thymus as an organ of the reticulo-endothelial system. An antibody was postulated to act against the
end-plate protein of muscle or alternatively against the nerve terminals. The concept was shown to be compatible with the pharmacological evidence and a special point was made of its ability to account for the fact of neonatal myasthenia. This seems to indicate transplacental passage of a toxic substance, yet cross-transfusion to unrelated individuals has never induced myasthenia in the recipient (Simpson, 1960b). Meanwhile the American group were following up their observation of cytolysis of frog muscle. Almost coinciding with my publication (1960b) they reported identification of a complement-fixing muscle-binding globulin in myasthenic serum (Strauss et al, 1960). Their results were confirmed by Beutner et al (1962) and Feltkamp et al (1963). Different types of antibody reaction against muscle are described by these authors, some non-specific, but none appears to localize at the muscle end-plates or nerve terminals. We have not been successful in repeating these experiments. Strauss and his colleagues (1960) interpreted their observations as indicating the presence of anti-muscle antibodies in myasthenic serum but did not associate them with the thymus. In the following year Marshall and White (1961) showed that direct injection of bacterial antigen into the guinea-pig thymus produced a histological reaction which was essentially the same as that seen in myasthenia gravis. In the next 2 years experimental biologists were coming to the conclusion that the thymus played a very important role in the development of immunological response at least in the foetus (Burnet, 1962; Miller, 1961).

One question which immediately arises is whether the immunological response is organ- and species-specific. Space does not permit full review of the evidence (see Tables 1 and 3). The studies of the writer (Simpson, 1963) and other workers suggest that (i) the anti-skeletal muscle globulins are not species-specific, (ii) many myasthenic patients have abnormal serum antibodies against thyroid and gastric mucosa and antinuclear factor is commonly present in their serum. The clinical correlations which originally suggested the auto-immune hypothesis may be taken as indication that other antibodies remain to be detected.

The rapid support given to this part of the hypothesis may justify my drawing attention to the other part based on the genetic and endocrinal aspects. It was suggested that a hypothalamo-pituitary mechanism might act on the thymus (as has been suggested before in a different context), controlling an activity directed to cellular differentiation, particularly in the reticulo-endothelial system (Simpson, 1960b). This is not the place to go into detail about the mechanisms of immunological responses, but at least one plausible theory requires control of the thymus by a
homoeostatic mechanism (Burnet, 1962). Perhaps the control required is imposed by the pituitary gland (Simpson, 1963).

**TREATMENT**

The auto-immune hypothesis will have served its purpose so long as it stimulates new looks at the nature of myasthenia gravis. Meanwhile not the least of its attractions is that, for the first time, it provides a rational basis for the treatment of the disease by thymectomy. The initial favourable claims for this operation were not supported by subsequent American experience (Eaton and Clagett, 1950). On numerous occasions Keynes (1946, 1954) pointed out that this was due to the failure to separate those patients with thymic tumours from the remainder, as the prognosis was worse in these cases. His own results appeared to show a beneficial response to removal of the non-tumorous thymus. The writer independently reviewed the cases operated on by Keynes and other London surgeons and compared them with medically treated cases (Simpson, 1956, 1958). This review substantially confirmed Keynes's claims and showed that, in fact, the experience of three American centres was closely similar if the same criteria were used. It was concluded that there was a substantial chance of improvement after thymectomy in all cases (Fig. 6). This is most evident, and the saving in life is greatest, when the duration of illness is less than 5 years and when no thymoma is present. The mortality from myasthenia is reduced to half in males and a quarter in females compared to cases treated medically for a similar period (in statistically comparable cases).

**Table 3**

<table>
<thead>
<tr>
<th>Antibodies specific against tissues and anticellular substances in serum of myasthenic patients. The reactions against muscle were of low titre and considered non-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Antinuclear factor (ANF)</td>
</tr>
<tr>
<td>Sensitized sheep cell test (SSCT)</td>
</tr>
<tr>
<td>Thyroid</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
</tbody>
</table>

This is most evident, and the saving in life is greatest, when the duration of illness is less than 5 years and when no thymoma is present. The mortality from myasthenia is reduced to half in males and a quarter in females compared to cases treated medically for a similar period (in statistically comparable cases).
The post-operative mortality shown in Fig. 6 has now dropped to negligible figures. In the last 8 years in Edinburgh only one case from a series of twenty-eight operated on by Mr Andrew Logan has died post-operatively. There is often a remarkable remission in the hours immediately after the operation. This may be so great that a patient who has previously had persistent ptosis will show tonic retraction of the eyelids for 2 or 3 days. A more important consideration is that the remission may so lower the requirements for anticholinesterase medication that the dose required before operation is now dangerously excessive. An observer unfamiliar with this hazard will interpret the increasing weakness as indicative of a myasthenic crisis when in fact it is due to a so called 'cholinergic crisis' in which further medication may be fatal. In my personal opinion the very low mortality post-operatively in my Edinburgh series is due to recognition of this fact. I cannot emphasize too much the absolute necessity for close collaboration between a skilled thoracic surgeon and a physician experienced in the treatment of myasthenia gravis, and the latter has the more difficult task. The immediate response may persist but many cases relapse a little in the next few days. They then improve slowly. It is often 3 years later before they feel really well but, in retrospect, they then realize that the 'tide turned' after the operation.

Thymectomy benefits both sexes, the ultimate status being similar for both, but the extent of improvement is greatest for women who would
otherwise have a worse prognosis than men (Simpson, 1958). Benefit may occur at any time. It is less probable if the disease has been present for more than 7 years and operation is less justifiable then as the unmodified disease tends to become less active after that period. Nevertheless, a patient who is deteriorating despite optimum medication has nothing to lose in the hands of an experienced thymus surgeon and may show improvement. Certainly the result does not appear to be influenced by the severity of the disease. Young patients may show most benefit, but short pre-operative duration of myasthenia is the more important factor.

The prognosis for life is worse if a thymoma is present. Even after thymectomy two out of three cases die within 5 years. Nevertheless the survivors may benefit to the same extent as the non-tumour cases. This may happen even without pre-operative radiation of the thymus (Simpson, 1955). The desirability of radiotherapy was stressed by Keynes (1955) but I feel that this requires substantiation. Radiotherapy has been recommended as an alternative to thymectomy but there is a danger that myasthenic symptoms will be aggravated initially (Jones et al., 1955). Other indirect ways of reducing the activity of the thymus are the operation of bilateral denervation of the carotid sinuses (Thévenard, 1954), and administration of ACTH or corticosteroids (Torda and Wolff, 1951). Any improvement due to ACTH may be preceded by dangerous relapse (Westerberg and Magee, 1955). There can be few indications for these indirect methods of destroying the thymus now that operative mortality is so low.

The drug treatment of myasthenia gravis is a subject for a lecture by itself. I have reviewed this recently (Simpson, 1964) and so will omit it from this chapter but there are one or two observations I must make because of their bearing on Dr McArdle’s chapter on the physiology of the neuromuscular junction (Chapter 4). The first is that the beneficial action of a cholinesterase inhibitor does not favour any particular one of the several possible mechanisms of transmission failure (Simpson, 1960b). The second point requiring emphasis is that there is a wide variation in the duration of action of an anticholinesterase drug from one patient to another. Neostigmine may have a useful action for 8 hours in one patient and only 1-2 hours in another, when taken orally. For this reason it is essential to establish the duration of effective action in each patient. A simple ergograph is a useful adjunct to this (Fig. 3).

Neostigmine acts rapidly. Its most powerful action lasts for only 1 to 1½ hours and is followed by a carry over for a further 1–6 hours. The activity then tends to fall off rapidly. This ‘let down’ can be most discouraging
70

Biochemical Aspects of Neurological Disorders

to a patient. Pyridostigmine does not have the early marked effect of Neostigmine. The duration of the 'plateau' of useful activity is little longer than that of Neostigmine but the fall off is more gradual so a smoother control can be established if the tablets are correctly timed. In fact the majority of patients find Pyridostigmine the drug of choice but many patients will value the 'boost' which only Neostigmine can give.

Theoretically it seems desirable to use a long-acting preparation to avoid the need for frequent dosage. All anticholinesterases tend to be cumulative and this is more marked the longer the duration of the plateau activity (Fig. 7). This may not be obvious for weeks when using Neostigmine or Pyridostigmine so that the physician may be lulled into a false sense of security. The third point I want to make about drug treatment of myasthenia is my growing conviction that one of the most common

![Duration of activity of three anticholinesterase drugs: --- Edrophonium; ---- Neostigmine; - - - - Pyridostigmine.](image)

causes of death in myasthenics is depolarization block ('cholinergic crisis') mistakenly diagnosed as Neostigmine resistance. This state may be difficult to recognize. I have come to rely on pupillary constriction. It is a very valuable sign provided that the patient is not receiving large amounts of Atropine. The use of edrophonium to test for the cholinergic state has been a major advance (Osserman and Kaplan, 1953) but certain warnings must be given: (i) it is dangerous in the presence of cholinergic crisis and may be fatal, preparation for assisted respiration must be made before the drug is injected, (ii) the test must be carried out at the most informative time, that is when the drug in use is most active (e.g. 2 hours after an oral dose of Neostigmine), (iii) the patient's subjective assessment should never be relied on, if objective improvement does not occur no further anticholinesterase medication should be given until later testing shows un-
equivocal improvement after edrophonium, (iv) test the response of the respiratory and bulbar muscles first. The reason for this warning, based on personal experience (Simpson, 1961a) is that since myasthenia gravis affects muscles differentially the tolerance of anticholinesterase drugs will also vary. Muscles which are only slightly or not at all affected by the myasthenic process may therefore suffer from depolarization block with a drug level which is still inadequate to control the severely myasthenic muscles. Thus a favourable response to edrophonium may be found in extra-ocular or shoulder girdle muscles when the respiratory muscles are already over-dosed. These must be the first to be tested. If there is any serious doubt it is wiser to initiate assisted ventilation and to withhold further medication until the patient is obviously myasthenic again. If cholinergic paralysis is confirmed, atropine sulphate (2 mg) should be injected intravenously every hour until signs of atropine toxicity develop. Grob and Johns (1958) have recommended the use of oxime drugs in the treatment of over-dosage of quaternary ammonium anticholinesterase drugs. My personal experience is limited to pyridine-2-aldoxime (2 PAM) and methane sulphonate (P2S) and I have found these to act too slowly, too briefly, and too weakly to be able to recommend them (Simpson, 1961b). The differential diagnosis and management of myasthenic and cholinergic crises are more fully described by Simpson (1964) and all who treat myasthenia gravis must be familiar with detail and not just with general principles. To all others I would give one closing advice—a tracheostomy is often life saving and if respiration is in doubt it should never be delayed. If combined with positive pressure ventilation it is safe to stop medication and watch for changes in the clinical state. Unfortunately, even in the most experienced hands, myasthenia gravis remains a very serious disease with a persisting risk of death.

REFERENCES

Alajouanine T., Castaigne P., Nick J., Contamin I. and Libermite F. (1957) Rev. neurol. 96, 243
Burzard E.F. (1953) Brain 28, 438
Campbell H. and Bramwell E. (1950) Brain 23, 277
Colin C. and Dymott J.E. (1959) Acta neurol. belg. 59, 539
Biochemical Aspects of Neurological Disorders

Fraser P. and Turner J.W.A. (1953) Lancet ii, 417
GroB D. (1951) J. Amer. med. Ass. 151, 519
Harnes A. de and Roussel J. (1955) Acta neur. psychiat. belg. 55, 364
Harvey A.M. (1948) Bull. N.Y. Acad. Med. 24, 8
Keynes G. (1954) Lancet i, 1197
MacDermot V. (1950) Brain 83, 24
MacEachën D. and Parnell J. (1948) J. clin. Endocr. 8, 842
Russell D.S. (1913) J. Path. Bact. 4, 279
Simpson J.A. (1958) Brain 81, 112
Simpson J.A. (1960b) Scot. med. J. 5, 410
Sloan H.E. (1943) Surgery 14, 154
Symonds C.P. (1921) Clin. J. 51, 604
Thévenard A. (1952) Rev. neur. suppl. 90, 107
Weigt C. (1901) Neur. Zbl. 20, 597
MYASTHENIA GRAVIS AS AN AUTOIMMUNE DISEASE: CLINICAL ASPECTS

John A. Simpson*
University of Glasgow, Scotland

Introduction

The factors which may be associated with the first attack or later relapses of myasthenia gravis are emotional disturbance, infection, particularly of the upper respiratory tract, and pregnancy. In this respect, and also in the sex incidence, age of onset and remittent course, there are striking resemblances with systemic lupus erythematosus (S.L.E.) and multiple sclerosis. In drawing attention to this, Simpson (1960) first pointed out that certain disorders of nonmuscular tissues may be related to the neuromuscular syndrome.

Neurological Disorders

While reviewing a large series of myasthenic patients in 1953 to 1955 with a view to determining the value of thymectomy in treatment (Simpson, 1958), I was interested to note the occasional occurrence of sensory symptoms, epilepsy, or psychosis and certain other disorders in these patients (TABLE 1).

Some of the sensory symptoms such as paraesthesia, deafness or hyperacusis could be accounted for by the mechanical effects of muscular weakness, but numbness of the face and loss of taste or smell sensation could not be explained in this way, and it seemed necessary to consider the possibility of associated damage to sensory receptors or nerve fibers (Simpson, 1960, 1964a, 1965). The previous reports by other authors are too numerous to list here but have been analysed by Simpson (1964a, 1965). It appears that sensory symptoms were widely recognized until summarily dismissed in the classical review by Campbell and Bramwell (1900). They have been particularly ignored since the concept of a defect of transmission at the neuromuscular junction has dominated all thinking on myasthenia gravis, but occasional reports still appear in the literature (Harvey, 1948; Russell, 1953; Alajouanine et al., 1957).

Cerebral disorders such as epilepsy and psychosis are not commonly associated with myasthenia gravis though Hoefer and coauthors (1958) found a surprising incidence of convulsive and syncopal attacks. Nevertheless, the occurrence of these disorders in each of three large series suggests that it may not be coincidental (Osserman, 1958; Simpson, 1960; Storm-Mathisen, 1961).

*Attendance at the Conference was made possible by a Welcome Research Grant.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid (all types)</td>
<td>67</td>
<td>19†</td>
<td>17</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Diabetes and glycosuria</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>&quot;Rheumatoid&quot; arthritis</td>
<td>12</td>
<td>7</td>
<td>15</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Systemic L. E.</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cutaneous L. E.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Haemolytic anemia</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephritis</td>
<td>2</td>
<td>1</td>
<td>1(?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raynaud's syndrome</td>
<td>10</td>
<td>8</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Epilepsy and &quot;Blackouts&quot;</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychosis</td>
<td>9</td>
<td>2</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td><strong>407</strong></td>
<td><strong>94</strong></td>
<td><strong>325</strong></td>
<td><strong>90</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

*The series reported by Simpson (1960) includes all the London cases and the first 33 from Scotland.
†Includes one case of Hashimoto's disease and two of lymphadenoid thyroid at autopsy.
‡Considered to be caused by medication.
§Includes series previously reported by Van der Geld and colleagues (1963).
Hematology

The occasional relationship between erythroid aplasia of the bone marrow and thymic tumours has been recognized for more than thirty years (Matras & Priesel, 1928). The aplasia is not always confined to the red cells of the blood but may be pancytopenic, as in the first case reported in association with myasthenia gravis (Wintrobe, 1946). The nature of this relationship is uncertain, but the recent finding by Holborow et al. (1963) of antinuclear factor (A.N.F.) in the blood of a patient with the syndrome of thymoma and red cell aplasia and by Anderson and Ladefoged (1963) of the Coombs test transiently positive support the possibility of an immunological mechanism. The correlation with A.N.F. has not yet been reported in the similar cases with myasthenia gravis, but in one such case Oosterhuis (1963) noted a positive direct Coombs test.

I have previously reported an association between myasthenia gravis and macrocytic anemia (Simpson, 1960). It is now certain that some, if not all, of these cases were suffering from pernicious anemia (Simpson, 1964a). Single cases of myasthenia with pernicious anemia have been reported by earlier writers and were considered to be coincidental, but it will be apparent that this association is more common than that between myasthenia gravis and erythroid aplasia. When I mentioned this finding in the 1960 paper, the relationship was merely used to support the hypothesis that myasthenia gravis was a diffuse systemic disorder though the concept was developed along immunological lines. At that time, however, evidence began to accumulate that pernicious anemia was associated with the production of an organ-specific antibody to the intrinsic factor and to the parietal cells of the stomach (Schwartz, 1960; Irvine et al., 1962; Taylor et al., 1962). It has also been reported recently that pernicious anemia may be associated with rheumatoid arthritis, autoimmune thyroiditis, systemic lupus erythematosus, thyrotoxicosis and diabetes mellitus, all of which may be associated with myasthenia gravis (Simpson, 1960).

One of my cases had megaloblastic anaemia with low serum vitamin B.12 assay in the eleventh year of myasthenia due to thymic tumour. He also had an episode of severe hemolysis, but I am uncertain whether this was spontaneous or because of the intravenous injection of pyridine-2-aldoxime methiodide (P.A.M.) for cholinergic crisis. Storm-Mathisen (1961) described a patient who had been treated for hemolytic jaundice before myasthenia gravis began at the age of 32. Eight years later, after remission of the myasthenia, she had myxoedema and arthritis with a raised erythrocyte sedimentation rate (E.S.R.). The author does not comment on the case and does not appear to have considered a diagnosis of systemic lupus erythematosus.

Reticuloendothelial System

I have never been able to palpate the spleen of a myasthenic patient as reported by Montesano (1898), but the spleen was found to be enlarged at
post-mortem examination in four cases. Two young patients had a generalized lymphadenopathy without adequate explanation, and another one developed reticulum cell sarcomatosis eight years after thymectomy. J. Lesny (Prague) and C. W. M. Whitty (Oxford) have informed me of two similar cases under their care. Both were young children who had neostigmine-responsive myasthenia gravis associated with lymphosarcoma. (Simpson, 1964a). One of my patients had typical myasthenia gravis associated with sarcoidosis. Alter and Osnato (1930) described a case of myasthenia gravis with generalized lymphadenopathy. The thymus was “granulomatous” and considered by the authors to resemble Hodgkin’s disease. Senator (1892) reported a case of myasthenia with “multiple sarcomata,” anemia, albumosuria and “chronic parenchymatous changes in the kidney.” The diagnosis appears to have been multiple myelomatosis. It is doubtful whether the muscular weakness was truly of myasthenic type. Generalized lymphadenopathy occurred in the case of myasthenia associated with S.L.E. reported by Galbraith and coworkers (1964).

These disorders of the blood and reticuloendothelial system are exceptional and may not be related directly to myasthenia gravis, but it should be remembered that lymphadenopathy associated with rheumatoid arthritis is largely confined to young children. If the relationship is more than coincidental, I believe that it is likely to be an association of immunological disorders (Simpson, 1960, 1964a, b).

The suggestion would be more generally acceptable for systemic lupus erythematosus. This diagnosis was made in one case in my series (Simpson, 1960, 1964a), and Rowland (1955) found L. E. cells in the blood of one of his patients. Harvey et al. (1954) described myasthenic symptoms in three cases in their classical paper on systemic lupus erythematosus. Since then the latter has been considered to be one cause of “symptomatic myasthenia,” but it is now apparent that there is no sharp dividing line between this disease and “true” myasthenia gravis. Space does not permit me to examine the problem of the other “symptomatic myasthenias” (polymyositis, dermatomyositis and carcinomatous myasthenia), but I believe that they are all parts of an immunological spectrum (Simpson, 1964b).

Acrocyanosis

An interesting clinical link between these neuromuscular diseases is acrocyanosis. Although not previously reported in myasthenia gravis, I have found this condition in 3.5 per cent of cases (Simpson, 1960). The true incidence may be higher since it was not specifically inquired for in the first 400 of my 501 cases. It is interesting to note that acrocyanosis appeared to correlate with a poor response to neostigmine though the response at an earlier date was always sufficient to justify the diagnosis of myasthenia gravis. The possible role of autoagglutinins, found in the blood in some types of acrocyanosis, has not yet been explored.
"Rheumatoid" Arthritis

The author was the first to draw attention to an unusual incidence of arthritis in patients with myasthenia gravis (Simpson, 1960). In six patients the painful swelling involved peripheral joints and was transient. Two patients were considered to have ankylosing spondylitis. In 11 more cases the joint changes have been permanent.

The arthritis is indistinguishable clinically and radiologically from true rheumatoid arthritis, but the modifying quotation marks were used in the original and subsequent papers to indicate that identity could not be assumed (Simpson, 1964). It will be shown below that the Rose-Waaler test is often negative. White and Marshall (1962) and Van der Geld and colleagues (1963) have also found a high incidence of rheumatoid arthritis (sic).

Other Immunological Disorders

Ulcerative colitis and hepatic cirrhosis were associated with systemic lupus erythematosus which followed thymectomy for myasthenia gravis in a patient recently reported by Alarcón-Segovia et al. (1963) and by Galbraith et al. (1964). I have seen two myasthenic patients with acute hepatitis but considered that this was caused by chlorpromazine. The only case of systemic lupus erythematosus in my series had pericarditis, angioneurotic oedema and myxoedema. The latter was attributed to previous roentgen therapy for suspected carcinoma of the larynx. In 1955 when I saw this patient the possible association between myasthenia gravis, S. L. E. and thyroiditis was not known.

Endocrine Disorders

It has been known for many years that thyrotoxicosis is related to myasthenia gravis. A discussion of this theme would not be appropriate to this paper, but a review of the literature and of my case material shows that there is also a relationship with nontoxic goiter and myxoedema. Studies of the family histories suggest that the relationship may be a genetic defect of a hormonally-controlled immunological mechanism (Simpson, 1960, 1964, 1965).

The debate on the possible role of hyperthyroidism in myasthenia gravis has obscured the fact that pathologists have in the main described nontoxic goiters, often lymphadenoid, in association with myasthenia gravis, though the changes were usually considered to be involutionary in a previous toxic goiter (Norris, 1936; Miller, 1940; Giordano & Haymond, 1944). Rowland et al. (1956) found 4 nontoxic goiters in an autopsy series of 26 myasthenics. Ringerz (1951) found 5 abnormal thyroids in 18 autopsies. One of these was a nontoxic goiter (without lymphoid infiltration), one gland was considered to be of toxic type, and three showed patchy parenchymal atrophy with cellular disintegration associated with dense lymphoid infiltration. Two of these had
lymphoid follicles in the thyroid "giving to the picture a certain resemblance to that of lymphadenoid goiter." Both of these patients had a thymic tumour.

Case MN. 2257 of my own series, was found at autopsy to have a lymphadenoid goiter. The section (FIGURE 1) was shown and discussed in a Honeyman Gillespie Lecture but was omitted from the published version (Simpson, 1960) as an expert on the pathology of Hashimoto's disease had reservations about the diagnosis. The spleen of this patient, who had a thymic tumour, also showed lymphoid and reticular hyperplasia.

Case MN. 2327 died seven months after incomplete thymectomy. Thyroid disease had not been suspected during life, but typical Hashimoto's struma was found at autopsy and the spleen was distinctly enlarged.

Case MN. 5503 developed pernicious anemia in 1935 and myasthenia gravis in 1961. Although clinically euthyroid, her plasma level of protein-bound iodine was 2.4 μg/100 ml., and the serum antibody titers were 1/250 against thyroid (by T. C. H. test) and 1/16 against gastric tissue (by complement fixation test) with strongly positive immunofluorescence against gastric parietal cells. At autopsy the thyroid gland was typical of Hashimoto's disease.

Case MN. 7390 became myasthenic in 1943 and had thymectomy in 1955. He developed nontoxic goiter and pigmentation of the skin in 1960. A radio-iodine uptake test gave a T. P. I. of 1.6, and the protein-bound iodine was
Annals New York Academy of Sciences

14.0 μg/100 ml. Antibody titers against thyroid tissue were T. C. H. 1/25,000 and C. F. T. 1/16. Electrophoresis showed hypergammaglobulinemia.

The last case is more fully described by Simpson (1964b) and the others are described earlier by Simpson (1964a). In the same year the coincidence of myasthenia gravis and Hashimoto’s disease was reported by Daly and Jackson (1964) and by Becker and coauthors (1964).

**Plasma and Cerebrospinal Fluid Protein**

Space does not permit full presentation of my studies on the globulins of the serum and cerebrospinal fluid (C. S. F.) (Simpson, 1960, 1964a). These will be reported elsewhere, but a small number of cases and a review of the literature by Oosterhuis and colleagues (1964) shows that hypergammaglobulinemia is not uncommon in myasthenia gravis and the protein content of the C. S. F. may be increased.

**Summary of Hypothesis**

In my original paper the hypothesis of a multiple system disease analogous to systemic lupus erythematosus was based on the demonstration of a characteristic spectrum of associated disorders of tissues other than muscle (Simpson, 1960). Since then it has been shown that there is a significant relationship with pernicious anemia and Hashimoto’s disease (Simpson, 1964a, b). During this period it has become firmly established that both of these conditions are associated with defective immunological tolerance, and Miller (1961) has demonstrated the role of the thymus gland in immunological disease.

**Antibody Against Muscle**

In the early days of this work in Glasgow, I collaborated with J. R. Anderson (1955, unpublished) in an attempt to produce an experimental myasthenia in mice by injection of emulsified homologous muscle with Freund’s adjuvant. The experiment failed. I was postulating that an antibody fixed reversibly to the receptor site of the muscle end plate would act as a neuromuscular blocking substance of competitive type. It would also be capable of placental transmission, accounting for neonatal myasthenia and yet explaining the failure of cross-transfusion experiments (Simpson, 1960).

We were also unsuccessful in demonstrating antimuscle antibodies. Later studies with W. R. M. Alexander in Edinburgh were equally unsuccessful. We tried to reproduce the more successful methods of Strauss et al. (1960) but again failed. We abandoned this aspect of the investigation though other workers have confirmed their results (Beutner et al. 1962; Feltkamp et al. 1963; Geld et al. 1963; Djanian et al. 1964; Thivolet & Kratchko, 1964).

The specificity of the antimuscle substance or substances will not be reviewed as it has been discussed by Simpson (1964a, b) and in this monograph, but it is clear that there is disagreement between workers. In searching for a
possible explanation of our failure to reproduce their results, it was noticed that Strausser et al. (1960) obtained their positive results from the pooled sera of 10 myasthenic patients of whom 6 had a thymoma, whereas many other patients investigated by them showed no muscle-binding globulin in their serum. Beutner et al. (1962) and Thivolet and Kratchko (1964) confirmed their results with sera from 2 of 10 and 3 of 9 myasthenic patients, respectively, and each of the positive reactors had a thymoma. Van der Geld et al. (1963) found that the incidence of antimuscle antibodies was consistently higher in cases with thymoma. It is not clear whether their nontumour cases included examples of fluorescence of the A bands of muscle fibers or only the less specific sarcolemmal or nuclear fluorescence. The type of thymus pathology in the cases of Djanian et al. (1964) is not recorded. Since noticing this correlation I have not had another case with a thymic tumour but hope to resume this work in Glasgow with the collaboration of J. Vetters, who has successfully demonstrated A-band fluorescence with some of my sera which were previously considered inactive. Positive results have not been confined to sera from cases with a thymoma.

Antibodies against Other Tissues

Antinuclear factor (A. N. F.) is commonly found in the serum of patients with myasthenia gravis (White & Marshall, 1962; Feltkamp et al., 1963; Simpson, 1964b) (TABLE 2). The rheumatoid factor is sometimes present in the blood but does not correlate closely with a history of arthritis (Simpson, 1964a, b).

Van der Geld et al. (1963b) and Simpson (1964b) have found antithyroid antibodies in the sera of 30 per cent of cases. W. J. Irvine, who is responsible

| TABLE 2 | ANTIBODIES DETECTED IN SERA FROM PATIENTS WITH MYASTHENIA GRAVIS |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Present Series | A.N.F. | R.F.‡ | Muscle | Thyroid | Stomach | Liver |
| Positive | 10 | 0 | 1 | 12 | 3 | 0 |
| Total | 49 | 8 | 10 | 45 | 44 | 36 |
| White & Marshall | Positive | 6 | 2 | 1 |
| (1962) | Total | 15* | 15 | | |
| Van der Geld | Positive | 11 | 5 | 38 | 36 |
| et al. (1963) | Total | 111 | 111 | 98 | 111 |

*One case of carcinomatous myasthenia excluded.

‡Rheumatoid factor—S.S.C.T. in present series, latex test in others.
for these tests in my cases, has also found specific antigastric substances in 3 of 35 of these cases, 1 of which had pernicious anemia. Unfortunately the other patients with pernicious anemia reported by Simpson (1964a) are no longer available for immunological studies.

CONCLUSION

The natural history of myasthenia gravis including its age and sex incidence, the course of the disease, the phenomena of neonatal myasthenia and particularly the occasional association with neuropathic disease, aplastic and pernicious anemia, reticulosis, acrocyanosis, "rheumatoid" arthritis, ulcerative colitis, hepatitis, thyrotoxicosis and Hashimoto's disease support the hypothesis that the neuromuscular disorder is part of an immunological disorder which may occasionally involve other tissues (Simpson, 1960, 1964a, b). The pathological changes of the thymus, muscles, thyroid and occasionally of other tissues support this suggestion (Smithers, 1959; Simpson, 1960). Strauss et al. (1960) independently reached a similar conclusion from different premises. Their demonstration of serum antibodies against human muscle has been confirmed, but it is suggested that the titer is higher in cases with a thymoma. Cross-reactions suggest that more than one antimuscle substance may be produced, some reacting with cardiac muscle or with thymic tissue (Beutner et al., 1962; van der Geld & Oosterhuis, 1963). The further demonstration of antinuclear factor and organ-specific antibody against thyroid and stomach suggests that there is a major defect of immunological homeostasis. I have suggested that this may be genetically determined and have an endocrinological controlling mechanism (Simpson, 1960, 1964a).

REFERENCES


GALBRAITH, R. SENATOR. 
H. A. GIORDANO, A. GELD, H. VAN DER & H. J. G. VAN DER,
J. SIMPSON. SIMPSON, J. M. SCHWARTZ. K. OSSERMAN,
OOSTERHUIS. H. J. G. NORRIS, E. H. MILLER,
MASSACHUSETTS GENERAL HOSPITAL.
IRVINE, W. J., S. A. HARVEY,
FELTKAMP, T. RUSSELL.
L. P. ROWLAND,
N. 1951. MONTESANO,
G. H. MILLER,
A. PRIESEL.
1892. KOENDERS, Amsterdam, Holland.
LE P. K. DAVIES.
B. L. CONLEY & E. H. SHULMAN.
ARTHERSON, G. D. A. ASHIERSON, G. D. JOHNSON,
R. D. BARNES & D. S. CARMICHAEL. 1963. Antinuclear factor and other antibodies in blood and liver
80: 520.
MONTESEANO, G. 1898. Rivist di Psychiatria. Cited by Campbell & Bramwell (1900)
lac. cit.
NORRIS, E. H. 1937. A thymoma (adenoma of the thymus) from an unusual case of
myasthenia gravis, with observations on the general pathology. Amer. J. Cancer 30: 308.
de Myasthenia Gravis. Koenders, Amsterdam, Holland.
Myasthenia gravis with hypergammaglobulinaemia and antibodies. J. Neurol.
Neurosurg. Psychiat. 27: 345.
RINOFERTZ, N. 1951. Pathology of the thymus and other organs in myasthenia gravis.
ROWLAND, L. P. 1955. Prostigmine-responsiveness and the diagnosis of myasthenia
RUSSELL, D. S. 1953. Histological changes in the striped muscles in myasthenia gravis.
J. Path. 65: 279.
SCHWARTZ, M. 1960. Intrinsic factor antibody in serum from patients with pernicious
Scotland.
Discussion of the Paper

A. STORM-MATHISEN (Gainsford Hospital, Oslo, Norway): Dr. Simpson mentioned one of my patients. The patient mentioned, case No. 56, is completely free from myasthenic symptoms. Her myxedema is controlled with thyroid preparation. She is working, has married and has a child. The remission has lasted 12 years. It was not symptoms of S. L. E.
supramaximal and the corded with surface conventional. The study of the strength-duration relationships of the lower motor neurones, point to a primary lesion of the lower motor neurones, the neurologist would like to have further information about the site of the lesion – is it poliomyelitis, axonal or at the neuromuscular junction? Some help may be obtained from a study of the muscular response to repeated stimulation of its motor nerve (Simpson & Lenman 1959, Simpson 1960a).

Stimulation and recording techniques are conventional. The stimulus must be brief and supramaximal and the muscle response is recorded with surface electrodes to integrate the electrical activity of a volume of muscle since the area of the response is assumed to be proportional to the number of responding muscle fibres for any particular rate of stimulation. If the muscle tension is recorded isometrically by a strain gauge it will be seen that the amplitude of the evoked potential does not bear the same relationship to the twitch tension at all rates of stimulation. The decreased amplitude at fast rates of stimulation is due to restimulation within the refractory period of the muscle (Farmer et al. 1960). Movement artifacts are minimized by splinting the limb during stimulation but some artifact of this type is almost inevitable in routine diagnostic studies.

Normal Neuromuscular System

Stimulation of a motor nerve at rates above 12/sec in the normal subject causes the evoked muscle action potential to increase progressively with the first 3–5 stimuli. This is accompanied by progressive shortening of the duration of the action potential, particularly the second phase of the diphasic deflection. It is probably due to improved synchronisation of the muscle response (Harvey & Masland 1941, Simpson & Lenman 1959, Farmer et al. 1960). At 0.1–0.2 sec from the start (irrespective of the rate of stimulation) there is often a temporary decrease in amplitude of about 30% which is due to an artifact of movement. The action potential then remains at a constant level for a considerable period which depends on the frequency of stimulation. These normal increments and decrements must not be mistaken for the pathological types described below.

If fast stimulation is continued the muscle potential slowly decrements but does not disappear although the muscle contraction may cease from fatigue (Merton 1954). Decrement to 50% may occur in 30 seconds with supramaximal stimulation at 50 shocks per second but often takes much longer. If stimulation is interrupted and resumed there is no obvious post-tetanic potentiation. When tetanization is repeated with rests of 1–2 seconds there is no recovery of the earlier amplitude unless the stimulus frequency is reduced. This decrement is absent or long delayed at rates below 15/sec unless this is preceded by 'fatigue' induced by more rapid stimulation.

Pathological Decrement of Response

In partial denervation the amplitude of the evoked muscular response is always diminished in proportion to the fall out of motor units. Premature progressive decrement of the response is less common and occurs in several disorders.

Professor John A Simpson
(University of Glasgow)

Disorders of Neuromuscular Transmission

Electrodiagnostic techniques should be used to explore the physiological properties of normal and pathological states of the lower motor neurones and nerve fibres and to make a nosological diagnosis. Measurement of the strength-duration relationships of electrical excitability and conventional electromyography provide information about (a) degeneration of peripheral nerve axons and muscle fibres, (b) the recruitment and frequency pattern of motoneurones, and (c) the muscle fibre constitution of motor units. From these data it is possible to decide whether muscular weakness is neural or myal (Simpson 1962). Measurement of nerve conduction velocity adds another parameter. Pathological slowing of conduction is probably due in the main to abnormality of the myelin-sheath of peripheral nerve fibres (Simpson 1964). If these studies, with the electromyographic evidence of axonal branching, point to a primary lesion of the lower motor neurones, the neurologist would like to have further information about the site of the lesion – is it poliomyelitis, axonal or at the neuromuscular junction? Some help may be obtained from a study of the muscular response to repeated stimulation of its motor nerve (Simpson & Lenman 1959, Simpson 1960a).
Myasthenic Reaction

The most striking feature of the evoked muscle potentials in myasthenia gravis is a rapid decrement in amplitude which occurs earlier than in normal subjects and may be visible in the first few responses even at rates of stimulation as low as 3/sec. This is not an invariable finding and it may not be marked until tetanization has been repeated several times within a short period or after a similar period of voluntary contraction of the muscle (Fig 1). When it appears, the time constant of the decrement in amplitude decreases progressively with successive tetani. The rate of fall is faster with more rapid stimulation.

Other responses which are commonly seen in myasthenia gravis are: (1) Initial rapid decrement with continuation of nondecrementing response at submarginal level (Fig 2a). (2) Initial rapid decrement followed by recovery of amplitude for 2-3 sec then continuing decrement (Fig 2c). (3) Progressive decrement at slow rates of stimulation (3-10 c/s) but progressive increment at faster rates of stimulation (Fig 2b). (4) Progressive increment from the start of tetanization or after a decrementing response to the first 3-5 shocks (Simpson 1960b).

Types (3) and (4) are usually seen in muscles without clinical evidence of myasthenia in an early stage of the disease or in remission. If stimulation is continued these muscles usually show the delayed decrement sooner than normal muscle.

After tetanization the amplitude of the action potential of a single twitch response recovers within a second and may be greater than the first response to the original train of stimuli (post-tetanic facilitation), but after successive faradizations with brief rest periods (about one second) the recovery becomes progressively slower and even twitch responses may be decreased (Fig 1). Desmedt (1958) has named this 'post-activation exhaustion' and considers that it is the cause of the weakness seen clinically in the myasthenic muscle.
It will be seen that the post-activation response of the myasthenic neuromuscular system shows facilitation as well as exhaustion components which combine in different ways as they respond individually to different rates of stimulation. The decrementing response may be abolished by administration of anticholinesterase compounds but the greatest response obtained may remain less than in an equivalent normal muscle.

**Symptomatic Myasthenia**

In dermatomyositis, polymyositis, and systemic lupus erythematosus there are similar muscular responses to neural stimulation but they differ quantitatively from true myasthenia gravis. The most common finding is a rapid decrement of the evoked action potential but this is rarely seen with stimulation at rates less than 10/sec (Fig 3). The decrement may be delayed by injection of edrophonium or neostigmine in some cases, but in my experience the improvement has never been so spectacular as in myasthenia gravis and it is often absent. On resuming slower rates of stimulation, the recovery is usually slower and post-tetanic facilitation usually absent. On the other hand the early facilitation effect during faradization is often marked and in a few cases exceeds anything seen in myasthenia gravis. On voluntary contraction the same slow augmentation of the electromyogram may be seen, corresponding with increased tension of the dynogram (Simpson & Lenman 1959, Simpson 1960a).

The nature of the acquired myopathy in the following case is obscure.

**Case 1** (MN 2803) Man aged 64 when seen in 1957.

In 1951 he had thyrotoxicosis treated with methylthiouracil. In 1952, following acute respiratory infection, he developed increasing weakness and wasting of all limbs, mainly proximal, and bilateral ptosis. Tendon reflexes virtually disappeared. Equivocal response to neostigmine. No improvement with ephedrine, potassium or guanidine. Subjective improvement on pyridostigmine 240 mg daily. Worsened by ACTH or cortisol.

1953: No response to faradism. Galvanic threshold raised.

1954: Normal biopsy of right deltoid muscle.

1957: EMG (right biceps): marked insertion activity; myopathic pattern; normal conduction velocity of right ulnar nerve; normal silent period.

Harvey-Masland test (right abductor digiti minimi) showed no abnormal fatigability although the potential evoked by 5/sec stimulation was lower than that at 0.5/sec. On supermaximal stimulation at 50/sec the evoked potential increased rapidly to 300% of the original level. With continued stimulation the decrement to about 50% but when the stimulus frequency was suddenly reduced the potential rapidly increased again to 200%. When stimulation was stopped for 10 sec the potential returned to 80% of the original level (Fig 4).

1964: He had gradually recovered and was now normal for his age. Harvey-Masland test normal. Anticholinesterase medication discontinued.

1965: Remains well. No evidence of neoplasm of any organ.
Carcinomatous Myasthenia

Eaton & Lambert (1957) and Rooke et al. (1960) have used the term 'myasthenic syndrome' for the muscular fatigability which may be associated with carcinoma of the bronchus. They showed that the electromyographic response to a single maximal nerve stimulus is greatly reduced in amplitude. A transient further depression may occur during slow rates of stimulation but a marked incrementing response is found at higher rates of stimulation (Fig 5). Post-tetanic facilitation is a prominent feature and this is followed by a period of post-tetanic exhaustion similar to that seen in myasthenia gravis. The response to anticholinesterase drugs is inferior to myasthenia gravis and often disappears as the disease progresses. The electromyographic and pharmacological reactions do not appear to differ except in degree from polymyositis (Simpson & Lenman 1959).

Neuropathies

In the majority of cases of partial denervation the muscular response to faradization is normal. In rare cases of peripheral neuropathy a decrementing response to serial stimulation may be seen (Pinelli 1957). We have reported this in diabetic neuropathy, Guillain-Barré syndrome, and in post-zoster motor neuropathy (Simpson & Lenman 1959, Simpson 1962) (Fig 6).

During conventional electromyographic investigation of the atrophic small muscles of a hand affected by amyotrophic lateral sclerosis, one frequently observes that some of the remaining motor units (identified by coaxial needle electrodes) show rapid decrement in amplitude during voluntary contraction (Fig 7a) (Simpson & Lenman 1959, Lambert & Mulder 1957). In some cases faradization of the ulnar nerve at the wrist with supramaximal stimuli causes rapid decrement of the action potential of a single unit. The action potential of the ulnar nerve recorded simultaneously at the elbow shows no decrement even when the muscle potential has disappeared (Fig 7b) indicating that the latter is not caused by failure to stimulate the nerve.

Similar decrementing response to serial stimulation has been reported in anterior poliomyelitis (Buchthal & Honcke 1944, Hodes 1948). Pinelli (1957) reported a decrementing response to faradization of a peripheral nerve in one case of syringomyelia and one of multiple sclerosis but only with very rapid stimulation (100/sec).

Recruiting or incrementing responses to faradization are not widely recognized in diseases of the lower motor neurone or muscle. Simpson & Lenman (1959) described an unusual case of bulbar and shoulder girdle palsy.
Case 2 (MN 2801) Woman aged 58
In 1957 she had progressive dysarthria after pharyngitis. Four months later she had slight facial weakness and loss of power in all limbs, mainly proximal. No sensory loss; tendon reflexes brisk, plantar reflexes normal. Occasional fasciculation. Not improved by edrophonium, neostigmine, ephedrine, prednisolone, or z-tocopherol. Thyroid function normal. Chest X-ray normal.

1957: EMG (right deltoid and biceps): no spontaneous activity; myopathic pattern. Normal conduction velocity (left ulnar nerve). Stimulation at 50/sec caused progressive increase of potential evoked in abductor digiti minimi to 160% then progressive decrement to 50% in the next 65 seconds but resumed its original amplitude almost immediately when the rate of stimulation was reduced to 0.5/sec (Fig 8). Muscle biopsy was normal.

1958: Progressive muscular atrophy, mainly distally. Tendon jerks became exaggerated and plantar reflexes became extensor.


**Hereditary and Metabolic Myopathy**
In hereditary muscular dystrophy the amplitude of the evoked action potential of muscle may be lower than normal at rapid rates of stimulation but the difference is probably not significant and the time course of the later decrement is normal if faradization is continued.

In chronic hypoxia the decrement may occur earlier than normal and the amplitude fluctuates irregularly. The antidromic nerve action potential simultaneously recorded does not fluctuate so the variability is unlikely to be caused by movement of the stimulating electrode. I have not studied this condition sufficiently to be dogmatic about these conditions.
findings but if recording artifacts can be excluded the findings suggest intermittent response of muscle fibres rather than a systematic disorder of junctional transmission (Simpson 1960a). In chronic hypothalamic states there is abrupt loss of response of a proportion of the muscle fibres followed by abnormally early decrement of the remainder (Simpson & Lenman 1959).

**DISCUSSION**

Stimulation of a normal motor neurone releases more acetylcholine than is required to cause maximal endplate depolarization. This provides a ‘safety-factor’ so that the amount released remains adequate for full response despite gradual reduction with each of a train of stimuli unless this is continued for a long period (the duration depending on the frequency of stimulation). The amount released presumably depends on the stores of preformed acetylcholine in the nerve terminals, possibly in a bound form, and on the rate of synthesis by choline acetylase.

Synthesis may be affected throughout the whole length of the nerve fibre from its cell of origin, or it may be formed in the cell body and transported distally along the axon by protoplasmic flow (Hebb & Waites 1956). Defective synthesis or release of acetylcholine might therefore be expected in lower neurone disorders whether the pathology is mainly poliomyelitic or distal. The comparative rarity of the myasthenic phenomenon in disease of the lower motor neurone probably indicates that the critical state of ‘absent safety factor’ is present during a relatively short period of neuronal degeneration. This would also account for the occasional reports of favourable response to neostigmine in disorders of the lower motor neurone.

The excess of acetylcholine over that required for full muscular response produced by faradization of a normal nerve obscures the facilitation effect of a tetanus. The potentiation is believed to be a pre-junctional effect which increases the efficiency of ejection of acetylcholine by the nerve terminals (Liley 1956). It is, accordingly, of some interest that early tetanic and post-tetanic facilitation have been reported in amyotrophic lateral sclerosis (Simpson & Lenman 1959), and poliomyelitis (Pinelli & Buchthal 1951), but not, so far as I am aware, in peripheral neuropathy. The possible use of this fact as an electrodiagnostic test for the integrity of the motor nerve terminals has been suggested (Simpson 1960a, 1964).

The nature of the abnormality of junctional transmission in myasthenia gravis and polymyositis is still uncertain. The safety factor of transmission could be reduced by a post-junctional abnormality of the endplate. Clinical and pharmacological studies do not permit a firm decision (Simpson 1960b). Nevertheless it is clear from this study of metabolic myopathy that the changes in myasthenia gravis and polymyositis cannot be accounted for by a post endplate lesion of muscle fibres and the balance of probability favours a pre-junctional mechanism in myasthenia gravis (Desmedt 1958, Elmqvist 1965). If this be accepted, one is driven to the conclusion that the myasthenic syndrome of polymyositis, and the recruitment effect described in that disease by Simpson & Lenman (1959) also point to a pre-junctional lesion. Have we discarded too readily the term ‘poliomyositis’? This is of more than semantic importance for electrodiagnostic tests it could imply a restoration of fibrillation to its former status as a pathognomonic sign of denervation.

**REFERENCES**


Eaton L M & Lambert E H (1957) J. Amer. med. Ass. 163, 1117

Elmqvist D (1965) Acta physiol. scand. 64, Suppl. 499, 1


Harvey A M & Maudsley R L (1941) Bull. Johns Hopk. Hosp. 69, 1

Hebb C O & Waites G M H (1956) J. Physiol. 132, 662

Hodes R (1948) Arch. Neurol. Psychiat., Chicago 60, 457


Liley A W (1956) J. Physiol. 133, 571

Merton P A (1954) J. Physiol. 123, 553

Pinelli P (1957) Riv. Pat. nerv. ment. 78, 121


(1959) Develop. Med. child Neurol. 1, 55

(1964) Brit. med. J. ii, 709

The Biochemistry of Myasthenia Gravis

John A. Simpson

It is a signal honour to be invited to discuss myasthenia gravis in Heidelberg. Though earlier physicians may have described cases we now recognize as myasthenia gravis, there is no doubt that Erb (1879) gave the first clear account of the disease and differentiated it from other causes of bulbar palsy, and his name will ever be
linked with it and honoured wherever myasthenia gravis is studied. None will dispute that the selection of subjects for research in medicine depends on the contemporary view of disease. For this reason there has been little study of the biochemical state of the myasthenic patient since interest has been focussed on the neuromuscular junction.

Biochemistry of Muscle

Analysis of muscle tissue has been particularly neglected. Nevin (1934) found no abnormality of bound phosphorus compounds such as creatine phosphoric acid and adenosine triphosphoric acid which are known to play a role in the contraction of muscle. Comings (1939) found a high potassium content in a biopsy specimen of myasthenic muscle. The work was done at a time when the importance was not realised of referring all estimations of tissue constituents to the level of non-collagen nitrogen, to eliminate discrepancies due to the large amount of connective tissue in muscle. He subsequently conducted potassium balance studies which appeared to demonstrate that neostigmine caused liberation of potassium into the blood-stream which returned to the muscle as weakness reappeared, though the urinary excretion of potassium was not increased (Comings, 1940, 1941). These results have not been confirmed.

Numerous studies of the metabolism of carbohydrate, nitrogen, creatine, creatinine, calcium and magnesium were reviewed and shown to be contradictory by Adams et al. (1936). They made personal studies of these items and also of sodium, potassium, phosphorus, sugar, urea, amino acids and uric acid. Their results were within normal limits. Since no later authors have shown significantly abnormal results and personal studies of creatine and electrolytes have been uniformly negative, it is not proposed to review the literature further. Creatininuria may be found, especially in women, but the incidence is no greater than in other diseases associated with difficulty in taking food or with muscular wasting. Milhorat and Wolff (1936) showed that creatininuria in myasthenic patients was increased when the disease was rapidly progressive and this has been the writer's experience.

In recent years interest in the chemistry of muscle has centred on the enzymes which may escape from damaged muscle and appear in greater concentration than normal in the blood. The serum levels of glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), aldolase, and creatine phosphokinase showed no significant elevation in 22 myasthenic patients studied by the author. It may be concluded that myolysis is not a regular feature of myasthenia gravis although there is pathological and electromyographic evidence of a minor degree of polymyositis (Simpson, 1960). A former theory that the defect of neuromuscular transmission in myasthenia gravis might be due to a pathologically high level of cholinesterase ("true" or "pseudo") in the blood was abandoned when it was shown that the serum level was low (Stedman and Russell, 1937) or normal (Wilson and Stoner, 1944). The amount of cholinesterase in muscle is also normal (Goodman et al., 1939). The author has not carried out personal studies since histochemical methods support this conclusion.

Chemical aspects of junctional transmission with possible relevance to myasthenia gravis have recently been reviewed by McArdle (1964). It is unlikely that current biochemical techniques have anything to contribute with regard to the fundamental
nature of the disorder of neuromuscular function in myasthenia gravis such as defective synthesis of acetylcholine. Nevertheless a biochemical study may throw some light on the pathogenesis of the disease.

Serum Protein Abnormalities

In a paper drawing attention to the occurrence of pathological changes in organs other than muscle in myasthenia gravis, the writer mentioned that hyperglobulinemia was found in 4 cases and the protein was raised in the cerebrospinal fluid (C.S.F.) of 9 others in a series of 440 cases (Simpson, 1960). The series, consisting of cases from the National Hospital for Nervous Diseases, London, the neurological unit of the Northern General Hospital, Edinburgh, and the Western Infirmary, Glasgow, has now been extended to 500 cases. This paper reports results of some studies on plasma proteins in 55 cases. It is unfortunate that some of the tests were omitted in various cases. The available data are tabulated in Tab. 1 and Fig. 1.

For the present purposes the upper limits of normal levels for serum globulins were taken as: \( \alpha_1 \), 0.5 g; \( \alpha_2 \), 0.8 g; \( \beta \), 1.15 g; \( \gamma \), 1.6 g/100 ml serum. These figures exceed the upper limits usually adopted for the electrophoretic method of estimation so that it is safe to assume that higher levels are pathological.

Definite hyper-\( \gamma \)-globulinemia was found in 12 of 55 cases (including the 4 previously reported by Simpson, 1960) and in 5 others the electrophoretic profile showed a prominent peak in the \( \alpha_2 \) or \( \beta \)-globulin bands.

Three cases had 2.0 gm/100 ml or more of \( \gamma \)-globulin. Cases MN 2327 and MN 7390 had Hashimoto's disease complicating myasthenia gravis (Simpson, 1964a).
<table>
<thead>
<tr>
<th>Case</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Associated Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN 294</td>
<td>6.75</td>
<td>3.5</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>MN 243</td>
<td>6.5</td>
<td>2.5</td>
<td>Thymoma</td>
</tr>
<tr>
<td>MN 278</td>
<td>5.8</td>
<td>2.5</td>
<td>Hashimoto's disease</td>
</tr>
<tr>
<td>MN 258</td>
<td>7.6</td>
<td>2.5</td>
<td>Hashimoto's disease</td>
</tr>
<tr>
<td>MN 304</td>
<td>6.5</td>
<td>2.5</td>
<td>Non-toxic goitre</td>
</tr>
<tr>
<td>MN 408</td>
<td>6.5</td>
<td>2.5</td>
<td>Previous thyrotoxicosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Associated Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN 430</td>
<td>7.0</td>
<td>2.5</td>
<td>Antinuclear factor</td>
</tr>
<tr>
<td>MN 461</td>
<td>7.5</td>
<td>2.5</td>
<td>Antinuclear factor</td>
</tr>
<tr>
<td>MN 431</td>
<td>7.5</td>
<td>2.5</td>
<td>Antinuclear factor</td>
</tr>
<tr>
<td>MN 490</td>
<td>7.5</td>
<td>2.5</td>
<td>Antinuclear factor</td>
</tr>
<tr>
<td>MN 452</td>
<td>7.5</td>
<td>2.5</td>
<td>Antinuclear factor</td>
</tr>
</tbody>
</table>
to be continued Table 1. Serum Proteins, and Associated Disorders

<table>
<thead>
<tr>
<th>Case</th>
<th>Total Protein g/100 ml</th>
<th>Albumin g/100 ml</th>
<th>Total Globulin g/100 ml</th>
<th>α1  g/100 ml</th>
<th>α2  g/100 ml</th>
<th>γ  g/100 ml</th>
<th>γ Glob. Pl. %</th>
<th>Total Turbidity</th>
<th>E.S.R. mm 1st hr.</th>
<th>Associated disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN 6522</td>
<td>8.0</td>
<td>4.0</td>
<td>4.0</td>
<td>0.2</td>
<td>1.1</td>
<td>1.7</td>
<td>3</td>
<td>2</td>
<td>Thymoma</td>
<td></td>
</tr>
<tr>
<td>MN 6780</td>
<td>8.0</td>
<td>4.0</td>
<td>3.7</td>
<td>0.4</td>
<td>0.6</td>
<td>0.75</td>
<td>3</td>
<td>2</td>
<td>Hashimoto's disease</td>
<td></td>
</tr>
<tr>
<td>MN 6807</td>
<td>8.0</td>
<td>6.0</td>
<td>2.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.65</td>
<td>_</td>
<td>_</td>
<td>Thyrotoxicosis, Antinuclear factor +</td>
<td></td>
</tr>
<tr>
<td>MN 6933</td>
<td>7.1</td>
<td>5.2</td>
<td>1.9</td>
<td>0.3</td>
<td>0.4</td>
<td>0.8</td>
<td>_</td>
<td>_</td>
<td>Antinuclear factor +</td>
<td></td>
</tr>
<tr>
<td>MN 7345</td>
<td>7.25</td>
<td>5.3</td>
<td>1.95</td>
<td>0.25</td>
<td>0.5</td>
<td>0.6</td>
<td>_</td>
<td>_</td>
<td>Pernicious anemia</td>
<td></td>
</tr>
<tr>
<td>MN 7390</td>
<td>8.2</td>
<td>3.3</td>
<td>4.9</td>
<td>0.2</td>
<td>0.5</td>
<td>1.4</td>
<td>1</td>
<td>2</td>
<td>Thyrotoxicosis later</td>
<td></td>
</tr>
<tr>
<td>MN 7476</td>
<td>7.25</td>
<td>4.35</td>
<td>2.9</td>
<td>0.15</td>
<td>0.65</td>
<td>0.9</td>
<td>_</td>
<td>_</td>
<td>Rheumatoid arthritis, Sjögren's syndrome, Hashimoto's disease, gastric antibody</td>
<td></td>
</tr>
</tbody>
</table>

The Biochemistry of Myasthenia Gravis
Abnormal cephalin-cholesterol flocculation and thymol-turbidity tests were found in 10 of the cases. Some of these had electrophoretic patterns within normal limits but these tests may indicate the presence of specific though unidentified proteins.

The plasma proteins in cases of myasthenia gravis were investigated by Tin- venard and Mercier (1955). In 5 of their cases there was considered to be a decrease of \( \gamma \)-globulin and in one case this fraction was increased. Their work was criticised on technical grounds by Lowenthal and Van Sande (1956) who pointed out the necessity to use absolute values rather than percentages. These authors found raised \( \gamma \)-globulin and lowered albumin in the serum of 12 of 16 myasthenic patients. (The albumin change was less constant and should be ignored as it alters secondarily to changes in the globulins.)

Single cases of myasthenia gravis with hyperglobulinaemia are included in the reports of Castaigne et al. (1961), Struik et al. (1962) and Oosterhuis (1963). The latter author and his collaborators have recently reported three patients with myasthenia gravis and hyper-\( \gamma \)-globulinaemia (Oosterhuis et al., 1964). On the other hand Osserman (1958) and Corridoni (1960) found no change in the electrophoretic pattern. Kornfield (1964) found no electrophoretic abnormality in 61 cases of myasthenia gravis but he excluded all those with associated diseases which might affect serum proteins and does not present absolute values.

These results are not necessarily contradictory since both hyperglobulinaemia and \( \alpha \)-\( \gamma \)-globulinaemia may be associated with thymic tumours. The patient with hyperglobulinaemia reported by Castaigne et al. (1961) had a thymoma and aplastic anaemia. Oosterhuis et al. (1964) found hyperglobulinaemia in 3 of 15 patients investigated and two of these had a thymoma. In the present series of cases there were four known to have thymic tumours.

Case MN 2237 was found at autopsy to have Hashimoto’s disease (illustrated in Simpson, 1966). The electrophoretic pattern was within normal limits (see Tab. 1 for the values in this and succeeding cases).

Case MN 5095 had a partial removal of a thymoma 15 years before the protein analysis. In the terminal stage he developed megaloblastic and haemolytic anaemia. The cephalin-cholesterol and thymol turbidity tests were abnormal and there was a high erythrocyte sedimentation rate but the absolute concentrations of the serum globulin fractions were normal.

Case MN 6476 had a slight increase of the \( \alpha \)-globulin fraction only.

Case MN 6522 had slight increase of the \( \alpha \)- and \( \gamma \)-globulin fractions. The abnormalities in these cases with a thymoma were slight and of doubtful significance. The first two cases were complicated by Hashimoto’s disease or megaloblastic haemolytic anaemia. On the other hand significant hyper-\( \gamma \)-globulinaemia was found in four cases without tumours (Tab. 1).

Case MN 2237, with 2.0 g of \( \gamma \)-globulin per 100 ml serum, had Hashimoto’s disease.

Case MN 7390, with 2.8 g of \( \gamma \)-globulin per 100 ml serum also had Hashimoto’s disease.

Case MN 7550 had 2.8 g of \( \gamma \)-globulin per 100 ml serum but no complicating immunological disorder has been detected up to the time of reporting.

Case MN 7788 with a \( \gamma \)-globulin level of 2.1 g per 100 ml has no obvious complication either.
Case Rh 000177 has myasthenia gravis complicated by rheumatoid arthritis, Sjogren's syndrome and myxoedema and has high titres of serum antibodies against thyroid and stomach with positive Rose-Waaler, Coomb's, antinuclear factor and Sjogren's antibody. The total globulin level is raised (4.3 g/100 ml) and the laboratory-reported "increased y-globulin fraction" but unfortunately no measurements were made.

With regard to the two cases without clinical explanation of the hyper y-globulinemia it should be observed that Hashimoto's disease was not detected until post-mortem examination in cases MN 2257 and MN 2327 (Staspoon, 1964a, 1966). Inspection of the tables shows that hyperglobulinemia or alterations of the cephalin-cholesterol flocculation or thymol turbidity tests are often associated with a history of goitre, lymphadenopathy, "rheumatoid" arthritis, or megaloblastic anaemia.

The erythrocyte sedimentation rate was usually normal but showed a tendency to be raised in association with Hashimoto's disease (MN 2327, MN 5503, MN 7390), pernicious anaemia and haemolytic anaemia (MN 5095, MN 5503, MN 7780) but not with uncomplicated thymoma. Case MN 4636 had a borderline level of y-globulin and a raised ESR. Her serum also contained antibodies against thyroid cells in a titre of 1 in 16 and there was a previous history of thyrotoxicosis. She may have unrecognised thyroiditis though the protein bound iodine level in her blood is at present within normal limits (5.1 μg/100 ml).

These correlations suggest that the increased globulins are associated with the production of auto-antibodies rather than with a thymoma per se and the cases of Oosterhuis et al. (1964) support this proposition.

**Protein of cerebrospinal fluid**

Few patients of this series have had analysis of the C.S.F. as lumbar puncture is rarely carried out in myasthenia gravis. For this reason it is impossible to comment on the frequency with which the protein content is raised. Nevertheless, 10 of the patients in whom this investigation was carried out showed a protein level of 50 mg/100 ml or more (Tab. 2).

<table>
<thead>
<tr>
<th>Case</th>
<th>Protein mg/100 ml</th>
<th>Family Test</th>
<th>Large Curve</th>
<th>Other Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH/PS/JF .</td>
<td>125</td>
<td>+</td>
<td>000000000</td>
<td>Acroparesthesia</td>
</tr>
<tr>
<td>NH/MC/AW</td>
<td>50</td>
<td>±</td>
<td>000000000</td>
<td>Raynaud's syndrome; Minor fits</td>
</tr>
<tr>
<td>NH/PM/WB</td>
<td>80</td>
<td>+</td>
<td>000000000</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>NH 6127 .</td>
<td>220</td>
<td></td>
<td></td>
<td>Non-toxic goitre; G-B syndrome after 16 years myasthenia</td>
</tr>
<tr>
<td>NH 61423 .</td>
<td>60</td>
<td>+</td>
<td>000000000</td>
<td>Thyrotoxicosis; Nystagmus</td>
</tr>
<tr>
<td>NH 61491 .</td>
<td>80</td>
<td>+</td>
<td>000112110</td>
<td>Arthropathy; Prominent eyes</td>
</tr>
<tr>
<td>NH 62044 .</td>
<td>80</td>
<td>+</td>
<td>000000000</td>
<td>Arthropathy; Prominent eyes</td>
</tr>
<tr>
<td>MN 675</td>
<td>55</td>
<td></td>
<td>000000000</td>
<td>Arthropathy; Prominent eyes</td>
</tr>
<tr>
<td>MN 4649</td>
<td>100</td>
<td></td>
<td>554421000</td>
<td>Arthropathy; Prominent eyes</td>
</tr>
<tr>
<td>MN 7778</td>
<td>56</td>
<td></td>
<td>000000000</td>
<td>Arthropathy; Prominent eyes</td>
</tr>
</tbody>
</table>
This series includes the 9 cases reported previously (Simpson, 1960). In all of these cases a positive neostigmine test justified the diagnosis of myasthenia gravis. There was often an associated condition (shown in Tab. 2) which might have been related to the abnormal C.S.F. and it is possible that these cases were only incidentally associated with myasthenia gravis. A possibility which was not considered when the earlier data were obtained is that a raised level of protein in the C.S.F. may be associated with myxoedema. This can certainly be excluded in the last three cases in Tab. 2.

Previous references to the C.S.F. in myasthenia gravis have usually described normal protein levels, but 1 of 32 patients with myasthenia gravis reported by Kennedy and Moersch (1937) had a high level of protein in the C.S.F. A case described by de Haen and Roussel (1953) also had hyperglobulinaemia. One of the cases of thymoma with myasthenia gravis, hyperglobulinaemia, and multiple serum antibodies reported by Oosterhuis et al. (1964) had 80 mg/100 ml of protein in the C.S.F. with abnormal colloidal reactions. One may reach the same conclusions as with the serum proteins, viz. that most cases of myasthenia gravis have no abnormality of C.S.F. protein content but that increased globulin may be found in the presence of associated disease which may be of immunological type.

Disorders of Endocrine Function

There is a high incidence of clinically recognisable disorders of endocrine function in myasthenic patients. The question that now arises is whether these are the cause of the neuromuscular condition, as has been suggested in the past, or related entities as suggested by Simpson (1960, 1964a). If an endocrinological or other biochemical lesion were causative it would be present in the majority of cases though frequently in a subclinical degree. For economy of space and since no regular abnormality was found the results are presented as a diagram (Fig. 2).

**Disorders of Endocrine Function**

<table>
<thead>
<tr>
<th>Myasthenia gravis - Endocrinology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>131I Uptake</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>5----------</td>
</tr>
<tr>
<td>3----------</td>
</tr>
<tr>
<td>1----------</td>
</tr>
<tr>
<td>0----------</td>
</tr>
</tbody>
</table>

Fig. 2. Endocrinological tests: 131I uptake, N = normal, + = increased, — = decreased. P.B.I. = protein-bound iodine in blood, Ca = serum calcium, P = serum inorganic phosphate, 17-OHCS = 17-hydroxycorticosteroids in urine, 17-KS = 17-ketosteroids in urine. The scale in column 2 also refers to columns 3—6.
Thyroid function

Thyroid function tests were carried out on 35 cases. As the technique of radioiodine uptake and clearance tests varied in the different hospitals with which the author has been associated, the results are charted according to the interpretation of the laboratory. Normal values were recorded in 26 cases and there was evidence of hyperthyroidism in 4 cases. Some of the patients who had normal tests of thyroid function had a previous history of goitre or of possible hyperthyroidism. Hypothyroidism was found in 5 cases. The first two have been described elsewhere (Simpson, 1964a).

Case MN 5503 had myasthenia gravis and pernicious anaemia. She also had low uptake of $^{131}$I and the blood protein-bound iodine was subnormal (2.4 µg/100 ml). The basal metabolic rate was -8%. Hashimoto's disease was revealed at autopsy (Simpson, 1964a).

Case MN 7390 developed Hashimoto's disease five years after thymectomy for myasthenia gravis of 12 years' duration. A radioiodine uptake test gave a T.P.I. of 1.6 and the protein-bound iodine in the blood was 1.4 µg per 100 ml.

Case MN 6476 had a thymoma. Although there was no clinical evidence of myxoedema there was reduced uptake of $^{131}$I. The protein-bound iodine level was 3.8 µg/100 ml. He died in another hospital and no post-mortem examination was made.

Case WIG 153614 has had myasthenia gravis for more than 20 years. She now complains of tiredness of a different type and has a low uptake of radioiodine. This case is currently under investigation.

Case Rh 000177 has a protein-bound iodine level of 1.3 µg/100 ml. This is the patient described above with rheumatoid arthritis and multiple antibodies including antithyroid.

These results show that there is a high incidence of disorders of thyroid function in patients with myasthenia gravis, but as this may be either hyperfunction or hypofunction and as the majority of cases have normal thyroid function it must be concluded that these are para-phenomena rather than causative of the neuromuscular disorder. Hashimoto's disease may be a frequent complication.

Glucose Tolerance

The same comment may be made on carbohydrate metabolism as measured by the standard glucose tolerance test. Prior to the introduction of neostigmine, many authors reported that there was glycosuria and abnormal sugar tolerance in a high proportion of cases of myasthenia (Williams and Dyke, 1922; Cohen and King, 1932). In the author's series 5% of myasthenic patients have had glycosuria and eight patients have had confirmed diabetes mellitus (Simpson, 1960, 1964b). Osserman (1958) found the same incidence and Frankel (1963) has recently reported abnormal glucose tolerance in 9 of 11 myasthenic patients. In the present series there was pathological reduction of glucose tolerance in 8 of 35 cases though only 1 of these had clinical symptoms of diabetes mellitus. A further 6 had a family history of diabetes (Simpson, 1960).
Parathyroid Function

The serum levels of calcium and inorganic phosphate showed no significant deviation from normal in 39 cases. It is concluded that the parathyroid glands function normally in myasthenia gravis. There was no confirmation of the report by Schwarz et al. (1953) of a persistent elevation of the fasting serum phosphate level.

Adrenocortical Function

There are some reports of adrenocortical insufficiency in myasthenia gravis (Thomset et al., 1961). Only one case of Addison's disease occurred in the present series (case MN 5409) and this was shown at autopsy to have been caused by tuberculosis of the adrenal glands. The excretion of 17-hydroxy-corticosteroids (17-OHCS) and 17-ketosteroids (17-KS) was estimated in 34 cases. It will be seen from Fig. 2 that most of the results are within the normal range. High values are evidently due to stress. There is, however, an unexplained group of subnormal values. These patients showed no clinical evidence of Addison's disease but no water-loading or salt-deprivation tests were carried out so their significance is unknown. Grasschenkov and Perelman (1965) have reported adrenal insufficiency in myasthenia gravis but do not give details of their findings. In view of the occurrence of thymic hyperplasia in Addison's disease and the immunological basis of some cases of adrenal insufficiency (Anderson et al., 1957) it is suggested that more attention should be paid to adrenal function in the future. Nevertheless it seems clear that any association with myasthenia gravis is likely to be secondary, and possibly analogous with thyroiditis (Simpson, 1964b).

Gonadal Function

The well known effects of menstruation and pregnancy on the strength of myasthenic patients has caused many workers to investigate ovarian function. Schurr (1959) reported an unusually low recovery of pregnanediol from the urine of female patients. Dr. K. Fothergill of the Medical Research Council Clinical Endocrinology Unit in Edinburgh kindly carried out some investigations on the author's patients. He studied four women during different phases of the menstrual cycle, one pregnant woman, and one male patient. In every case he considered that the pregnanediol excretion was normal for the appropriate condition. He also tested the response to injections of ACTH and of progesterone, again with normal findings.

Conclusions

From these results it may be concluded:

1. There is no evidence of gross myopathic disease.

2. There is a high incidence of disorders of plasma and cerebrospinal fluid protein fractions, associated with disorders of other systems, and consistent with an immunological disorder.

3. There is no regular disorder of endocrine function, but many cases show evidence of clinical or subclinical disturbance of thyroid function or of carbohydrate metabolism. Low rates of excretion of steroids are reported but the significance is uncertain.

The nature of the relationship between myasthenia and endocrine disease is
obscure. Some of the endocrinopathies are probably due to immunological damage but other considerations suggest a common genetic factor (Simpson, 1960; 1961a, b). If this expresses itself through a hypothalamic-pituitary-thymic mechanism, as the author has previously suggested, it would reconcile the autoimmune hypothesis with the work of Graschenkov whose recent death has prevented him from contributing to this symposium. His gentle courtesy will be missed.

Acknowledgements

I am grateful to the Medical Committee of the National Hospital, Queen Square, London and colleagues in Edinburgh and Glasgow for permission to study their cases: Biochemical studies were carried out in several laboratories but most of the work was done under the direction of Dr. S. L. Tomsett at the Northern General Hospital, Edinburgh, to whom I express my sincere thanks.

References

Nevin, S.: Brain 57, 239 (1934).
Disorders of Voluntary Muscle

By Various Authors

EDITED BY
JOHN N. WALTON
T.D. M.D., F.R.C.P.
Professor of Neurology, University of Newcastle upon Tyne; Neurologist, Regional Neurological Centre, Newcastle General Hospital; Physician in Neurology, Royal Victoria Infirmary, Newcastle upon Tyne

Second Edition
With 198 Illustrations

J. & A. CHURCHILL LTD
104 Gloucester Place, London
1969
CHAPTER 16

Myasthenia Gravis and Myasthenic Syndromes

John A. Simpson

Historical Introduction

Myasthenia gravis is a specific muscular disease characterised by the development of an abnormal amount of muscular weakness in voluntary muscles following repetitive activation or prolonged tension, with a marked...
tendency to recovery of motor power after a period of inactivity or lessened muscular tension (Viets, 1958). Some authorities consider that a therapeutic response to anticholinesterase drugs is also necessary in the definition.

A case described by Thomas Willis (1672) is generally accepted as the first description of the disease and later landmarks are the papers by Erb, Goldflam and Jolly two centuries later (Viets, 1953; Keynes, 1961). The clinical picture was established by the review of Campbell and Bramwell (1900) which abstracted every case reported up to that date. This masterly review contains most of the facts known about the disease, including some that are difficult to reconcile with the concept of neuromuscular block which has been current since Walker (1934) demonstrated the beneficial action of physostigmine. Laquer and Weigert (1901) first noted the relationship between myasthenia gravis and the thymus gland. Simpson (1960) suggested that these two facets of the disease and many of the forgotten or overlooked clinical features can be accounted for by postulating that the thymus produces an antibody against the end-plate protein of muscle, and occasionally against other organs.

![Graph showing distribution of age at first myasthenic symptom]

**Fig. 1.** Distribution of age at first myasthenic symptom.

**Natural History**

Myasthenia gravis affects all races. Estimates of prevalence range from 1 in 50,000 to 1 in 10,000 of the population. Females are affected twice as often as males, the disproportion being 4:5:1 in the first decade, but reversing in later life (Fig. 1). The modal age of onset is about 20 years for each sex but because of the different distribution curves the mean age is a little lower for women (26 years) than men (31 years) (Simpson, 1958, 1960).

The onset of symptoms may be insidious but is often sudden, being precipitated by an emotional upset or a febrile disease, usually an upper
respiratory infection. The role of psychological disturbance is not sufficiently recognised and this causes many failures to recognise the disease at an early stage. Symptoms may first appear during pregnancy or in the puerperium or the first recognition may be due to an abnormal response to a relaxant drug used during anaesthesia (often induced for otolaryngological investigation of dysphonia or dysphagia). In other cases there is no obvious precipitating cause and the diagnosis may then be extremely difficult if the initial weakness is of an unusual type (e.g. unilateral facial palsy or sudden dyspnoea).

The further course is equally variable. There may be a rapid spread of weakness from one muscle to another or an interval of months before the next symptom. Remissions of more than a month occur in fewer than half of the cases, and usually in the early years only of medically-treated patients, becoming less frequent and less prolonged as time goes on. More than one long remission is uncommon, and if myasthenic symptoms return after an absence of a year or more they usually become progressive. Relapses are precipitated by the same factors as the first attacks but additional causes are menstruation, extremes of cold or heat (especially a hot bath or a stuffy atmosphere), inoculation or vaccination and, occasionally, allergy. Bright
sunlight causes ptosis and blurred vision, and a few patients declare that it also causes generalised weakness.

The clinical state is most labile during the first 5 years. Most of the deaths directly attributable to the disease occur in this period, particularly during the first year, with a second danger period in the progressive cases from 4 to 7 years after the onset (Simpson, 1958). Deaths from myasthenia per se rarely occur after 10 years though the patient may be constantly at risk of asphyxiation from inhaled foreign bodies because of the diminished expiratory reserve. Undoubtedly some cases progress for many years, but in the average case the 'active' stage of the disease is limited to a period of 4–7 years and the subsequent course depends on the extent of damage occurring during that period. If thymectomy is to be beneficial the operation must as a rule be carried out during this 'active' period (Simpson, 1958, 1960).

This conclusion is based on analysis of many cases but it is difficult to prognosticate for the individual. Sometimes signs are confined to the extra-ocular muscles and, if others are not involved after 2 years, there is an excellent chance that the further course will be benign. Grob (1953) and Ferguson, Hutchinson and Liversedge (1955) report this type of history in 20–30 per cent. of cases but it has been rare in my experience except in males. Perhaps for this reason there is disagreement regarding prognosis, for Grob (1958) considers that it is worse in the male whereas in my experience women have had a poorer prognosis than men if treated without thymectomy (Simpson, 1958).

Myasthenia is associated with a thymic tumour in 10–20 per cent. of cases and about 60 per cent. of these patients are male (Schwab and Leland, 1953; Simpson, 1958) (Fig. 2). This type of myasthenia tends to appear at a later age and is rare under the age of 30 (Keynes, 1955). Muscular weakness is usually severe and difficult to control with neostigmine. Thymectomy is also less successful than in non-tumour cases though some patients with a thymoma respond excellently to its removal.

**Symptoms and Signs**

A characteristic feature of myasthenia gravis is the variability in muscular strength from day to day or even from hour to hour. Short-term weakness is often due to physical exertion but, as in long term relapses, the emotional state is an important factor. Weakness of an affected muscle increases if it is contracted repeatedly or if a contraction is maintained. The contracting muscle may lengthen gradually if it is supporting a load, or a coarse tremor develops with increasing 'rest periods' until the attempt to sustain the contraction ceases. Gradual 'fatigue' is not always seen and failure of contraction may be sudden. Recovery with rest or full medication is often incomplete. Permanent weakness with wasting of muscles ('myasthenic myopathy') is commoner than generally believed, particularly in the extra-ocular muscles, triceps brachii and quadriceps femoris, being found in 10 per cent. of female and about 20 per cent. of male cases (Simpson, 1958).
Nevertheless it is the recoverable weakness which is characteristic of myasthenia gravis and the diagnosis cannot reasonably be contemplated unless this feature is present at some stage. Weakness usually increases in the afternoon and evening even though the patient has made little physical effort, but this is not invariable and many patients are weakest on first waking in the morning. Another unfamiliar paradox is that a few patients reach a stage of weakness which is greatest after rest and relieved by moderate exercise. Most of these are males with 'myopathic' muscles which have ceased to respond to neostigmine after many years of treatment (personal unpublished observations).

Any muscle may be involved, usually several at a time but sometimes only one or even part of a compound muscle such as extensor digitorum. The extra-ocular muscles and levatores palpebrarum are most frequently involved (more than 50 per cent. of cases) and this is usually associated with weakness of orbicularis oculi. The flexors and extensors of the neck, the shoulder girdle muscles, and the flexors of the hip are next most commonly affected, closely followed by the muscles of facial expression, chewing, swallowing and speech. It will be seen from Fig. 3 that the relative incidence of involvement of muscles at some time during the course of a large series of cases follows closely the probability that these muscles will be the first to show myasthenic signs (Simpson, 1960). As in other muscular diseases there is a strong tendency for the proximal muscles of the limbs to be more severely affected than the distal. The upper limbs are more often involved than the lower. The extensors are more severely affected than the flexors in the upper limbs but the flexors are most affected in the lower limbs where weakness, if present at all, tends to be confined to the thigh flexors. The erector spinae group is frequently involved but other trunk muscles usually escape in the milder cases. Weakness of the diaphragm, intercostal or abdominal musculature usually accompanies widespread myasthenia but occasionally they may be the first affected. Many inexperienced physicians refuse to diagnose myasthenia gravis in the absence of ocular or bulbar symptoms. There is statistical justification for this point of view but there is no doubt that severe myasthenia may spare these muscles and present in unusual ways. Commonly the complaint is related to a muscle which is fatigued by a particular movement required by the patient’s work or daily activity and a craft palsy or cramp may be simulated. Systematic examination may reveal unsuspected weakness or fatigability of other muscles, but examination must be thorough and must include contraction maintained against resistance for an adequate period.

The symptoms are those associated with the appropriate muscle weakness. Paresis may be induced by bright sunlight or by looking fixedly at an object above eye level, but it is equally often precipitated by emotional disturbance such as the entry into a crowded room of a patient lacking self-confidence. Paresis may be unilateral or bilateral and it is quite common for the affected side to be changed suddenly for no apparent reason. Double vision is brought
on by reading or by embarrassment. The two images may separate steadily or, very characteristically, one image may appear to 'slip' suddenly. Facial weakness is rarely so severe as to cause symptoms but friends notice that the smile is distorted into a vertical 'snarl' (Fig. 4), whistling may be impossible, and women find difficulty in applying lipstick because of failure to pout the lips and to roll them in the customary manner. Weakness of the masseters may only be noticeable when chewing meat but may be so severe

Fig. 3. Percentage of cases in which various muscle groups are affected at the onset (left of key) and at some time during the illness (right of key).

that the mouth cannot be closed and the diagnosis may often be suspected by the patient's habit of sitting with a hand supporting the jaw, especially if the posterior nuchal muscles are also weak. Swallowing may be normal at the beginning of a meal but becomes impossible after a few mouthfuls. Involvement of the tongue leads to progressive thickening of speech, and if laryngeal and respiratory muscles are affected there is characteristic fading of the voice after speaking for a short time.
**Figure 17.4.** (a) The myasthenic 'snarl'.

**Figure 17.4 (b)** The triple furrowed tongue (same patient).
Abnormal fatigability of the shoulder girdle muscles is first noticed by women when they are unable to complete fixing their hair because the arms have to be rested, or they are unable to hang out clothes on a line or do similar work above shoulder level. If a patient with these symptoms is asked to hold the arms outstretched they gradually sag in less than a minute and a coarse tremor often develops; then the arms are dropped for a second, raised again immediately, and dropped once more—the cycle becoming shorter and shorter until further arm-raising is impossible. (To the unaccustomed eye, the sudden drop of the arms and renewed effort has the appearance of a hysterical phenomenon, but it is a genuine paresis and the prompt restoration is due to post-tetanic potentiation, vide infra.) Fatigue tests appropriate to the various muscle groups will readily suggest themselves and it is important to test all muscles in this way before classifying a case of myasthenia as 'localised'. It may be possible to record a progressive decrease of muscle power with a dynamometer or ergogram. Performance tests are conveniently combined with the pharmacological tests described below. Diaphragmatic movement or the ability to swallow a mouthful of barium before and after intramuscular injection of neostigmine (1 mg.) may be observed on the fluoroscope and the objective measurement of diplopia with prisms, etc., may be valuable in demonstrating the changing extent of weakness. Tests depending on subjective assessment by the patient are unreliable and diagnosis should not be based on them. Since the electromyographic and pharmacological tests give important evidence regarding the nature of myasthenic weakness they are more conveniently described in a later section (pp. 553-557).

In very severe cases the external sphincters of the bowel or bladder may be affected giving rise to stress incontinence, but smooth muscle is never affected. Fatigability of the pupil sphincters has been reported. It must be extremely rare but fatigue of visual accommodation may be more common. On the whole, the association of normal pupils with external ophthalmoplegia and weakness of eye closure is characteristic and easily distinguished from other causes of ophthalmoplegia.

Tendon reflexes are usually present and may be so brisk that clonus is present. If a reflex is elicited repetitively the jerk may decrease progressively until it disappears. Persistent absence of many reflexes should suggest that the weakness is due to carcinomatous myasthenia rather than myasthenia gravis but a localised absence may be due to myopathic change in the muscle. This is most common in late cases but is occasionally present within months of the onset. The extra-ocular muscles, triceps brachii, and quadriceps femoris are most often affected, in that order. Atrophy of the tongue is curiously selective, giving rise to a triple longitudinal furrowing which, though rare, is never seen in any other condition (Fig. 4). It appears to have been described first by Buzzard in 1905 but was emphasised as typical of myasthenia gravis by Wilson (1954).
Abnormalities of Sensation

Pain may be complained of in weak muscles, especially in the neck, the back and around the eyes. This is usually due to the extra effort required to maintain posture and sometimes there appears to be true myositis. More common is a sensation of 'stiffness' and there may be paraesthesias of hands, thighs or face (Harvey, 1948; Simpson, 1960). There are, however, some reports of transitory trigeminal anaesthesia; anosmia (Alajouanine, Castaigne, Nick, Contamin and Lhermitte, 1957) and ageusia (personal observation) which are difficult to explain as secondary to motor weakness and may indicate occasional involvement of sensory neurones or end-organs.

Endocrinological Relationships

A relationship between myasthenia gravis and disorders of the thyroid gland is beyond doubt. Many muscular syndromes are associated with thyrotoxicosis and there may sometimes be difficulty in deciding whether the muscular disorder is true myasthenia or not (see Chapter 18) but if the correlation is examined from the other point of view there can be no doubt that disorders of the thyroid occur more frequently in myasthenics than in normal subjects. Millikan and Haines (1953) found that the incidence of hyperthyroidism before, during, or after detectable myasthenia gravis is about 5 per cent. If all thyroid disorders including non-toxic goitre and primary myxoedema are added, the incidence may be as high as 9 per cent. in males and 18 per cent. in females (Simpson, 1958; Downes, Greenwood and Wray, 1966). Many of these cases have thyrotoxic symptoms or signs for only a few months and they may precede myasthenic symptoms by many years. Thyrotoxic symptoms may be subsiding when myasthenia appears and vice versa. In a limited period of observation this may lead to the conclusion that the two disorders have a 'see-saw' relationship to each other (McEachern and Parnell, 1948). This is certainly exceptional.

There are a few reports of myasthenia in cases of spontaneous myxoedema (Feinberg, Underdahl and Eaton, 1957; Sahay, Blendis and Greene, 1965) but there is little recognition of the frequency of non-toxic goitre in association with myasthenia gravis (Rowland, Hoefer, Aranow and Merritt, 1956; Simpson, 1958). Clinical or latent Hashimoto's thyroiditis is not uncommon in myasthenia gravis and may develop after thymectomy (Simpson, 1960, 1964, 1966a; Becker, Titus, McConahey and Woolner, 1964). Many myasthenics resemble thyrotoxic patients in other ways. Slight exophthalmos and thickening of the upper eyelids is common (Simpson, 1960) and many patients complain of excessive sweating even before the use of anticholinesterase medication. A possible explanation for these apparently paradoxical findings is that the thyroid and myasthenic disorders are independently associated through a common factor which may be a genetically-determined hypthalamo-pituitary abnormality. In favour of this suggestion is my finding that myasthenics often have relatives with
goitre even though their own thyroid is normal. This family history is mainly on the maternal side (Simpson, 1960).

A pituitary mechanism would also account for the rare association between myasthenia gravis and pituitary adenoma and the occasional beneficial effect of X-ray treatment of the pituitary (Zondek and Ticho, 1931). Also difficult to assay but probably directly related in this way are the reported association of myasthenia gravis with adrenal insufficiency or glycosuria. The latter was present in only 2 per cent. of my cases but others had a family history of diabetes mellitus (Simpson, 1962). A pituitary factor may also be involved in the changes in the myasthenic state associated with menstruation and pregnancy. It is most common for weakness to increase in the premenstruum but the time differs in each patient. The effect of pregnancy is equally variable (Harvey, 1948). Exacerbation usually occurs during the first trimester with later remission and then relapse in the puerperium (Turner and Fraser, 1953) but the course may be quite different in successive pregnancies. The most regular association is for the myasthenia to be exacerbated at or soon after delivery. I have not been able to confirm the finding of Schrire (1959) who reported low pregnandiol excretion in non-pregnant myasthenic women and stated that this was corrected by thymectomy.

**Blood and Reticulo-endothelial System**

Pure red cell aplasia associated with a thymic tumour is an occasional occurrence in myasthenic patients (Green, 1958). Less well-known though numerically more frequent is the coincidence of pernicious anaemia and myasthenia gravis (Simpson, 1960, 1964, 1966a). Pernicious anaemia has a well established link with thyrotoxicosis and there is growing evidence that an auto-immune mechanism may be involved in its aetiology; this may also be true for aplastic anaemia. Aplasia of other marrow cells was also present in some reported cases. Thymic tumours are sometimes associated with agammaglobulinaemia but this has not yet been reported in myasthenia gravis. On the contrary, hyperglobulinaemia is an occasional finding (Lowenthal and van Sande, 1956; Simpson, 1960). It is more likely to occur in the presence of a thymoma or with associated auto-immune disease (Oosterhuis, van der Geld, Feltkamp, and Peetoom, 1964; Simpson, 1966b). I have also noted cases with generalised lymphadenopathy, splenomegaly and lymphosarcoma. The impression gained is that in a small proportion of cases the thymus is not the only lymph organ showing pathological changes and that abnormalities of the blood cells and proteins may occur of a nature consistent with auto-immune disease (Simpson, 1960).

**Associated Disorders**

Thyrotoxicosis is the only disease which is commonly associated with myasthenia gravis. It is difficult to discover correlations which are ‘not statistically significant’, especially if the doctor preparing a case history fails
to record data which he considers to be irrelevant. This has often been the case in myasthenia since the idea of defective neuromuscular transmission became dominant, but it is interesting to compare the commoner associated disorders in five large series (Table 1). The most common of these is arthritis of rheumatoid type which may be transient. An association with Sjögren’s disease has been reported (Downes, Greenwood and Wray, 1966; Simpson, 1966b).

It is possible that these associated diseases are coincidental, but the similarity between the series is surprising. Hoefer, Aranow and Rowland (1958) have reported eight cases of epilepsy and a patient with syncopal attacks in a series of 180 cases of myasthenia. Taking account of the disorders of the blood and reticuloendothelial system, and the occasional disturbances of sensation and of the cerebrospinal fluid protein seen in some cases, Simpson (1960) has suggested that the neuromuscular syndrome may be only the most prominent part of a widespread tissue disorder analogous to (but not identical with) systemic lupus erythematosus.

Symptomatic Myasthenia

The suggestion of an autoimmune basis for myasthenia gravis links it with systemic lupus erythematosus, polymyositis and dermatomyositis, in all of which a myasthenic state is an occasional occurrence (Chapter 12). There may be a therapeutic response to neostigmine at first but this is rarely so striking as in myasthenia gravis and is soon lost. A myasthenic syndrome is also associated with carcinoma (Anderson, Churchill-Davidson and Richardson, 1953). A striking difference is that the tendon jerks are usually absent and the neostigmine response is lost at an early stage. Although these syndromes are important in considering the possibility of an autoimmune basis for myasthenia gravis it must not be overlooked that a similar muscular ‘fatigability’ may result from pre- and post-junctional causes. Some patients with lower motor neurone lesions show a positive Jolly reaction (Simpson, 1966c) and Churchill-Davidson and Wise (1963) have shown that most of the neuromuscular phenomena of myasthenia (including resistance to depolarising substances) may be demonstrated in the normal newborn infant though clinical myasthenia is not seen.

Neonatal Myasthenia

About one in seven live-born children of myasthenic mothers shows evidence of myasthenia at birth and, if the affected child survives, there is complete recovery in 1 to 12 weeks without later relapse. The reported cases are summarised by Osserman (1958). There is no correlation between the severity of the infant’s symptoms and the duration of the mother’s illness or the severity of the mother’s myasthenia during pregnancy. It is extremely rare for a myasthenic mother to have more than one affected child. Previous thymectomy does not remove the possibility that the baby will be myasthenic, but the transient nature of neonatal myasthenia suggests that the child is affected.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma (non-thymic)</td>
<td>3</td>
<td>90</td>
<td>325</td>
<td>180</td>
<td>74</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sjogren's syndrome</td>
<td>2(?)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nephritis</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Raynaud's syndrome</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Psychosis</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epilepsy, etc.</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pemphigus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table I**
by some factor transmitted from the mother. The duration of action is long for a simple toxic ‘curare-like’ substance but would be compatible with an immunity reaction in which the infant’s muscles might be presumed to react to a maternal antibody transmitted through the placenta, because of antigenic relationships between the muscles of mother and child (Simpson, 1960). Stern, Hall and Robinson, (1964) and Oosterhuis, Felskamp and van der Geld (1966) found that there is no correlation between the presence of neonatal myasthenia and of antimuscular antibodies in the infant’s serum.

Genetic Factors

Myasthenia gravis occasionally affects related individuals. Most of the recorded cases are of the ophthalmoplegic type. I have studied two families of affected sisters and feel that their disability differs from that of ordinary myasthenia gravis and is more closely related to ocular myopathy. There are several examples of myasthenia affecting only one of a pair of identical twins; thus any genetic factor is unlikely to be direct. Simpson (1960) has drawn attention to the increased frequency of thyroid disorders (and possibly of diabetes mellitus) in the family history of patients with myasthenia and has suggested that there may be a genetic factor involving the hypothalamo-pituitary axis with variable expression. Thyroid disorder may be the commoner but both expressions sometimes occur in the same person.

Neuromuscular Transmission

In 1895 Jolly showed that the pathological fatigability of myasthenia could be reproduced by faradic stimulation of a motor nerve while the ‘fatigued’ muscle would still respond fully to locally-applied galvanism. Electromyographic recording shows that the loss of power when the motor nerve is repetitively stimulated supramaximally is accompanied by a decrement of the evoked action potential of the muscle while the antidromically conducted nerve action potential is unchanged in amplitude. The decrement is often temporarily reversed if faradisation is continued, probably due to facilitated release of acetylcholine from the motor nerve endings. The same mechanism is considered to explain the post-tetanic facilitation which is sometimes seen immediately after a voluntary or indirectly-stimulated tetanic contraction (Fig. 5a) The amplitude of the integrated action potential evoked by the first stimulus of a train is usually normal but may be subnormal, the unstimulated muscle fibres being available for recruitment during post-tetanic facilitation. In some muscles tetanisation with supramaximal stimuli causes only recruitment (Fig. 5b) (Simpson, 1960, 1966c). In other muscles a normal response is found with rates of stimulation up to 50/sec. for repeated trains of stimuli lasting a few seconds with brief rests between, but often an identical train will produce a typical myasthenic decrement after several applications or after a prolonged voluntary contraction. Desmedt (1957) considers that a short-lived facilitation occurs in the period 1–20 seconds after (voluntary or evoked) tetanic
contraction which is followed by profound transmission failure and that this 'post-activation exhaustion' with a half-time of 10-15 minutes is characteristic of myasthenic block, differentiating it from the block caused by curare (but resembling the transmission failure caused by hemicholinium which is considered to inhibit release of acetylcholine at the nerve endings). Some anomalies are difficult to account for. Sometimes nerve stimulation at slow rates (3-10/sec.) causes typical myasthenic decrement of the integrated muscle action potential whereas recruitment occurs at fast
rates (20–50/sec.). The voluntary electromyogram (EMG) recorded with needle electrodes may be equally difficult to explain. During a sustained voluntary contraction the EMG gradually reduces in amplitude and tends to synchronise, causing a coarse tremor with alternating periods of rest and facilitated innervation until complete ‘exhaustion’ occurs. At this stage the EMG is sufficiently reduced to make it possible to observe single units. Some of these can be seen to decrease in amplitude but, more commonly, units suddenly cease firing as in normal fatigue (Lundervold, 1954).

Many of the single units identifiable in myasthenic muscle are of the ‘myopathic’ type even after rest. This could be due to the missing fibres available for recruitment as described above, but is suggestive of a structural disorder of muscle in addition to any abnormality of neuromuscular transmission which may be present. This feature may make it difficult to exclude one of the symptomatic myasthenias referred to above but spontaneous fibrillation or positive sharp potentials are common in myositis but rare in myasthenia gravis. Eaton and Lambert (1957) and Wise and MacDermot (1962) have stated that supramaximal faradisation evokes an incremenating muscle response which is diagnostic of carcinomatous myasthenia. I have found exactly the same response in acquired myopathies unrelated to carcinoma (Simpson, 1966) and, as stated above, a similar though quantitatively smaller phenomenon is sometimes seen in myasthenia gravis, usually in muscles which are not clinically affected (Pinelli, 1957; Simpson, 1966).

Pharmacological Tests

The most widely used tests are those involving a therapeutic response to cholinergic drugs, confirming that weakness is of myasthenic type. Conversely, increased sensitivity to curare and similar substances may be used to detect latent myasthenia. This group of tests will be described first. They are dangerous and should not be used where performance tests or therapeutic tests indicate the diagnosis. Their uses should be confined to cases who give a clinical history suggestive of myasthenia gravis but who are in remission at the time of examination or who do not show convincing fatigability. All anticholinesterase medication should be withdrawn for 24 hours. Facilities for artificial respiration and syringes loaded with edrophonium (10 mg.) and neostigmine (1.5 mg.) should be prepared before carrying out the test.

Curare Test. The curarising dose for normal individuals is taken as 3 mg. d-tubocurarine intravenously per 40 lb. of body weight. The suspected myasthenic patient is given 2 per cent. of the normal curarising dose and observed for 3 to 5 minutes. If no weakness appears in that time, 5 per cent. of the estimated normal curarising dose is injected (Bennett and Cash, 1943). Bigger doses of curare should not be used, as muscle weakness may occur in some normal subjects with the 10 per cent. dose originally recommended.

Quinine Test. Quinine bisulphate 0.65 g. (10 gr.), 2-hourly by mouth to a maximum of three doses, may be used to precipitate weakness in suspected
myasthenics (Eaton, 1943). In this dose no neuromuscular effect can be detected in normal individuals but myasthenics may become very weak. This may be dangerous as the paralysis cannot be reversed by anticholinesterase drugs.

**Decamethonium Test.** The action of depolarising neuromuscular-blocking drugs in myasthenics is different from normal, and not simply an increased sensitivity as with curare and quinine, since they tolerate an unusually large dose (Churchill-Davidson and Richardson, 1952a). Tolerance is particularly marked in clinically-affected muscles. Muscles showing myasthenic weakness are first to be blocked by decamethonium so the test should be used with caution in the presence of bulbar or respiratory weakness and resuscitative measures should always be at hand. This test is also performed after withdrawal of anticholinesterase drugs for 24 hours. Decamethonium iodide is injected intravenously in an initial dose of 1.5 mg. Four minutes later 0.5 mg. is injected and this dose is repeated at 2 minute intervals until muscle weakness is seen or there is a drop in the muscle action-potential evoked by supramaximal nerve stimulation at 10 per second when tested every half-minute. In normal subjects the block caused by a depolarising drug is non-decremental and is potentiated by neostigmine, but in the myasthenic subject the depolarisation block is brief and followed by a longer competitive (curare-like) type of block (‘dual response’).

The next group of tests depends on the observation of a temporary therapeutic response to an anticholinesterase drug. It will be convenient to describe the further use of edrophonium to determine the cholinergic status during treatment.

**Edrophonium Test.** The short duration antmyasthenic effect of edrophonium chloride (‘Tensilon’) makes it very suitable for a diagnostic test. A syringe is loaded with 1 ml. (10 mg.) for intravenous injection. Initially 2 mg. should be injected to detect abnormal sensitivity but if there is no response the remaining 8 mg. is injected after 30 seconds (Osserman and Kaplan, 1953). Within ½ to 1 minute there is improvement if weakness is due to myasthenia gravis but weakness returns in 4–5 minutes. Some normal subjects experience no obvious effects while others feel a tight sensation around the eyes and fasciculation may be seen for a few seconds, particularly in the orbicularis oculi. Adequately treated myasthenic patients show this normal reaction. If weakness is due to cholinergic crisis it is transiently increased and fasciculation may occur. Unfortunately respiratory weakness may be increased to an extent endangering life.

The positive responses described are very valuable and reliable when present but failure to obtain improvement, or fasciculation, does not indicate that weakness is not myasthenic or that it is cholinergic respectively. Negative responses are sometimes found but false-positive results are extremely rare provided that only objective criteria are used. Subjective ‘improvement’ should never be relied on, especially when the test is used to
determine the adequacy of treatment. The test is best performed at the time of greatest activity of the therapeutic drug. Where neostigmine is being used the test should be performed 2 hours after a dose. A second test at 3 hours may be necessary when the response is equivocal (Osserman and Kaplan, 1953).

Because of the differing degree of involvement of different muscles, it is extremely important to test the respiratory and bulbar muscles and not the ocular when the test is carried out to differentiate between myasthenic and cholinergic weakness. It is possible for these vital muscles to be overdosed while the ocular muscles are still underdosed, and failure to recognise this may lead to fatal overdosage (Simpson, 1961). An equivocal or ‘adequate’ response should always be taken to mean that further dosage may be dangerous.

Neostigmine Test. Though the latency is greater, a favourable response to injection of neostigmine remains the most convincing evidence of myasthenia gravis since its duration is sufficient to permit repeated testing of all muscles (Viets and Schwab, 1935). The duration makes it unsuitable when cholinergic crisis is suspected. Neostigmine methylsulphate is injected intramuscularly (1.5 mg.) alone or combined with 0.6 mg. of atropine sulphate. Improvement begins in 10–15 minutes but is most obvious after 30 minutes. The same preparation may be used intravenously (0.5 mg.) when the response is more rapid but the danger of ventricular fibrillation or arrest is greater. The drug should never be given by this route unless accompanied or preceded by atropine. The response to 15 mg. of neostigmine bromide orally may be sufficient to make the diagnosis clear. If any of the parenteral tests are equivocal and the diagnosis of myasthenia gravis seems highly probable on clinical grounds it is worth carrying out a therapeutic trial with oral medication for a week.

Other Pharmacological Investigations

The abnormal response to decamethonium suggested to Churchill-Davidson and Richardson (1952b) that the motor end-plate was altered in myasthenia and the response to acetylcholine would be expected to be altered similarly, in view of its similar mode of action.

Acetylcholine Sensitivity

The response of myasthenic muscle to proximal intra-arterial injection of acetylcholine has been a matter of argument and reports range from subnormal to hypersensitive. The conflicting findings were resolved by Engbaek (1951) who showed that most were obtained with unphysiologically large dosage of acetylcholine. With suitable dosage it could be demonstrated that the myasthenic muscle has a raised threshold to acetylcholine. Grob, Johns and Harvey (1956a) confirmed this conclusion, though the difference from normal was slight, and they found that the immediate stimulation and its following ‘prompt’ depression (depolarisation block) were decreased. On
the other hand the ‘late’ depression of end-plate excitability was more marked in myasthenic patients than in normal subjects and the properties of the ‘late’ depression were different in the two groups. In the myasthenic patients they were characteristic of a competitive type of block, in contrast to the non-competitive type that occurred in normal subjects. Choline was found to have a similar but more prolonged action and Grob and his colleagues suggested that the ‘late’ depression of neuromuscular transmission may be due to choline released by hydrolysis of acetylcholine. The dose-effect relationship of acetylcholine and choline were the reverse of the expected, but it was suggested that acetylcholine may penetrate more readily into the region of the end-plate by virtue of its ionic interaction. The abnormal response of the myasthenic muscle could be due to an alteration in the end-plate, or to the formation of an abnormal product of acetylcholine or choline which has competitive blocking activity.

It is unnecessary to postulate an abnormal product since the effect of quaternary ammonium compounds on the motor end-plate depends on the dose (Grob, Johns and Harvey, 1956b). If the effective condition for depolarisation of the end-plate membrane is the density (or the rate of change) of ionic charges attached to the postulated receptor substance there would be a dose-dependent sequence of competition, stimulation, depolarisation block, ‘desensitisation’ block (followed by brief facilitation if desensitisation is short lasting) and finally competitive block (Simpson, 1960). The time factors would differ with different compounds and the relative duration of each phase would depend on the compound used, the dose, and the animal species. Anything which decreased the charge-density at the receptor sites would reduce the depolarisation block and prolong the competitive block. This dual response could be caused by abnormal structure of the end-plate as suggested above but would be expected if the receptor sites were partially occupied by molecules with a negligible charge. If this hypothesis is accepted the concept of a competitive blocking myasthenic toxin cannot be excluded. Furthermore, this concept would be compatible with the facilitation phenomena described above and with the possibility of transplacental transmission of a myasthenia-causing substance, both of which are difficult to account for on any other theory.

‘Myasthenic Toxin’

Earlier workers looking for a ‘curare-like substance’ have considered that it must either be liberated in contracting muscle or secreted by the thymus. Many have described ptosis occurring after release of a tourniquet which had occluded venous return from an exercised limb of a myasthenic patient (‘the Walker effect’). This test is difficult to evaluate because of the long latency of the response. A modification of the test is to exercise one limb by electrical stimulation distal to a tourniquet and to observe the effect of release of the tourniquet on the indirectly evoked muscle response of the other arm. Johns, Grob and Harvey (1956) found no evidence of release of a
blocking substance and my experience is similar, but Tsukiyama, Nakai, Mine and Kitani (1959) reported a decrease of the indirectly evoked muscle response in one arm 1-4 minutes after release of a cuff from the other arm; the cuff had been inflated during 5 minutes of supramaximal nerve stimulation at 10 per sec. They also reported suppression of the indirectly stimulated muscle response after intra-arterial injection of 'myasthenic serum'. Their report refers only to reinjection in the same (myasthenic) subject. Transfer to a normal subject has never been demonstrated.

Many workers have tested the effect of adding serum from myasthenic patients to a nerve-muscle preparation of another animal, usually the frog, and many have claimed to demonstrate a curare like effect if the serum is withdrawn after exercise, especially exercise in ischaemic conditions (Strüppler, 1954). Others have found no evidence of a blocking substance (e.g. Nastuk, Strauss and Osserman, 1959).

Attempts to demonstrate the presence of a thymic secretion have also been conflicting but usually negative. They are reviewed by Wilson, Obrist and Wilson (1953). These workers assayed extracts of human thymus obtained at operation from myasthenic patients and claimed that the extracts showed a blocking effect on the rat or frog neuromuscular junction which was related to the degree of benefit the operation had provided for the patients from whom the glands were removed. The same group later showed that thymus glands obtained from children at necropsy, or from other young animals, including the whale foetus, had a similar effect but it was transient and not reversed by neostigmine. (It has been suggested that potassium in the extract might be responsible.)

In any event, if the postulated thymic blocking substance is humoral it should be detectable in serum and this has not been done convincingly. Nastuk, Strauss and Osserman (1959) only rarely found that myasthenic serum caused reduced response of a frog muscle to indirect stimulation and in these cases the depression was virtually irreversible, being accounted for by lysis of surface fibres of the test muscle. This observation led these authors to a study of immunity reactions in myasthenia culminating in the demonstration of a muscle-binding globulin in myasthenic serum (Strauss, Seegal, Hsu, Burkholder, Nastuk and Osserman, 1960). At the same time Simpson (1960) had concluded from a clinico-pathological analysis that myasthenia gravis could best be accounted for on a basis of auto-immunity and that an anti-muscle substance would have the properties required of a competitive blocking substance which would be specific to the individual or to a genetically similar infant. Later immunological studies will be reviewed after the section on Pathology (p. 564).

The Defect in Neuromuscular Transmission

The nature of the block remains undetermined but four possibilities may be considered.
Pre-synaptic Defect. Desmedt (1957) makes a strong case for a pre-synaptic lesion in myasthenia gravis since the functional disturbance is closely simulated by the effect of hemicholinium which impairs acetylcholine synthesis. Elmqvist (1965) records conflicting findings on end-plate potentials of muscle obtained by biopsy from myasthenic patients and concludes that there is a deficiency of acetylcholine in the quanta released at motor nerve terminals. He does not comment on the fact that the end-plate potentials showed no decrement during a period of high frequency nerve stimulation. It is difficult to account for neonatal myasthenia by this theory unless acetylcholine synthesis is blocked by a toxic substance. In the writer’s view this type of lesion probably accounts for the myasthenic syndromes sometimes associated with lower motor neurone disorders (Simpson, 1966c).

Excessive Hydrolysis of Transmitter. This possibility can be dismissed, as cholinesterase in blood and muscle, including the subneural apparatus, does not appear to be excessive.

Post-synaptic Defect. The anomalous responses to substances acting at the motor end-plate receptors led Churchill-Davidson and Richardson (1952b) to postulate changes in the latter. There is no evidence that the receptor substance is abnormal in the elongated end-plates described below but by dispersing the ionic charge carried by the transmitter the activity of the latter could be reduced (Simpson, 1960). The main difficulties in accepting this theory are the fact that complete but temporary remissions can occur, and the facts of neonatal myasthenia which require to be explained.

Competitive Block. The possibility that a substance derived from acetylcholine or secreted by the thymus might compete for receptor sites on the motor end-plates has been discussed above. The electro-physiological and pharmacological data are almost equally compatible with each theory. Only this theory can explain transplacental transmission of myasthenia but the duration of the latter suggests that the hypothetical blocking substance must have a large molecule. Furthermore, it is necessary to account for the strong negative evidence of transfusion experiments. A logical reconciliation would be to presume that it is an antibody protein which is able to ‘recognise’ a particular tissue (muscle end-plate or nerve terminal) in a limited number of genetically-related individuals. Simpson (1960) formulated this hypothesis in an attempt to find a role for the thymus gland. An alternative hypothesis is that a ‘myasthenic toxin’ is released by a thymus gland damaged by immunological reaction (Goldstein and Whittingham, 1966).

Pathology

Muscle and Nerve

Despite frequent statements in recent years that myasthenia gravis is a disease without morbid anatomy, the lymphocyte infiltrations of muscle described by Weigert (1901) and termed lymphorrhages by Buzzard (1905)
have been repeatedly found in such cases. Buzzard (1905) also reported degenerative changes of muscle fibres. These were re-investigated and classified by Russell (1953) into the following types:

Type I (Fig. 6a), acute coagulative necrosis of a muscle fibre with eosinophilic change, loss of cross-striation, and inflammatory cellular reaction
leading to phagocytic removal of the fragmented fibre. This process may be limited to one fibre or can be so widespread as to cause naked-eye changes in the muscle.

Type II (Fig. 6b), the lymphorrhage, is considered by Professor Russell to be secondary to solitary muscle atrophy with basophilia of the cytoplasm and loss of cross-striation.
Type III (Fig. 6c), simple focal muscle change with eosinophilia and swelling but without loss of striation or inflammatory reaction.

Russell (1953) doubts the specificity of any of these changes since they are found in certain rheumatic-type diseases and endocrine myopathies, but it is possible that this may be due to a common 'auto-immune' mechanism (Simpson, 1960).

Special supravital staining techniques applied to the motor nerve endings by Coers and Desmedt (1959) have shown that the neuromuscular junctions may often be abnormal in myasthenia gravis. They described two changes in the terminal arborisation (Fig. 7). In one, the 'dystrophic' type, there is sprouting of the subterminal axons, the terminal knobs are shrunken and distributed over a wider area of the muscle fibre than usual. This type is probably reactive as the related muscle fibre is usually abnormal (often related to a lymphorrhage) and the same type of end-plate has been found in other neuromuscular disorders. In the other type, the 'dysplastic', there are few terminal knobs and these are arranged serially along a scanty number of terminal branches ending in a remarkably elongated end-plate region. Bickerstaff and Woolf (1960) and MacDermot (1960) confirm these findings and also describe proliferation of minute nerve fibres from the terminal knobs. The specificity of these changes is not yet established (Woolf, 1966). They seem to bear no direct relationship to the severity of the loss of function but it is obvious that myasthenia gravis cannot be considered only in terms of defective neuromuscular transmission. Minor abnormalities of ultra-structure of the end-plate are described by various authors. There is no general acceptance of their significance (Woolf, 1966).

**Thymus Gland**

There are pathological changes in the thymus in 70–80 per cent of patients with myasthenia gravis. Lymphoid hyperplasia of cortex and medulla is
JOHN A. SIMPSON

associated with numerous 'germinal centres' in the medulla (Castleman and Norris, 1949) (Fig. 6d). This is indicative of an active lymph organ and is not an arrest of involution as suggested by earlier observers. Even where neoplastic changes are present, lymphoid follicle formation can usually be identified within the tumour or in the surrounding gland tissue.

A thymoma is usually encapsulated and may be cystic and calcified, but it is sometimes malignant. Invasiveness is limited, spread being usually confined to the thorax and occasionally to the lymph nodes of the neck. An account of the histological types described is unnecessary here and the reader is referred to Iverson (1956). It is, however, of some interest to record that it is only in tumour material that the 'gland' ever assumes the histological appearance of a secretory tissue. In thenon-thymoma cases there is no proliferation of the epithelial cells of the thymus. There is in fact little evidence that the thymus has any endocrinological function. Histologically it has the appearance of a reticulo-endothelial organ actively participating in an 'auto-immune' type of reaction (Simpson, 1960). Similar changes can be produced in the guinea-pig thymus by injection of bacterial antigens and recent work on the thymus supports this hypothesis (Marshall and White, 1961). Occasionally the spleen and lymph glands may be abnormal (Simpson, 1960, 1966a).

Immunological Abnormalities

The antibody against skeletal muscle was demonstrated by Strauss, Seegal, Hsu, Burkholder, Nastuk and Osserman (1960) by an immunofluorescence technique. It is a 7S gamma globulin which binds specifically to the A bands of skeletal muscle. This has been confirmed, using similar techniques, by Beutner, Witebsky, Ricken and Adler (1962); van der Geld Feltkamp and Oosterhuis (1964); and by Djanian, Beutner and Witebsky (1964). The reported incidence varies from 10 to 47 per cent. The titre of antibody is highest in cases of myasthenia gravis with a thymic tumour in which it is nearly always found. Normal sera may show skeletal muscle binding activity but only in titres greater than 1 : 60 (Strauss, Smith, Cage, van der Geld, McFarlin and Barlow, 1966) and they bind to the I bands; A-band staining is seen only with myasthenic sera (Vetters, 1965) (Fig. 8). Fluorescence of subsarcolemmal nuclei is not specific. The specific antibody binding to A band also reacts with certain thymic cells. Previously considered 'epithelial', these are now recognized as myoid cells (Feltkamp-Vroom, 1966).

Correlation between clinical severity and titre of antimuscle globulin is claimed by Weiner and Osserman (1966) and denied by Oosterhuis, van der Geld and Feltkamp (1967). Thus the presence of an immunological disorder involving thymus and muscle is undoubted but its role in the pathogenesis of myasthenia is uncertain. McFarlin, Engel and Strauss (1966) find no evidence that myasthenic serum binds to the neuromuscular
Figure 17.8. Immunofluorescent staining of skeletal muscle and thymus. (a) A-band staining. (b) 'I-band' staining. (c) Myoid cell staining in thymus.
junction. Other antibodies commonly found in sera from myasthenic patients are antinuclear factor, antithyroid and anti gastric substances. Rheumatoid factor may also be present in the blood but does not correlate closely with a history of arthritis (Simpson, 1964, 1966a). These findings support the writer's hypothesis of a multi-organ involvement of immunological type in myasthenia gravis. How this is related to the neuromuscular disorder remains unknown.

Treatment

Thymectomy

The value of thymectomy has been obscured by failure to appreciate that results are different in cases with a thymoma (Keynes, 1955). Simpson (1958) made a personal assessment of the London series, the largest available, and reviewed the other major series with this distinction and showed that there was substantial agreement on the following points. When compared with a medically-treated series, operated cases have a much lower mortality and a greater chance of complete remission or substantial improvement. This is most evident, and the saving in life is greatest, when the duration of myasthenia is less than 5 years and no thymoma is present. After 7 years from the onset there is less chance of improvement from thymectomy though it may still occur. (The risk of death from myasthenia is less after that time whether the thymus is removed or not.) There is a general impression that males do not benefit in the same way as females but the statistical trend is the same although the improved prognosis is less obvious. Without thymectomy women have a slightly poorer prognosis than men; after operation this comparison is reversed. Simpson (1958) confirmed that the prognosis was worse if a thymoma was present, whether it was removed or not (only one case in three was still alive at the follow-up) but nevertheless reported that some patients with thymoma may be benefited by thymectomy to an extent which was as great as in non-tumour cases. These conclusions are supported by a recent survey of 1,355 patients by Perlo, Poskanzer, Schwab, Viets, Osserman and Genkins (1966).

It is not at present possible to select patients who are likely to benefit from operation. Failure of operation to prevent myasthenic death occurs more often in patients requiring a large dose of neostigmine but if they do survive the ultimate state is not apparently correlated with the pre-operative severity. Patients with severe bulbar weakness are most likely to die despite operation. On the other hand, if myasthenia remains confined to the extraocular muscles for 2 years the prognosis for life is so good that thymectomy is not justified (Grob, 1953; Ferguson, Hutchinson and Liversedge, 1955). Schwab and Leland (1953) considered that the younger the patient at the onset of myasthenia the better was the response to thymectomy but this conclusion was influenced by the high proportion of older women with thymomas in their series. Simpson (1958) considered that there was some evidence to support their opinion but the difference in age was insufficient
to influence the selection of cases for operation and duration of illness was the more important factor.

The response to thymectomy is unpredictable. It may be immediate or delayed. The improvement is often so gradual that it is only appreciated in retrospect that the tide turned after operation. The maximum benefit is then seen at about the third year. It is very rare for further relapse to occur. There is often a temporary improvement during the first few days which may be so rapid that it is obvious as soon as the patient recovers from the anaesthetic though no neostigmine has been injected throughout the operation. A previous ptosis may be replaced by lid retraction. Unfortunately the dose of neostigmine may require progressive increase on the third or fourth day but it is extremely important to recognise the extent of the temporary remission as during this period a previously ineffective dose of neostigmine may cause a cholinergic crisis. It is the writer's opinion that many post-operative deaths have been caused in this way. It is essential that a physician experienced in the care of myasthenics should co-operate closely with the surgeon throughout, but especially at this time.

Radiotherapy

Radiation of the thymus is not a satisfactory alternative to surgery. Its value is doubtful and there is a risk of temporary exacerbation which contraindicates its use in the patient who is 'unfit for surgery'. Keynes (1955) stresses the desirability of pre-operative radiotherapy before removal of a thymoma and Perlo, Schwab and Castleman (1966) agree that it increases the duration of post-operative survival but this aspect requires further appraisal. The writer doubts if it is necessary or valuable.

Carotid Sinus Denervation

In view of the early favourable reports of an anti-myasthenic action of corticotrophin and some evidence from animal experiments that adrenocortical hypertrophy occurred after bilateral denervation of a carotid sinus, French workers have carried out this operation on myasthenic patients (Thévenard, 1954). The favourable effect claimed is not seen for about a year. Mertens (1955) was not impressed with his results and the operation has not found favour, since improvement (if indeed it is attributable to the operation) is apparently inferior to that following thymectomy. It does not seem rational to seek atrophy of the thymus by such an indirect method when thymectomy is such a safe operation in experienced hands.

Medical Treatment

The patient will usually require treatment with anticholinesterase drugs for at least a period after operation but it is desirable to reduce the dose slowly but steadily. Many patients are unwilling to give up the drug they have come to depend on but the writer has the impression that the long-term prognosis is best for those who are gradually able to withdraw.
medication, though there is no evidence to support Harvey (1948) in his opinion that the use of neostigmine prevents remission. Many patients are best treated by medical means and the response is usually very gratifying. Nevertheless, even in an early case the response is rarely complete and the extra-ocular muscles in particular often cease to respond, no doubt due to the development of myopathic changes (Keynes, 1961). Rowland (1955) has drawn attention to a group of patients with 'clinical myasthenia gravis' showing little or no response to neostigmine. Their nosological position is still obscure.

Various drugs with anticholinesterase activity are available. Some may have additional actions on the end-plate directly or on pre-synaptic release of acetylcholine. They will be discussed in order of duration of action.

**Edrophonium Chloride ('Tensilon').** This cholinergic drug may be given intramuscularly or intravenously when it has a prompt action which is valuable as a diagnostic test (Osserman and Kaplan, 1953). Its maximum effect is completed in 5 minutes and, although some effect can still be demonstrated 20–30 minutes later, this is too brief for therapeutic purposes. Details of this test will be found in the section on pharmacological tests.

**Neostigmine Bromide ('Prostigmin').** This drug has stood the test of time. The 15 mg. tablet has a cholinergic activity which varies from 2–6 hours in different patients. The total and the spacing of doses must be established by individual trial and may vary from ½ tablet t.d.s. (22.5 mg/day) to three tablets every 2 hours (540 mg/day). Exceptional cases requiring even bigger dosage have been reported but an average dose is ten tablets daily (150 mg.) (Simpson, 1958). Neostigmine has the advantage of rapid action which gives the patient the feeling of a 'surge of power', but the loss of strength when its effect wears off may be equally rapid and many patients find this let-down disagreeable since the timing of the next dose must be judged very accurately. It is usual practice to take some of the tablets 30 minutes before a meal when there is bulbar weakness. Absorption is erratic in some patients and it is then necessary to rely on subcutaneous injection of neostigmine methylsulphate. The dose may be calculated on a basis of 1 mg. by injection having an effect equivalent to 15 mg. orally.

**Pyridostigmine Bromide ('Mestinon').** This drug avoids the main disadvantage of neostigmine and is preferred by the majority of patients (Simpson, unreported controlled trial). The duration of activity is sometimes longer than neostigmine (3–8 hours) though the difference is less marked than originally reported. Its main advantage is that its activity wanes more slowly, thus allowing a sustained level of activity to be maintained by regular 3–8 hourly dosage (the frequency must again be established by trial). Some patients miss the 'boost' of neostigmine and are best treated with a combination of the two drugs. The 60 mg. tablet of pyridostigmine is approximately equivalent in action to the 15 mg. tablet of neostigmine. The slightly longer
action makes it a useful drug for overnight medication, but when pyridostigmine is used as the sole medication care must be taken to avoid cumulative effect.

**Slow Release Neostigmine and Pyridostigmine.** In an attempt to prolong the action of these drugs, tablets containing an attenuated outer layer and concentrated core have been on trial in the U.S.A. Preliminary reports are promising, especially for the elimination of the need of frequent interruption of sleep, and cumulative effects have not been prominent (Schwab, Osserman and Tether, 1957).

**Ambenonium Chloride (WIN-8007, Mysuran, Mylelase).** The drug is dispensed in 10 mg. and 25 mg. tablets, the latter being approximately equal in potency to 15 mg. of neostigmine. The duration of action is slightly longer than that of 60 mg. pyridostigmine. Muscarinic side-effects are less frequent than with neostigmine or pyridostigmine but central reactions are more common and the onset of cholinergic crisis may be more difficult to detect.

**Bis-neostigmine Compounds (BC-40 etc).** The cholinesterase inhibiting power of neostigmine may be increased and prolonged by combining in one molecule two neostigmine radicles separated by a polymethylene chain of various lengths, Pateisky, Herzfeld and Stumpf (1955) and Herzfeld Kraupp, Pateisky and Stumpf (1957) have investigated the actions of BC-40 (Hexadistigmin), BC-47, BC-48, and BC-51 and report durations of activity exceeding one day. The Vienna group have found BC-51 the most useful of the series but noted wide variation in absorption when the drugs were given orally. An average oral dose of BC-51 is 7·5 mg. every 4 days. More frequent dosage is hindered by cumulative effects, yet the carry-over is inadequate and neostigmine is usually required in addition. In the writer's limited experience the cumulative action which this duration entails has made these drugs unsatisfactory for domiciliary use.

**Alkyl-Phosphates.** This group of anticholinesterase preparations which includes di-isopropylfluorophosphate (DFP), tetrachlylypyrophosphate (TEPP), hexamethyltriphosphate (HETP), and octamethylpyrophosphoramid (OMPA), had a vogue in the treatment of myasthenia because their long duration of activity was considered desirable. This very property leads to inflexibility of control. Furthermore they have a greater effect on central synapses than the quaternary ammonium compounds, giving rise to headache, nightmares, and personality disturbance. Some workers are still using TEPP but there is a general tendency to abandon these drugs (Westerberg and Magee, 1955).

**Adrenocorticotropic Hormone (ACTH).** The use of ACTH in myasthenia gravis was suggested because of its effect in shrinking thymic and lymphatic tissue. Torda and Wolff (1951) reported remissions of myasthenia following its use but the drug is rarely prescribed because initial deterioration is common (and may be fatal) and the favourable response may not occur until the drug is withdrawn (Westerberg and Magee, 1955). Both effects may be
considered to support the auto-immune hypothesis. Cortisone and its derivatives are much less effective and have no place in practical therapeutics.

**Immunosuppressive Drugs.** Delwaide, Salmon and van Cauwenberge (1967) report improvement of myasthenia after treatment with azathioprine.

**Aldosterone Inhibitors.** The use of spironolactone (400 mg. daily) was suggested by Gottlieb and Laurent (1961) to potentiate neostigmine by conserving potassium. Evaluation is not yet possible but preliminary experience suggests that it is of limited value though it gives "a sensation of well-being and self-confidence".

**Potassium Chloride.** Though useless as a primary therapy, potassium has a role as an adjuvant in dosage of 5 gr. b.d. or q.i.d. (Laurent and Walther, 1935). The nausea and diarrhoea which it may cause may be difficult to distinguish from cholinergic crisis.

**Ephedrine Sulphate.** This drug, introduced by Edgeworth (1930) from her personal experience, is still used as an adjuvant in a dose of 25 mg. t.d.s. by mouth. Its value is questioned and its mode of action unknown (it may be an Orbeli effect), but many experienced clinicians continue to use it.

**Guanidine Hydrochloride.** This drug has a limited anti-myasthenic action attributed by Desmedt (1956) to sensitisation of chemoceptors to acetylcholine. It is given orally in a dose of 20-50 mg. per kilogram body weight, divided into three doses. It tends to cause paraesthesiae (Dodd, Riven and Minot, 1941). It is now rarely used for the treatment of myasthenia gravis but it is effective in the management of carcinomatous myasthenia (Lambert, 1966).

**Germinc Diacetate.** Flacke, Caviness and Samaha (1966) report that this veratrine alkaloid is as effective as anticholinesterase drugs. It causes iterative firing of muscle fibres.

**Galanthamine Hydrobromide** (*Nivalin*). This tertiary amine isolated from the Bulgarian snowdrop is an anticholinesterase which is gaining favour in the U.S.S.R. It is said to have a duration of action comparable to neostigmine (Uzunov, 1966).

**d-Tubocurarine.** Churchill-Davidson and Richardson (1957) and other workers have given paralysing doses of d-tubocurarine (while sustaining respiration mechanically) to patients who have become resistant to neostigmine in the belief that the end-plates may thus be rested and recover their sensitivity to neostigmine. The writer has used it to treat cholinergic crisis but it is difficult to judge the dose required.

**Myasthenic Crisis**

Sudden deterioration in the myasthenic patient's condition may occur because of unusual physical exertion, emotional upset, an infection, or childbirth, or simply by progression of the disease. Drugs which may cause neuromuscular block such as streptomycin and neomycin should be used with caution and it is best to avoid curare, quinine, quinidine, chloroform, morphine, or ether. ACTH, corticosteroids, thyroid compounds,
sedatives, and other respiratory depressants should be used with care (Osserman, 1958). The use of an enema has precipitated fatal myasthenic crisis and this should be remembered in the preparation for thymectomy. The potassium depletion caused by an enema may be responsible.

The absence of cholinergic signs and the presence of favourable response to the edrophonium test (see p. 556) indicate the cause of the severe weakness. It is necessary to increase the dose of anticholinesterase medication and the only suitable method in emergency is intramuscular injection of neostigmine. It is necessary to control respiration and there should be no hesitation in performing tracheostomy if pulmonary ventilation is failing or there is severe dysphagia. This procedure should never be delayed as once respiration is safe, treatment can proceed methodically without panic measures, the dose of neostigmine being regulated by repeated edrophonium titration (Osserman and Kaplan, 1953).

Cholinergic Crisis

Mild muscarinic effects of anticholinesterase medication (colic, diarrhoea, belching, nausea) are not uncommon in myasthenic patients though less prominent than in normal subjects taking the same dose. More severe muscarinic signs such as vomiting, sweating, hypersalivation, lachrymation, miosis and pallor are less common and indicate that the dose is nearing a dangerous level. The most valuable indication of impending danger is the size of the pupil. It should not be allowed to contract to less than 2 mm. diameter in normal room lighting. Bradycardia is very unusual with oral medication but may be prominent and lead to cardiac arrest with intravenous medication. Hypotension occurs with severe cholinergic poisoning. In the most severe cases confusion and coma indicate block of cerebral synapses. The use of an antagonist such as atropine sulphate (0·3–0·6 mg) is obligatory with intravenous dosage but need not be given if the cholinergic drug is administrated orally or by subcutaneous injection unless colic is intolerable. The disadvantage of suppressing the muscarinic symptoms is that more serious nicotinic signs may be overlooked (Schwab, 1954). There is no evidence that atropine inhibits the nicotinic signs. The earliest of these is fasciculation of muscles. This need not be a serious sign since it will first appear in muscles unaffected by myasthenia. Persistent fasciculation in the leg muscles is consistent with excellent clinical control. Conversely, a depolarisation block may be reached without preceding fasciculation, or the latter may be so transient as to be overlooked. A muscle may pass from myasthenic weakness to cholinergic block without passing through a stage of normal strength. Poisoning has reached a dangerous level (‘cholinergic crisis’) when weakness increases because of depolarisation block. This may be difficult to recognise and undoubtedly accounts for the majority of cases of ‘neostigmine resistance’ which cannot be attributed to myopathic change. It has not been emphasised that different muscles will reflect their degree of myasthenic involvement. Thus some muscles may suffer cholinergic
block while others still require further anticholinesterase medication. Since the muscles of respiration are often relatively spared by myasthenia they may be blocked by a dose of neostigmine which is insufficient for the ocular or limb muscles. It is extremely important to measure the effect of a test dose of edrophonium on the respiratory and bulbar muscles as well as on the more easily tested muscles. Even though short acting, the additional cholinergic effect of edrophonium may be fatal in cholinergic crisis. In these circumstances atropine should be injected first and there should be facilities for immediate assisted respiration. The test is described on p. 556.

Cholinergic paralysis requires urgent treatment. A cuffed endotracheal tube should be passed at once and positive-pressure respiration started. Tracheostomy may be necessary if this has to be prolonged. Atropine sulphate should be injected intravenously 2 mg, every hour until signs of atropine toxicity develop. Specific antidotes for anticholinesterase poisoning are not satisfactory in clinical practice. Drugs of the oxime group have some effect on overdosage of quaternary ammonium anticholinesterases (Grob and Johns, 1958). Personal experience is limited to pyridine-2-aldoxine (2-PAM) and methane sulphonate (P2S) but their latency has been found to be too long and their potency and duration of action inadequate for satisfactory treatment (Simpson, 1961). Physiological antagonism can be obtained by the use of d-tubocurarine. The author has used this form of treatment of cholinergic crisis but in view of the difficulty in judging the necessary dose it is not recommended unless respiration is artificially controlled. In these circumstances there is little need for an antidote other than atropine to protect the cardiovascular system. Controlled respiration and repeated atropine injection pending the recovery of neuromuscular response is the most satisfactory form of treatment at present available. Anticholinesterase medication should not be resumed until there is a clear 'myasthenic type' of response to edrophonium on two successive occasions at intervals of 1 hour. On resumption, neostigmine should be given by injection and an adequate dose discovered by trial, guided by edrophonium testing. Only when this has been done should oral medication be resumed, at first with neostigmine and then with longer-acting drugs by cautious substitution and prolongation of dose-interval.

**Differential Diagnosis**

Myasthenia gravis is commonly mistaken for hysteria because it is so often precipitated by emotional disturbances, and physical signs may be absent if the patient has rested before examination. The intermittent nature of the symptoms and the frequent occurrence of diplopia and dysarthria or other bulbar symptoms may suggest multiple sclerosis. Motor neurone disease, Parkinsonism, polyneuritis, and endocrine disorders, particularly thyrotoxicosis, may cause weakness which increases with effort and hypokalaemic states, periodic paralysis, paroxysmal myoglobinuria, craft palsies and other disorders causing transient paralysis may be confused with
myasthenia gravis. Symptomatic myasthenia in polymyositis, systemic lupus erythematosus, and other ‘collagen’ diseases may give a positive Jolly test but the response to edrophonium or neostigmine is never so marked as in myasthenia gravis and tends to disappear in a few weeks (Simpson, 1966c). Carcinomatous myasthenia tends to spare the bulbar muscles, areflexia is common, and the response to neostigmine is not sustained. The recruiting EMG response to indirect faradisation is not confined to carcinomatous myasthenia but is highly suggestive of it (see p. 555).

The most difficult disorder to differentiate from myasthenia gravis is the condition termed 'pseudoptosis' and the congenital syndromes with facio- and extra-ocular palsies including congenital ptosis, ocular myopathy and the Von Graefe-Moebius syndrome. None of these conditions respond favourably to anticholinesterase drugs. It is, in fact, rare for one of them to be seriously confused with myasthenia gravis. The more common error is to fail to recognise that disease when it is present, but once myasthenia is considered the diagnosis is rarely in doubt.

Acknowledgements

Figure 2 is reproduced by courtesy of the editor of *Nursing Times* and the other figures by courtesy of the editor of the *Scottish Medical Journal*. Dr. J. M. Vetter kindly provided Fig. 8.

References


JOHN A. SIMPSON


Myasthenia gravis occurs in all races with a modal age of onset about twenty years, mainly female except in late onset cases where males predominate. Thymoma is rare under the age of thirty but is present in 30% of cases starting over forty. Prognosis is poorer if there is a thymoma, regardless of treatment.

There is an "active stage" of 5-7 years during which most deaths and most remissions occur. Thymectomy is most beneficial during this period but is still worth trying in longer cases failing to respond adequately to medical treatment. (Simpson 1958).

Associated diseases include thyroid disorders, 'rheumatoid' arthropathy, pernicious anaemia, and a list of disorders having in common an 'autoimmune' disturbance. Drawing attention to this, Simpson (1960) suggested that myasthenia was due to immunological damage to muscle and that the thymus was involved in this way rather than by endocrine activity. The pathology of the thymus and muscle supported this proposition.

The beneficial results of early thymectomy were reviewed and shown to apply to non-tumour cases. In pre-operative care it is essential to avoid enemas, and some drugs may increase weakness e.g. curare, quinine, quindine, neomycin, streptomycin, polymyxin-B, chlorpromazine, ether and chloroform. Respiratory depressants should be avoided.

Tracheostomy is unnecessary for operation, the dose of neostigmine etc. should be continued up to and after the operation. Beware of diminished requirements about 48 hours after operation (danger of cholinergic crisis).

Differential diagnosis of myasthenic and cholinergic crisis was discussed and attention drawn to special diagnostic difficulties in presence of under- or over-ventilation. Most consistent sign of cholinergic state is pupil less than 3 mm in room lighting.

Use of Tensilon test in discriminating was discussed with management of crisis. In cholinergic crisis withdraw drugs, control ventilation and wait until Tensilon test becomes positive (emphasis on objective signs). Give atropine in big doses. Oximes of little value as antidotes. Atropine should be kept for this purpose, not given routinely.

Research work on the disorder of transmission was briefly reviewed. Theory of pre-junctional abnormality (Desmedt, Elmquist) and post-junctional abnormality (Croob et al, Churchill-Davidson) probably both unsatisfactory. All findings accountable by deformed end-plate structure shown by supravital staining.
Myasthenia Gravis—The Present Position

J. A. SIMPSON: For many years myasthenia gravis was the only disease believed to be closely connected with the thymus. There have been four eras.

1900–40 Clinico-pathological correlation: The high incidence of thymoma was recognized. Argument about the nature of the thymic abnormality in the remainder—hypertrophy, failure to atrophy. Castleman emphasized the importance of germinal centres. The production of curare-like substance was postulated. The main stream of medicine and experimental biology considered the thymus as an endocrine gland with a possible function in the growth of the young animal.

1940–60 Thymectomy: Acrimonious debate of value was resolved in favour of Keynes by Simpson (1958), who reviewed all large series and showed therapeutic value if (a) thymectomy was performed during the 'active' stage of the disease, and (b) thymoma was not present.

1960–68 Autoimmunity: Simpson (1960 et seq.) drew attention to associated disorders of other organs and suggested that all known clinical and pathological data pointed to an immunological disorder in myasthenia with the thymus as the important regulator. He was rapidly supported by the independent work of Strauss et al. (1960) and others who demonstrated anti-muscle antibodies. Miller (1961) confirmed the immunological role of the thymus—mechanism debatable.

1968—Biology of growth and repair: Renewed work on the thymus (Szent-Györgyi et al. (1962) and others) suggests the fundamental place of thymic immunological mechanisms in the control of normal growth, tissue differentiation, and repair.
The thymus has always been an intriguing organ - dismissed with the pineal gland and the vermiform appendix as a vestigial organ without recognized function in the human adult. Like these other organs it has been the source of a medical mythology which no doubt obscures some genuine facts.

Galen, in the 2nd century A.D., considered that it was present to provide padding between the superior vena cava and the sternum. In the 15th century Vesalius had to conclude that the function of the thymus was a mystery. In the 17th century Glisson believed that it was concerned with the growth of the foetus. This concept was supported in the 19th century by Astley Cooper and a related theory persists to this day.

Since the thymus is relatively (though not absolutely) larger in growing children than in adults it is not surprising that it has been considered to produce a growth hormone. This work was reviewed by Cameron (1945) and need not be recounted here as all claims made in the era 1920-40 were subsequently denied by other workers. Nevertheless one aspect of the work from this era may justify reappraisal. Rowntree (1935) gave daily intraperitoneal injections of acid watery extracts of young calf neck thymus to successive generations of rats. A growth acceleration associated with precocious differentiation of tissues was observed in the first seven generations only. This work seems to have been abandoned since Segaloff and Nelson (1940) and others were unable to confirm it.

From 1940 to 1960 published work falls into two classes. Many workers continued to study the role of the thymus in growth and its interrelationship with the endocrine system of which it was assumed to be an organ. The most prolific writer in this field is Comsa (1958a,b) who concluded that the
thymus mediates the growth effect of the anterior pituitary and suppresses the activity of thyroxine on target tissues such as muscle. Gyllensten (1953) is highly critical of Comsa's earlier work on statistical grounds but confirms that the thymus is a target organ for the thyrotropic hormone and has power to inactivate it. In his view there is a balance between the growth of thymic and lymphatic tissue which is regulated by the pituitary-thyroid axis. This conclusion converges with the second class of research during the same period.

The concept of a 'thymolymphatic' system acting together in infections, though not in exactly the same manner, was widely discussed more than thirty years ago. (Hammar, 1931). One difficulty in the acceptance of a reticuloendothelial role for the thymus was its apparent failure to produce antibodies. This objection became less valid when it was shown that lymphocytes entering the blood stream from the thoracic duct do not contain antibodies but are capable of producing them in vitro (Wesslén, 1952).

Since the thymus contains epithelial as well as lymphoid cells, any 'growth' and 'immunological' functions need not be linked, but Metcalf (1956) isolated a cell free factor from thymic epithelial cells which acted as a 'lymphocyte stimulating factor'.

This was the position in the years 1953-59 when I was investigating the clinical aspects of myasthenia gravis and establishing that thymectomy had a definite value in its treatment (Simpson, 1958). There seemed to be no connection between the clinical facts and the mainstream of biological work on the thymus. It is now necessary to take a synoptic view of the position that had been reached in that field.

1900-1940 Clinico-pathological Correlation

In 1901 Laquer & Weigert described a case of myasthenia gravis in
in which there was a thymic tumour. Bell (1917) and Norris (1937) reviewed the literature at different times and established that Weigert's case was not unique. They found that 45-48% of the reported cases had obvious thymus abnormalities and about 30% of these (15-20% of all cases) were thymic tumours. The remaining abnormalities were described as 'hyperplasia' and Norris (1937) considered that the lymphoepithelial thymoma associated with myasthenia was not a true neoplasm but rather an extreme form of hyperplasia.

At that time the mere persistence of the thymus in adult life was considered to be abnormal since the gland was generally assumed to atrophy during childhood though Hammar (1906) had found a wide variation in size and cellularity of the gland in non-myasthenic children and adults. Boyd (1936) and Sloan (1943) found that the thymus never underwent complete involution. Sloan (1943) pointed out that the thymus from myasthenic patients was not distinguished from the normal size (thymomas excluded) but by histological change. He noted lymphoid hyperplasia in both cortex and medulla of the gland and in particular the frequent presence of 'germinal centres' in the medullary portion of the gland.

The germinal centre (fig.1) consists of reticular cells, large lymphocytes and macrophages. Frequent mitotic figures indicate active cell division and the centre is considered to be forming lymphocytes (or thymocytes). The germinal centre is surrounded by darker staining, more mature small lymphocytes. The close correlation between thymic germinal centres and myasthenia gravis was confirmed by Bratton (1948), Castleman & Norris (1949) and Ringertz (1951). According to Castleman (1955) this appearance is present in 68% of myasthenic patients without thymoma.

Thymic tumours are classified according to cell type (Castleman &
Norris, 1949; Iverson, 1956; Lattes, 1962). Common to all types and differentiating them from the non-tumour type of pathology is hyperplasia of epithelial elements, though the lymphocytes (thymocytes) may be the main proliferating cell type. Myasthenia gravis is usually associated with a lymphoepithelial thymoma. It has never been reported with a predominantly spindle cell type. The tumour is usually encapsulated but may be locally malignant within the thoracic cavity. Spread may occur by lymphatics to the neck or diaphragm, or to the axillary and coeliac glands and to the omentum (Keynes, 1955). They do not metastasize by the blood stream.

The type of tumour, benign or malignant, seems to be of no consequence so far as the occurrence of myasthenia gravis is concerned. It may be more significant that many observers have noted germinal centres within the tumour or in the surrounding 'normal' thymic tissue beyond its capsule (Castleman & Norris, 1949). These observations led to the conclusion that the epithelial cells of the thymus were responsible for the myasthenic syndrome. Since the endocrine function postulated at that time seemed irrelevant, the conclusion was reached by most workers that the abnormal gland must produce a 'myasthenic toxin' related to curare.

A resemblance between myasthenia gravis and curare poisoning had been recognized since the 19th century but became the dominant hypothesis in the 1930's when the therapeutic action of anticholinesterase drugs was established. There have been many attempts to isolate a curare-like substance from the blood of myasthenic patients but none stands up to rigid scrutiny (Nastuk et al, 1959). In my opinion, all the known facts relevant to neuromuscular transmission in myasthenia can be explained by the physical structure of the endplate (Simpson 1960, 1968a) but the possibility of a myasthenic toxin released from a damaged thymus is still postulated (Goldstein & Whittingham,
1966). The concept was supported by the claims of Wilson et al (1953) to have extracted a neuromuscular blocking substance from thymuses removed at operation from myasthenic patients, the potency of the extract being greatest in glands from those patients who derived greatest benefit from thymectomy. Wilson and his colleagues made further studies on extracts from human and foetal whale thymus glands. Quaternary nitrogen compounds have been isolated which could interfere with the synthesis of acetylcholine but none has produced competitive block of neuromuscular transmission which is reversible by neostigmine (Nowell et al, 1959; Nowell & Wilson, 1962).

No supporter of the thymic curare-like toxin theory has been able to account for the fact that myasthenia may continue after thymectomy or even occur for the first time after removal of a thymoma except by the unsubstantiated assertion that there must have been ectopic thymic tissue remaining.

**1940-1960 - Value of Thymectomy**

It may be questioned whether the abnormal thymus is causally related to myasthenia or is, like thyrotoxicosis, an associated endocrine disorder. If it could be demonstrated that removal of the thymus relieved, arrested or cured myasthenia gravis it would be necessary to consider that it is causally related. Unfortunately the evidence in the 1940's seemed too equivocal to justify conclusions. American experience was unfavourable whereas Keynes in London published a series of papers in favour of the operation (Keynes, 1955). An independent assessment of 294 thymectomies (mainly by Keynes) and 110 non-operated cases of myasthenia supported Keynes' claim and showed that the published results from Baltimore, Boston and the Mayo Clinic were similar if their data were reclassified to differentiate between those with and without thymoma (Simpson, 1958). Failure to make this distinction,
which Keynes had insisted on, had obscured the value of the American operations on non-tumour cases. The prognosis for myasthenia is much worse in the presence of a thymic tumour and remains poorer even after removal of a tumour. Furthermore, the prompt improvement which might be expected from removal of a source of curare-like substance is only rarely seen. Simpson (1953) drew attention to the aspects of the natural history of myasthenia gravis indicating an 'active stage' of 5 - 7 years, and a later stage of non-progressive disorder of neuromuscular function, sometimes ending in a 'burned out' phase. Thymectomy was most beneficial during the 'active stage', suggesting that the thymus gland controlled the disease process but did not directly produce a neuromuscular blocking substance.

1960-68 - Autoimmunity

A personal study of 440 cases of myasthenia gravis from 1956 to 1959 showed that certain disorders of other systems occurred rather frequently in myasthenic patients. These included all thyroid diseases (not just thyrotoxicosis), diabetes mellitus, a 'rheumatoid' type of arthropathy, acrocyanosis, pernicious anaemia, reticulosis and hyperglobulinaemia. The hypothesis was suggested that myasthenia gravis was a multisystem disease associated with a breakdown of immunological tolerance (Simpson, 1960). Smithers (1959) had observed that the germinal centres of the myasthenic thymus suggested an autoimmune process and, independently, Strauss et al (1960) demonstrated the presence in serum from myasthenic patients of a complement-fixing globulin which appeared to be an antibody against muscle fibres.

Later contributions have been reviewed elsewhere (Simpson, 1966; Strauss et al, 1966) and the clinical and immunological observations have
been confirmed by other workers but a short discussion of the biological implications is necessary since the three contributions differ in their heuristic value. Smithers' observation does not lead directly to any conclusion about the neuromuscular disease. On the other hand the findings of Strauss and his colleagues did not imply any role for the thymus. A later finding by van der Geld et al (1964) that serum reactive with muscle might also react with thymic epithelial cells, some of which are myoid cells (Feltkamp-Vroom, 1966) leads to the speculation that the muscle antibody is a reflection of thymic disorder which also produces a circulating neurohumoral inhibitor (Strauss et al, 1966). A variant of this is that the thymus disease is itself an autoimmune thymitis as a response to antigen originating in skeletal muscle or thymic myoid cells (Goldstein, 1966). Neither of these theories would lead to the prediction of immune reactions against a variety of tissues of which muscle need not be the first; nor would they account for a familial relationship with thyroid disease (Simpson, 1960). The observations of Simpson (1960, 1966) and later workers cannot be accounted for by any 'leak' hypothesis of induced autoimmunity but demand a generalised breakdown of immunological tolerance.

The original hypothesis (Simpson, 1960) had to be formulated in tentative terms as an immunological function for the thymus was not generally accepted until the publication of the important studies by Miller (1961). Although at the time of writing there is still controversy about the exact mechanism, there can no longer be any doubt that the thymus plays an important regulating role in immunological reactions.
Biology of growth and repair

It is now necessary to return to the pre-1960 work on the thymus which has been widely neglected in the excitement generated by the findings of Miller and other immunologists. Surely it would be more profitable to attempt a synthesis than to reject the work on growth which indicated a role in foetal and neonatal life linked with the anterior pituitary and other endocrine glands. The factors which precipitate myasthenia gravis (emotional stress, infection, pregnancy), the genetic factors, and the associated endocrine disorders suggest that the thymus may be controlled by a hypothalamo-pituitary mechanism which is genetically determined (Simpson, 1960). The thymus might play a role in tissue differentiation during embryogenesis of which the control of blood cells and plasma proteins is a fraction which survives after birth. If organs are to develop normally it is obvious that there must be some mechanism for recognizing and inhibiting or destroying abnormal cells, for arresting growth, and for promoting the breakdown, scavenging, and replacement of cells damaged by 'wear and tear'. This could well be the fundamental role of the immunological mechanism and its role in prevention of infection an adaptation which has been selected for its survival value. A similar idea is implicit in the suggestion of Burnet (1962a) that the thymus is primarily concerned with the maintenance of the chemical integrity of the body, including the mechanism of specific protein synthesis, and that the development of the immunological competence of the body is part of the process of morphological and functional differentiation of the embryo and young animal. Weiss (1950) and Burwell (1963) have developed alternative theories of control by the reticuloendothelial system of cellular growth and differentiation. Recently Szent-Györgi et al (1962)
claim to have isolated two factors from normal calf thymus. One of these, 'promine', is said to be a growth-promoting hormone and the other, 'retine', a growth-inhibiting factor. Confirmation of this work is eagerly awaited because disturbance of a breakdown/regeneration cycle at the motor nerve terminals could account for the phenomena of myasthenia gravis (Simpson, 1968). It will be interesting to see whether it is under pituitary control.

Much of this is, in the nature of things, speculative. The 1960 paper was described as 'a new hypothesis', since it could not be dignified as a theory, and closed with a quotation from Hughlings Jackson.

'The use of hypotheses is the method of science. To suppose we can make discoveries by the Baconian method is a delusion. A hypothesis or supposition is not a conclusion; it is only a starting point for methodical observation and experiment, the endeavour being not only to prove it, but to disprove it.'

The earlier hypothesis led to the recognition that certain diseases are associated with myasthenia gravis and a prediction that antibodies would be found against more tissues than muscle was confirmed (Simpson, 1966). The inferences and suggestions for experiments outside of the field of immunology have not been exploited. These are the main justifications for restating the hypothesis. Myasthenia gravis still holds clues to the function of the thymus.
Myasthenia Gravis: Clinical Aspects

The first time I addressed this Society on the subject of myasthenia gravis was to give a preliminary report on an evaluation of thymectomy (Simpson 1956). That work gave me the chance to examine more than 400 patients with myasthenia in a comparatively short period of time. This provided a golden opportunity to study the natural history of the disease and its rarer manifestations. Some curious facts appeared which led me to propose that the neuromuscular disorder was only part of a multisystem disorder, probably based on a breakdown of immunological tolerance (Simpson 1960).

Time does not permit a review of all the evidence which led to this hypothesis and I shall only summarize some parts which seem to be relevant to the interests of this Section.

Natural History

Myasthenia gravis occurs at all ages but the modal age of onset is about 20 years, with a secondary peak for males in the fifth decade. Below that age females outnumber males 4:5:1, but in later life males predominate. The onset may be insidious or sudden. It is often precipitated by an emotional upset, and this fact accounts for the common failure to make the diagnosis in the first years, as the dramatic but reversible weakness is readily mistaken for hysteria. Infections or pregnancy are also precipitating factors – rarely physical exercise. A tendency to have remissions is well known, but prolonged complete remissions are in fact not common and rarely repeated. Most of the 'useful' remissions occur during the first seven years. Conversely most of the deaths due to progressive myasthenia also occur in the same period. The highest mortality is in the first year, with a second danger period from four to seven years after the onset (Simpson 1958). Most of the beneficial response to thymectomy is seen in patients operated on during this same period, which appears to be an active and labile stage of the disease. After that period remissions are less complete, thymectomy is less valuable, and there may be less response to anticholinesterase drugs. On the other hand, deterioration is less rapid or stops and deaths are fewer (though risk of asphyxia from aspiration may persist). One has the strong
impression that about the 5-7 year point the active disease process subsides, leaving more or less permanent sequelae.

I will not discuss the well-known symptoms of neuromuscular disease, but only draw attention to the distribution of weakness when a large population is studied. Certain muscle groups are more likely to show the earliest signs and the ranking order is the same if one lists the total incidence of involvement - extracocular, bulbar, neck, limb girdles, distal limbs and trunk. I draw attention to this because it is quite different from the distribution of a peripheral neuropathy of axonal type and much closer to the distribution of a myopathy. A clinical observation of this type must be kept in mind when deciding between prejunctional and postjunctional mechanisms.

**Associated Disorders**

Most people are aware that there is some connexion between myasthenia gravis and thyrotoxicosis. Studies of small numbers of cases have led to false conclusions such as a presumed 'see-saw' relationship between the two. In fact in my series of 510 cases it is obvious that all one can conclude is that both diseases may occur at some time or another in the same individual, with no obligatory temporal relationship. Furthermore, thyroid disease appears more often than one would expect in close relatives of myasthenic patients (Simpson 1960). Interestingly enough, if the earlier literature is reviewed it will be seen that there are more reports of 'lymphadenoid' goitre than of thyrotoxicosis (Ringerz 1951). In my experience nontoxic goitre and myxedema occur quite commonly, giving a total incidence of thyroid disease of 9% in males and 18% in females suffering from myasthenia gravis (Simpson 1958). Radioiodine studies show that the majority of myasthenic patients have normal thyroid function and there are as many substhroid as hyperthyroid among those without clinical manifestations of thyroid disorder (Simpson 1966b). Clinical or latent Hashimoto's thyroiditis is not uncommon and may develop after thymectomy (Simpson 1964).

The role of the other endocrine glands is less certain. Glycosuria or diabetes mellitus appear to be a little more common than expected. Premenstrual exacerbation of symptoms is common, and pregnancy has a definite though variable effect. Exacerbation usually occurs during the first trimester with later remission followed by relapse in the puerperium (Fraser & Turner 1953), but the course may be different in successive pregnancies. My study of the patients in the thymectomy series first drew attention to a relationship between myasthenia gravis and other disorders, notably an arthropathy resembling rheumatoid arthritis, pernicious anaemia, and other disorders now recognized as being associated with disorder ed immunological mechanisms (Simpson 1960). These findings have been confirmed by other workers (Downes et al. 1966). In postulating an immunological basis for the syndrome I was influenced by seven facts:

1. The age and sex incidence, the remitting course and the observed precipitating factors bore a marked resemblance to systemic lupus erythematosus (Harvey et al. 1954).
2. My observations suggested a multi-organ disorder.
3. The characteristic muscular pathology - the lymphorrhage - suggested an 'allergic' reaction. It had previously been discounted as 'nonspecific' because lymphorrhages were found in other conditions but all of these were disorders in which current theory was implicating 'autoimmune' disturbances.
4. It appeared to me that the thymus was more like an active lymphoid organ than an endocrine gland as it was then considered, and this conviction was enhanced by Smithers' (1959) paper.
5. Cortisone may cause remission after temporary relapse.
6. The infant born to a myasthenic mother may have myasthenic weakness. It does not occur in every pregnancy and the child is only affected for 4-8 weeks. Despite this, nobody has ever succeeded in making another adult myasthenic by transfusion of blood from a myasthenic patient. Obviously this could be accounted for if the 'toxin' presumed to cross the placental barrier was an antibody, and the duration of the neonatal disorder fitted rather well with this concept.
7. While my findings were being prepared for publication Nastuk et al. (1959) reported that myasthenic serum appeared to lyse frog muscle cells and this appeared to be a complement-fixing reaction.

From 1956 my colleagues in immunology had searched unsuccessfully for antimuscle antibodies, and we had failed to produce autoimmune muscle disease in mice. Working independently from the cytolytic effect Strauss et al. (1960) were able to demonstrate antimuscle antibodies in myasthenic serum. The volume of supporting work is now too great to review here (cf. Ann N. Y. Acad. Sci. 1966, 135, Art. 1). The multi-organ involvement is confirmed by the increased incidence of antibody against thyroid and stomach (Simpson 1966b). There is a significant incidence of hypergammaglobulinemia which tends to correlate with a thymoma, rheumatoid arthritis, Hashimoto's disease or pernicious anaemia (Simpson 1966b).
For the first time a logical connexion between the thymus and muscle has been established. It has been known for nearly sixty years that the thymus is usually abnormal in myasthenia gravis and that 10–15% of cases have a thymic tumour, the latter cases being clinically more severe. Now it has been shown that the titre of antimuscle antibody is greatest in the presence of a thymoma but that it need not necessarily be associated with clinical or latent myasthenia (Strauss et al, 1966).

Thus the present position is that there is no doubt that disordered immunological tolerance is common in myasthenia gravis but no evidence as to whether it is primary or secondary. When in 1960 I proposed this hypothesis Miller had not yet published his work establishing the immunological role of the thymus so it was necessary to suggest a possible mechanism for the neuromuscular disorder. At that time I favoured the idea that antibody against the receptor substance of the muscle endplate acted as a curare-like competitive blocking substance. At present there is no evidence to support this, and the nature of the defect remains obscure.

Neuromuscular Transmission

I cannot review here the mass of information on the functional disorder of neuromuscular transmission and abnormal responses to drugs acting at the endplate of muscle. I have recently analysed the data (Simpson 1968) and concluded that all can be accounted for by the structural changes demonstrated by Coers & Woolf (1959). These are presumably the result of a substance elaborated by the thymus and capable of passing the placental barrier. In my opinion it is a globulin of the antibody type.

Thymectomy

The first thymectomy for myasthenia gravis was performed by Sauerbruck in 1912 but there was little interest in the operation until Blalock et al. (1941) reported a series. Later American reports were discouraging although Keynes (1946, 1955) found it a valuable procedure. An independent study by Simpson (1958) confirmed the claims of Keynes that the operation was valuable if no thymoma was present, and review of the various American statistics showed that they were consistent with this. The operation was most effective if performed during the 'active' stage of disease and women gained most benefit. Other methods of destroying the thymus (radiotherapy, steroids, carotid sinus denervation) are less direct and hence inferior since the operation is so safe in skilled hands. Working closely with Mr Andrew Logan in Edinburgh and Mr Kenneth Fraser in Glasgow during the last twelve years there have been no post-operative deaths in patients under my care. For this reason I continue to rely on thymectomy, but it must be observed that in the last ten years there have been great improvements in medical management, notably in the wider use of pyridostigmine, better recognition of the difference between myasthenic and cholinergic crisis, greater familiarity with the use of edrophonium to evaluate the cause of weakness, and tremendous advance in the management of ventilatory failure. It might well be that these advances have restored the balance in favour of drug therapy.

REFERENCES

Blalock A, Harvey A M, Ford F R & Lilienthal J L (1941) J. Amer. med. Ass. 117, 1529


Frazier D & Turner J W A (1955) Lancet ii, 417


Keynes G

(1946) Brit. J. Surg. 33, 201


Ringertz N (1951) Aeta path. med. lund., scand. 29, 9

Simpson J A


(1958) Brain 81, 112

(1960) Scand. J. med. J. 5, 419


Smithers D W (1939) J. Fac. Radiol. (London) 10, 3


The Correlations Between Myasthenia Gravis and Disorders of the Thyroid Gland

J. A. SIMPSON

INTRODUCTION

An association between myasthenia gravis and thyrotoxicosis was first reported by Rennie (1908) who considered that it was coincidental. Since then the association has been noted too often to require further evidence, but the exact nature of the relationship remains in doubt. Cohen and King (1932) drew attention to some similarities existing between the two diseases, notably hypertrophy of lymphatic tissue, occasional lymphocytosis, occasionally glycosuria, and the presence of lymphorrhages in muscle tissue. More recently it has been shown that germinal centres in the thymus may be present in both diseases (Gunn, Michie & Irvine, 1964).

Myasthenia gravis is a rare complication of thyroid disease. Bartels and Kingsley (1949) found four cases of myasthenia gravis in a series of 12,962 cases of exophthalmic goitre and Sahay, Blendis and Greene (1965) found three in more than 12,000 cases of thyrotoxicosis. In the Gardiner Institute of Medicine, Glasgow, it has occurred only twice in 1019 cases. On the other hand it is not uncommon to find thyrotoxicosis in patients with myasthenia gravis. The main series in the literature, listed in Table I, show that about 5% of myasthenics have thyrotoxicosis at some time.

My own experience confirms the many early reports that thyrotoxicosis is closely associated with myasthenia gravis. At this date it is more important to examine the nature of the relationship. Engel (1961) reviewed the literature from the point of view of the temporal correlation. In 76% of 79 cases the hyperthyroidism preceded, or appeared simultaneously with, the myasthenia. He does not mention myasthenia preceding hyperthyroidism, though this is described by Millikan and Haines (1953) in 32% of cases with the combined syndrome. In 20% they occurred virtually simultaneously and in 48% the hyperthyroidism was first. In 1 in 4 of the last group the
hyperthyroidism preceded myasthenia by more than 5 years. My experience has been similar. It is evident that in some cases thyrotoxicosis will be subsiding as myasthenia increases, whereas in others the myasthenia is remitting as thyrotoxicosis increases (Thorner, 1939; McEachern & Parnell, 1948; Maclean & Wilson, 1954). In the majority of cases the 'see-saw' relationship suggested by these authors is absent (Bartels & Kingsley, 1949; Levy et al, 1951; Simpson, 1960; Engel, 1961). The first thymectomy for myasthenia gravis was performed by Sauerbruch on a patient who also had exophthalmic goitre. The latter was not affected by the operation (Schumacher & Roth, 1913). There is therefore no evidence of an inverse relationship between the two diseases.

**TABLE I**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Thyrotoxicosis</th>
<th>Myasthenia</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartels and Kingsley (1949)</td>
<td>4</td>
<td>90</td>
<td>4.4</td>
</tr>
<tr>
<td>Millikan and Haines (1953)</td>
<td>25</td>
<td>500</td>
<td>5.0</td>
</tr>
<tr>
<td>Grob and Harvey (1953)</td>
<td>8</td>
<td>270</td>
<td>3.0</td>
</tr>
<tr>
<td>Silver and Osserman (1957)</td>
<td>11</td>
<td>325</td>
<td>3.4</td>
</tr>
<tr>
<td>Oosterhuis (1964)</td>
<td>8</td>
<td>180</td>
<td>4.4</td>
</tr>
<tr>
<td>Rowland, Aranow and Hoefer</td>
<td>20</td>
<td>400</td>
<td>5.0</td>
</tr>
<tr>
<td>Simpson (1968)</td>
<td>37</td>
<td>518</td>
<td>6.1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>113</strong></td>
<td><strong>2283</strong></td>
<td><strong>4.9</strong></td>
</tr>
</tbody>
</table>

Other workers have taken the opposite point of view, that hyperthyroidism in some way 'causes' myasthenia gravis, to the extent of ordering thyroidectomy for control of myasthenia. This requires more serious consideration as it is found in general that myasthenia deteriorates as thyrotoxicosis increases despite the exceptions quoted above. Engel (1961) made some studies on five euthyroid patients with myasthenia gravis. Administration of 1-triiodothyronine in a dosage sufficient to suppress endogenous thyrotropin secretion without raising the basal metabolic rate (BMR) did not affect the myasthenic state. Larger dosage raised the BMR and this tended to increase myasthenic weakness. He then gave sodium iodide to block the release of thyroid hormone and so accelerate thyrotropin secretion. There was no effect on the myasthenia. If thyrotropin was administered weakness increased
as the metabolic rate increased. Blockage of thyroid hormone synthesis by methimazole with intermittent thyrotropin to deplete stored thyroid hormone led to improvement as the BMR fell, although endogenous secretion of thyrotropin increased. From these experiments Engel (1961) concluded that hypermetabolism made myasthenia worse no matter whether it was induced by 1-triiodothyronine or by thyrotropin, but thyrotropin had no effect if its action on the thyroid was blocked. There was no evidence of a primary effect of thyrotropin on muscle such as has been postulated for malignant exophthalmos. The deleterious effect of hypermetabolism seems in no way different from that of many other forms of stress.

PERSONAL EXPERIENCE

I have examined thyroid function in 66 myasthenic patients, including the selected series previously reported (Simpson, 1966a). Of the 66 patients there were 3 diagnosed clinically as having thyrotoxicosis, 4 as non-toxic goitre, and 2 as Hashimoto's disease. One patient had a previous history of thyrotoxicosis. Three patients without clinical or laboratory evidence of thyroid disease had a family history of thyrotoxicosis. The tests used were radio-iodine uptake and plasma protein-bound iodine with plasma cholesterol and BMR measurement. The results agreed with the clinical assessment in 59 but subclinical hyperthyroidism was found in 3 cases and hypothyroidism in 4 (Simpson, 1964b, 1966a). This confirms the finding of Sahay et al (1965) that latent hypothyroidism is at least as common as latent hyperthyroidism in myasthenia gravis.

I have previously reported (Simpson, 1958) a series of 404 myasthenics in which 21% of female and 9% of male patients had a thyroid disorder at some time in their life. The series has now increased to 518 patients but the incidence remains the same (Table II). These figures are not comparable with those of Millikan and Haines (1953) which refer only to thyrotoxicosis and a breakdown of my cases into different categories is shown in Table II, and the thyrotoxic patients are compared with those of other series in Table I. The incidence in the present series (6.1%) is a little higher than the others. Some of this is certainly due to different diagnostic criteria. Many of the cases occurred before radio-iodine uptake and protein-bound iodine estimation was available. It is probable that modern criteria would eliminate some and move others into the non-toxic goitre category. The high figure for total thyroid disease is supported by a smaller series examined in Edinburgh and Glasgow by modern diagnostic methods including
<table>
<thead>
<tr>
<th>Thyroid Disorder</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
<th>Total</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-tumour</td>
<td>Thymoma</td>
<td>Total</td>
<td>%</td>
<td>Non-tumour</td>
<td>Thymoma</td>
<td>Total</td>
<td>%</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>28</td>
<td>2</td>
<td>30</td>
<td>8.5</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>4.2</td>
</tr>
<tr>
<td>Non-toxic goitre</td>
<td>27*</td>
<td>4</td>
<td>31</td>
<td>8.8</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>3.6</td>
</tr>
<tr>
<td>Previous goitre</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>2.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Hashimoto/lymphadenoid goitre</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>1.7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Myxoedema</td>
<td>1**</td>
<td>0</td>
<td>1</td>
<td>0.3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Total thyroid disease</td>
<td>70</td>
<td>7</td>
<td>77</td>
<td>21.9</td>
<td>13</td>
<td>3</td>
<td>16</td>
<td>9.6</td>
</tr>
<tr>
<td>Number of myasthenics</td>
<td>321</td>
<td>30</td>
<td>351</td>
<td>100.0</td>
<td>147</td>
<td>20</td>
<td>167</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* 1 case recurrent thyroglossal cyst.

** Systemic lupus erythematosus and presumed radiation thyroid atrophy.
### TABLE III

**THYROID ANTIBODIES IN MYASTHENIC PATIENTS**

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-tumour</td>
<td>Thymoma</td>
</tr>
<tr>
<td>Clinical thyroid disease</td>
<td>10 (a)</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid antibodies with clinical thyroid disease</td>
<td>7 (a)</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid antibodies without clinical thyroid disease</td>
<td>7 (bd)</td>
<td>2</td>
</tr>
<tr>
<td>Number of myasthenic patients</td>
<td>40</td>
<td>2</td>
</tr>
</tbody>
</table>

(a) 1 case of Hashimoto's disease with Sjögren and rheumatoid arthritis. Gastric antibodies.
(b) 1 case pernicious anæmia. Hashimoto's disease at autopsy.
(c) 1 case Hashimoto's disease (Simpson, 1964).
(d) 1 case Sjögren's disease. No clinical thyroid disease.
(e) 1 case hypothyroid (biochemical).
immunological studies which also indicate as much subclinical as clinical involvement (Table III).

I have excluded patients with physical signs which might indicate previous transient thyrotoxicosis if there was no collateral evidence. This includes patients with a moderate degree of exophthalmos (Walsh, 1957) and others with a thick supraorbital pad resembling that seen in recovered thyrotoxicosis to which I have drawn attention (Figure 1) (Simpson, 1960). Klar (1930) has previously described oedema of the eyelids in a case of myasthenia gravis. An example is case MN.3912. Although there is no history of thyroid disease her serum has a high titre (1/128) of complement-fixing antibody against thyroid. She also has a raised titre (1/32) of antibody against gastric mucosa. Some patients complain of profuse sweating and weight loss and many are unusually nervous. These symptoms may precede the use of anticholinesterase drugs and so cannot be attributed to the muscarinic side-effects of treatment. None of these patients have been included.

The first point of interest in Table II is the number of patients who have had hyperthyroidism or simple goitre earlier in life but which has

Figure 1. Enlarged pads on medial side of upper eyelids.
(a) Case MN 4636. Transient thyrotoxicosis aged 45. No myasthenia until 9 years later. Thyroid slightly palpable: occasional lid lag. Serum PBI 5.1 ug/100 ml. Complement-fixing antibody against thyroid, titre 1:16.
(b) Case MN 1906. Long history of myasthenia gravis. No history of thyroid disease. (antibody studies not available).
subsided long before the onset of myasthenia. Secondly, there are the cases of non-toxic goitre and myxoedema which have been ignored in the recent past (Simpson, 1958, 1960). That this is unjustified is shown by a brief review of the literature from pathologists who, in the main, describe non-toxic goitres, often lymphadenoid (Norris, 1936; Miller, 1940; Giordano & Haymond, 1944). These authors usually considered that the changes were involutional in a previous exophthalmic goitre. Rowland et al (1956) found four non-toxic nodular goitres in a post-mortem series of 26 myasthenics. Ringertz (1951) found 5 abnormal thyroids in 18 autopsies. One of these was a non-toxic nodular goitre (without lymphoid infiltration), one gland (from a girl of 16) was considered to be of toxic type, and three showed patchy parenchymal atrophy with cellular disintegration associated with dense lymphoid infiltration. Two of these had lymphoid follicles in the thyroid 'giving to the picture a certain resemblance to that of lymphadenoid goitre'. Both of these patients had a thymic tumour.

My own experience has been similar. In drawing attention to it in a Honyman-Gillespie Lecture 1 discussed a slide which appeared to indicate unsuspected Hashimoto's disease in a myasthenic patient with a thymoma. This was omitted from the published version (Simpson, 1960) owing to some reservations about the diagnosis but it was presented with other cases in later papers (Simpson, 1966a, b) after Hashimoto's disease had been identified in life (Daly & Jackson, 1964; Simpson, 1964a) and at autopsy (Becker et al, 1964).

As pointed out above, diminished thyroid function is not uncommon in myasthenia gravis but myxoedema is rare.

**CASE HISTORY**

Case GK/ES first noticed weakness of the legs in September, 1946, when she was aged 49. This was followed by ptosis and diplopia and then by bulbar and upper limb weakness. Myasthenia gravis was diagnosed in October, 1948. There was a good response to neostigmine. In 1950 she had pericarditis and angioneurotic oedema and in 1952 was treated by radiotherapy for suspected carcinoma of the larynx. In 1954 she became myxoedematous. A diagnosis of chronic lupus erythematosus was made but the myxoedema was attributed to radiation of the thyroid. At that time the possible association between S.L.E., thyroiditis and myasthenia gravis was not known. When I examined her in 1955 she was able to lead a normal life if she took 10 tablets (150 mg) of neostigmine each day.

Storm-Mathison (1961) reported two myxoedematous myasthenics, one of whom
also had arthropathy and a previous history of haemolytic jaundice (Case 56). This patient was alive 12 years later with remission of myasthenia and well controlled myxoedema. The author did not consider that her patient had S. L. E. (discussion after Simpson, 1966b). Four cases of primary myxoedema associated with myasthenia gravis were reported by Feinberg et al (1957). The possibility of thyroiditis was not discussed. Sahay et al (1965) found 5 hypothyroid and 3 hyperthyroid patients in 260 cases of myasthenia gravis.

I do not wish to expand on the importance of a correlation between Hashimoto’s disease and myasthenia gravis as support for my autoimmune hypothesis of myasthenia (Simpson, 1960) which was presented to the First Symposium of this series (1961, unpublished), but rather to discuss these euthyroid or hypothyroid disorders of the thyroid gland in an endocrinological context. At this point it is already obvious that there is some connection between myasthenia gravis and thyroid disease in general and not specifically with hyperthyroidism. Certainly neither thyroid hormone, thyrotropin, or a hypermetabolic state can be causative factors in producing the neuromuscular disease as is implied by so many writers.

The clue to the relationship may be provided by an original observation reported by Simpson (1960). In 20 of 440 myasthenic cases there was a family history of goitre (toxic or simple) or of myxoedema. The affected relative was usually a sister or a female and could be on either side of the family (Simpson, 1960). The exact incidence is uncertain since patients have only been questioned systematically since I became aware of it at the end of 1953. Soon after this Macrae (1954) reported a myasthenic child whose mother had hyperthyroidism. Oosterhuis (1964) found a family history of thyroid disease in 9 of 116 myasthenic patients and 5 of these had personal evidence of thyroid disease. Greenberg (1964) reported two sisters with myasthenia and thyrotoxicosis. One later developed Hashimoto’s disease and the other became myxoedematous as the result of treatment. Rowland et al (1966) also report a familial incidence of both disorders. I have seen a male myasthenic (NH.4648) whose twin sister had goitre.

One feels that there is an important clue in these facts. It has been accepted for a long time that a constitutional or genetic defect may predispose to the development of Graves’ disease (Bartels, 1941). It was therefore suggested that a gene may have variable expression producing either thyroid disorder, myasthenia gravis, or both (Simpson, 1960). It could be responsible for altering immunological responses by an action on the thymus (Simpson, 1960, 1964b). A genetic factor has recently been demonstrated in the
autoimmune diseases including thyroiditis (Hall et al, 1962; Doniach & Rott, 1962), and there is increasing recognition of abnormal immunological reactions in thyrotoxicosis (Anderson et al, 1964). There is no valid evidence that the abnormality of thyroid function results from destruction of the gland by antibodies. In the same way there can be no doubt that there is a major disturbance of immunological reactions in myasthenia gravis but the original suggestion that antibody might block the endplate receptors has not been confirmed. The resolution of this problem seems tantalisingly near, but must wait for clarification of the links between genes, thyroid and thymus. For reasons which have been indicated previously (Simpson, 1960, 1966a, b) I feel that genetic control of both organs acts through a hypothalamo-pituitary mechanism. The recent finding that Graves' disease is caused by a long-acting thyroid stimulator resembling antibody globulin, and not by thyrotropin is another link in the chain. The picture remains incomplete but the clues are there for all to see.

SUMMARY

Myasthenia gravis is a rare complication of thyrotoxicosis (1 in 3,000) but thyrotoxicosis occurs in 5% of myasthenics. There is no regular temporal sequence and they may be widely dissociated in time. There is an even higher incidence of other thyroid disorders -- non-toxic goitre, Hashimoto's disease and myxoedema -- in 22% of female and 10% of male cases of myasthenia -- with an additional number (of similar dimensions) of subclinical cases shown by serum antibodies, radioiodine, and autopsy studies. Attention is drawn to a high incidence of thyroid disease in close relatives of myasthenics. It is suggested that disordered thyroid function does not cause myasthenia gravis but that both groups of disorders are associated by common genetic factors. Immunological tolerance is disturbed in both but may be a paraphenomenon. The link between genes, thymus, thyroid, and neuromuscular function cannot be defined but are probably convergent rather than linear.

REFERENCES

Daly, J. J. and Jackson, E. (1964) Brit. med. J., 1, 748
Greenberg, J. (1964) Archs Neurol. (Chicago), 11, 219
Gunn, A., Michie, D. and Irvine, W. J. (1964) Lancet, ii, 776
Maclean, B. and Wilson, J. A. C. (1954) Lancet, 1, 950
Macrae, D. (1954) Pediatrics (Springfield), 13, 511
McEachern, D. and Parnell, J. L. (1948) J. clin. Endocrinol., 8, 842
Miller, H. G. (1940) Archs Path., 29, 212
Simpson, J. A. (1958) Brain, 81, 112
I. Introduction

"Few diseases have been more satisfying to the teacher of medicine than myasthenia gravis for no better meeting ground exists for clinician, pathologist and pharmacologist. Ten years ago the standard teaching was that the myasthenic response must indicate one of three possible 'chemical lesions' at the neuromuscular junction: (i) insufficiency of acetylcholine, (ii) excess of cholinesterase, or (iii) a 'curariform' block of transmission, presumably due to a substance carried in the blood. More recently the logical fourth possibility of an abnormality of the motor endplates of muscle has been postulated. It seemed only a matter of time and of refinement of physiological and pharmacological techniques before the true lesion would be demonstrated. In that same period knowledge of the physiology of the neuromuscular junction has made its greatest advances, yet the solution of the problem of myasthenia evades us despite renewed and world-wide interest" (Simpson, 1958). Although the final solution remains missing ten years after that passage was written, there have been major reassessments. It is instructive to examine the reasons for these.

The most characteristic feature of medical thinking in the last 50 years has been the belief that all disease can be "explained" given an adequate knowledge of normal physiology or biochemistry. Each disorder then represented increased or decreased activity of a normal function. Detailed
knowledge of the function and pharmacology of the normal mammalian neuromuscular junction would therefore indicate the mechanisms which must be defective in myasthenia gravis. The thoughtful observer of disease may be forgiven for being increasingly sceptical of such an approach which ignores the fact that the geometry and molecular biology of the affected organs may be changed radically in disease. Nevertheless the "pathophysiological" approach has had the inevitable result that unthinking clinicians have begun to take note of only those things which fit that conceptual framework. All thinking about myasthenia gravis was concentrated on neuromuscular transmission in 1934 when Mary Walker's demonstration of the dramatic relief afforded by physostigmine coincided with Dale and Feldberg's confirmation of the role of acetylcholine in neuromuscular transmission. When patients were questioned without prejudice, Simpson (1960) rediscovered symptoms recognized by Buzzard (1905) which indicated that myasthenia gravis was not exclusively a disorder of neuromuscular transmission. From these observations an immunological hypothesis was constructed which has received widespread support from other workers. It is not the purpose of this chapter to review that aspect, or the clinical symptomatology of myasthenia gravis. For these the reader is referred to Simpson (1964a).

The purpose of this introduction is to emphasize for non-clinical readers that there are different levels of "causation" in medicine, and explanations of disease processes may concentrate on different levels without necessarily being incompatible. A corollary is that an "explanation" at the most superficial level will be adequate only in so far as it takes account of the deeper levels. Thus five levels of "causation" of a disorder may be recognized.

(i) Increased or decreased function of an organ ("pathophysiology").
(ii) The disorder of function may be secondary to a deformity of structure ("pathoanatomical").
(iii) Intracellular disorder of function ("molecular biological").
(iv) Cellular or body fluid disorders may be secondary to disturbance of a fundamental biological process ("dyshomoeostatic").
(v) First cause of each of the above (e.g. genetic, infective, psychosomatic).

Any "explanation" of myasthenia gravis in terms of the function of normal neuromuscular transmission is necessarily naive, being only at the first level of causation. Furthermore, unless it is recognized that there are geometrical and homoeostatic disorders which must be accounted for, even the first level explanation may be seriously misleading. It is the writer's conviction that many of the current arguments regarding the pre-synaptic or post-synaptic site of the myasthenic defect are meaningless because of failure to appreciate this. The present account will examine the available data from each of these points of view in turn.
II. Myasthenia

The term myasthenia is used in different senses in different countries. The writer uses it to mean that type of muscular weakness which increases progressively if a muscular contraction is maintained or if a movement is regularly repeated without adequate interval for rest. This phenomenon is often termed “pathological fatigue”. The term is graphic but undesirable as the increasing weakness has no close resemblance to normal fatigue of muscle or of its nervous control. It is unfortunate that there is no suitable word in the English language to describe the progressive loss of power of muscular contraction or of the associated action potentials of skeletal muscle. The myasthenic syndrome may be associated with a variety of neuromuscular diseases (Simpson, 1966a), but there is a clearly recognizable syndrome which is myasthenia gravis (Simpson, 1964a), and it is that disorder which will be analysed here.

A. The mechanical response of myasthenic muscle

The resemblance of myasthenia to curare poisoning occurred to Oppenheim (1887) and to Jolly (1895). The latter worker made a most significant contribution when he showed that the “fading” of muscle contraction seen during sustained voluntary effort could be reproduced if the motor point of a myasthenic muscle was stimulated by a burst of faradic electric current (i.e. a rapid train of oscillatory stimuli which stimulates the motor nerve entering the muscle) whereas the muscle continued to contract powerfully if a galvanic stimulus was used (which stimulates only muscle fibres). This important observation showed that the muscle was capable of contracting repeatedly without “fatigue” if its fibres were stimulated directly through its motor nerve. Later workers using electrical recording of the antidromic action potential of the motor nerve confirmed that the nerve continued to respond (Alajouanine et al., 1959) so the defect of response must be localized to the neuromuscular transmission mechanism. Slomic et al. (1968) using strain gauge recording showed that the normal “staircase phenomenon” evoked in muscle by trains of stimuli to its motor nerve is reduced or abolished in myasthenia gravis, even when there is no clinical evidence of weakness of the muscle tested. By applying a correction to allow for block of fibres derived from measurement of the action potentials they stated that the muscular mechanism believed to be responsible for the staircase phenomenon is also involved. Pritchard (1933) identified the cause of the myasthenic reaction as Wedensky inhibition. This concept still has some currency in the Soviet states. It can no longer be accepted in the sense usually understood as in the Wedensky phenomenon a partial block of a nerve so prolongs its refractory period that only the first of a rapid train of impulses will pass through the blocked area, though a slow train may pass without
difficulty. The result of a series of impulses at a critical frequency of stimulation will then be a single muscle twitch, not the decrementing series of twitches found in myasthenia gravis.

B. Electromyography of voluntary contraction

Lindsley (1935) made myographic and electromyographic studies using coaxial needle electrodes to record the activity of individual muscle fibres or of motor units. He found that the rhythm of discharge of the motor units on voluntary contraction was normal. The size of the potential recorded from each unit was subject to wide variation which was greater with fatigue of the muscle but abolished after injection of neostigmine. He concluded that the function of the motor neurone was normal but that there was intermittent block of neuromuscular transmission to some of the muscle fibres which it innervated.

With continued effort there is a progressive fall-out of motor units. If a special effort is requested a new burst of discharge occurs. This is usually poorly sustained, but in certain circumstances a surprising and well-sustained contraction is possible. Myasthenia gravis is quite commonly complicated by epilepsy or psychosis (Simpson, 1960). The motor convolution or the violent movements associated with mania may show no evidence of decrement and the desperate movements of the myasthenic patient asphyxiating from laryngeal obstruction may be extremely powerful. Denny-Brown (1953) states that the motor units producing the renewed discharge on special demand are not units which have recovered from the previous "fatigue" but are new units responding only to the special effort. This has not been the writer’s impression, though it is impossible to be dogmatic about it. It would, for instance, be quite impossible to state that the first units to drop out had not recovered since their morphology cannot be examined during full contraction by normal methods of recording. If the electromyogram (EMG) of a myasthenic muscle exerting a sustained isometric contraction is examined one commonly records cycles of sudden failure of contraction followed by renewed effort (Fig. 1). To the uninitiated, the clinical manifestation is very suggestive of hysterical failure of innervation, but close examination of the cycles shows that the confused recruitment pattern of the EMG becomes reduced in two ways. In addition to the amplitude decrement of motor units described by Lindsley (1935), the trace becomes simplified by sudden cessation of activity of some units (Denny-Brown, 1953; Lundervold, 1954; Simpson, 1956). At this stage the contraction becomes tremulous and the motor units tend to synchronize as in normal fatigue. Motor unit firing then stops abruptly but is resumed in about one second with greater intensity. The cycle is repeated, but with longer silent periods and decreasing re-recruitment. Simpson (1960) suggests that the phenomenon can be explained by short-lasting post-tetanic facilitation plus the purely muscular post-tetanic potentiation (Botelho and Cander, 1953) following the brief pauses and that it is unnecessary to postulate a hidden
11. DEFECT IN MYASTHENIA GRAVIS

Fig. 1. EMG with co-axial needle electrode from R. deltoid muscle while arm held out until posture could no longer be maintained. Records read continuously. Note development of synchronization and tremor leading to brief pauses of successively longer duration until further contraction is impossible. Each pause is followed by brief post-tetanic facilitation. The pattern reduces in amplitude but the units firing at the end of the contraction apparently stop firing suddenly, without decrement in amplitude. Calibrations 200 μV, 1 sec.

reservoir of high-threshold units. It is the same mechanism which abolishes induced ptosis if the myasthenic patient is allowed to blink during testing, and it is probably also the mechanism of the coarse monocular nystagmus which is sometimes present.

Many of the motor units in the affected muscles have the polyphasic action potentials of a "myopathic" motor unit (Fig. 2). This may only be seen in the last units remaining before final cessation of the weakening contraction, but in many cases similar units can be found at the beginning of contraction even after a period of rest, particularly in the deltoid and triceps humeri muscles. A minority of muscles affected in this way show scanty
Fig. 2. (a) End of contraction in Fig. 1. Time scale 100 msec. (b) Remaining motor unit potentials after (a) are small, brief, and polyphasic ('myopathic'). Time scale 20 msec. Amplitude calibration 200 μV for both records.
spontaneous fibrillation, positive sharp waves, and increased mechanical excitability (Fig. 3). All of these features are indistinguishable from the electromyographic pattern of polymyositis (Simpson, 1960).

Some conclusions may be drawn from these observations. First, that the early decrement in tension is associated with amplitude decrement of motor units, many of which develop the EMG pattern associated with an incomplete complement of muscle fibres. The defect could be either postjunctional, as suggested by the resemblance to polymyositis, or prejunctional in discrete branches of the telodendria of the motor axon. If it is the latter, the nerve terminals have not lost the property of post-tetanic facilitation of release of transmitter and this may recruit non-responsive muscle fibres (vide infra). Second, that the sudden arrest of unit firing without progressive decrement of the muscle unit potential suggests failure proximal to the terminal branching of the axon. This failure may be confined to the late stage of voluntary tetanus. And third, that evidence of denervation or of prolonged functional failure of acetylcholine release, as in botulism, is extremely rare. The occasional occurrence of spontaneous fibrillation is equally compatible with myositis.

![Fig. 3. Myasthenia gravis. R. triceps EMG. (a) Fibrillation, (b) slight voluntary activity with "myopathic" motor unit potentials, (c) full recruitment pattern on maximal effort. Calibration 200 μV, 20 msec.](image-url)
Unfortunately the existing techniques of electromyography of asynchronous tetanic contraction do not permit satisfactory isolation of a single motor unit to study the changes in its action potential throughout a strong voluntary effort. Considerable information of a statistical nature can, however, be obtained from the study of a population of motor units integrated by recording from the skin surface if each is stimulated more or less synchronously by means of its motor nerve.

C. Indirect stimulation of muscle

Early reports by Herzog (1917) and Eichler et al. (1935) on the changes in the action potential of a myasthenic muscle tetanized by repetitive stimulation of a motor nerve may be criticized on technical grounds. Harvey and Masland (1941b) drew attention to the importance of preventing displacement of the stimulating electrodes placed over the nerve and of using supramaximal stimulation (at least 30%) to ensure that every motor fibre in the mixed nerve is adequately stimulated. This can be a painful experience for the patient, and it may be preferable to block the nerve proximally by injected local anaesthetic. This has no immediate effect on distal function of the nerve but may complicate long term experiments. Special precautions must be taken to prevent the EMG record of the evoked muscle potential from being obscured by an artefact due to stimulus-escape (Pinelli, 1957).

Harvey and Masland (1941a) found that the action potential of the muscle after the second of a pair of supramaximal stimuli was depressed for 0.5–2.0 sec after the first (conditioning) stimulus (Fig. 4 (a)). With a train of stimuli ("tetanus") the effect varied with the interval between stimuli, but even with stimulation rates as low as 13 per sec there was an immediate and progressive decline in voltage for the first few responses. The voltage of the evoked potential then continued with little change at this lower level of response. The decline was more pronounced when the muscle was partially "fatigued" (sic) as a result of previous periods of stimulation (Fig. 4 (b)). The time course of recovery following a standard tetanus was tested with single test shocks. The compound muscle action-potential did not regain its initial voltage until 1–5 sec after the termination of the tetanus.

The possibility that a substance which blocks neuromuscular transmission is released by nerve stimulation is rendered unlikely by the observation of Harvey et al. (1941) that the first evoked potential is sometimes subnormal in myasthenic patients. This is sometimes followed by

![Fig. 4.](image-url)
transient facilitation or recruitment of neuromuscular transmission and in others by a profound degree of depression. They interpreted these findings as indicating that some muscle fibres were incapable of stimulation until neuromuscular transmission was facilitated by repetition of stimulation within a short period of time, or, in modern terms, until summation of endplate potentials reached the critical threshold. The increased voltage of the compound muscle potential could not be due to better synchronization of muscle response since it was accompanied by an increase in the duration of the potential. In one patient both depression and facilitation were seen. There was no evidence of repetitive response of muscle fibres.

These results were confirmed and extended by Johns et al. (1956). They noted that patients with generalized myasthenia gravis showed an abnormal response to repetitive nerve stimulation at rates as low as five per second. Initially the amplitude of the compound muscle action potentials declined rapidly and then returned toward the initial value. This was then followed by a slow exponential fall in amplitude. As the stimulus frequency was increased the initial trough deepened and the rate of the late exponential fall was increased (Fig. 5). Similar results were reported by Strüppler (1955) and Pinelli (1957).

Johns et al. (1956) noted that a facilitated response to a test stimulus
DEFECT IN MYASTHENIA GRAVIS

sometimes occurred 1-0 sec after a tetanus (Fig. 6). Such an increase was never seen in normal subjects and they concluded that there must be muscle fibres available for recruitment in the myasthenic, i.e. there is sometimes a partial block to neuromuscular transmission of a single impulse. The degree of post-tetanic facilitation may be increased by stimulating the nerve at frequencies faster than the muscle fibres can follow, or by continued stimulation after the muscle fibres have ceased to respond. Since all other examples of post-tetanic transjunctional potentiation which have been studied are accountable to increased output of junctional transmitter by rapidly stimulated nerve endings (Hutter, 1952; Liley and North, 1953), it is virtually certain that facilitation is a prejunctional phenomenon.

Personal studies from 1950 to date are in full agreement with the Baltimore workers, but one would emphasize that different degrees of decrementing response and facilitation may be present. Thus one may record a pure decrementing response [Fig. 7 (a)], a decrement to a new steady state [Fig. 7 (b)], initial decrement temporarily restored by facilitation [Fig. 7 (c)] and rarely facilitation without decrement. In some cases a decrementing response occurs at rates of stimulation below ten per second, but facilitation at faster rates (Fig. 7 (d) and Desmedt, 1957b). Further, the type of response may be completely different in different muscles from the same patient.

Fig. 6. (a) Response of muscle action potential to 121 supramaximal nerve stimuli at 50 per sec showing rapid decline, partial recovery, then progressive decline in amplitude in a case of myasthenia gravis. (b) Repeat 5 sec later showing post-tetanic facilitation of first four potentials, then more marked decline. (c), (d). After neostigmine (2 mg, i.a.). Less marked decline in amplitude of successive potentials, and disappearance of post-tetanic facilitation. (From Grob et al., 1956c.)
Fig. 7. Harvey-Masland test in myasthenia gravis. (a) Classical response. (b) Immediate decrement then maintained level of response. (c) Decrement temporarily restored by facilitation. (d) Decrement at 8 stimuli/sec, progressive increment at 50/sec. (From Simpson, 1966a.)
immediately. They (Desmedt et al., 1966) reported that nerve transmission is blocked by an exponential type of process, one of which results in "dual response" to decamethonium (vide infra) and that this response becomes greater in the "burned out" phase of the disease (Simpson, 1960). It is at this time that "myositis" changes may be prominent in the histopathology. This could account for the fact that some patients in this stage of the disease notice that muscular power improves after exercise (Simpson, 1960). In these patients the Jolly test, which records the power of contraction of the muscle when tetanized rather than its action potential, is misleading.

These phenomena are described at length as they are apparently unknown to some authors who have speculated on the nature of myasthenia gravis. Slemic et al. (1968) show that some of the phenomena may be due to recording artefacts if the stigmatic recording electrode picks up from irrelevant muscles, the indifferent electrode is also in a potential field, or if the muscle is moving under the electrodes. These factors require more rigid control than has hitherto been accorded to them, but there is no doubt that true facilitation in greater or lesser amounts may be demonstrable and must be accounted for in a discussion of the possible mechanism.

Desmedt (1957a, 1958) observed that post-activation facilitation is short-lived in myasthenic muscle. It disappears 20–30 sec after faradization and is succeeded by a stage of "post-activation exhaustion" (his terminology) during which the electrical and mechanical responses are greatly reduced and a short train of stimuli at rates as slow as three per second causes marked decrement of successive responses (Fig. 8). This post-activation "exhaustion" slowly disappears with a half time of 10–15 min and Desmedt considers that this, rather than the prompt decrement during a tetanus, is the real reason for the long-lasting component of the pathological fatigue seen clinically. Harvey et al. (1941) showed that the time course of recovery of junctional transmission following a single impulse differs from a single exponential type recovery curve in that the degree of block is maximal between 0.5 and 1.0 sec after the passage of the impulse, rather than immediately. They described the observed curves as a composite of two processes, one of which results in a facilitation of transmission, the other a block of transmission of considerably longer duration, and therefore of the same nature in the prompt depression and in the after-depression (Fig. 9). Since each process could be capable of summation and prolongation by a train of stimuli it remains to be demonstrated that the post-activation exhaustion of Desmedt is essentially different. It depends on the number of nerve impulses reaching the muscle stimulated and not on their frequency (Desmedt et al., 1963). Whatever its nature, there is no doubt that a
Fig. 8. Percentage reduction of voltage of 5th muscular response in a 3/sec indirect test-train (×100%, in (a)) for (b) myasthenia gravis, (c) cat poisoned with HC-3, and (d) cat with d-tubocurarine. Nerve faradized at 50/sec at time zero. (From Desmedt, 1958, see original for details of dosage and times.)
Defect in Myasthenia Gravis

"Myasthenic reaction" which cannot be demonstrated with a brief faradization of a motor nerve may sometimes be demonstrable after a prolonged voluntary contraction of the muscle even though there has been no obvious failure of the voluntary contraction. Similarly, although Desmedt (1958) and Slomic et al. (1968) almost invariably find a decrementing muscular response to supramaximal indirect stimulation at three per second, this has not always been the writer's experience and the myasthenic reaction may only be detectable after repeated applications of a fast tetanus with brief intermissions (Fig. 10). This is an important point when using the reaction as a diagnostic test.

Fig. 9. Response to test stimulus (as percentage of response to conditioning stimulus) at various time intervals between these stimuli. Curve P is a theoretical transmission curve derived from the decay curves for facilitation (F) and depression (D), two independent junctional processes assumed to follow transmission of one volley. Refer to original for theoretical formulation. "From Harvey et al., 1941.

D. The neuromuscular junction

Further consideration of the problem requires a brief review of the mechanism of transmission at the neuromuscular junction as it is at present understood. The motor nerve axis cylinder loses its myelin sheath as it branches into a terminal arborization (telodendria). The branches are covered by a continuation of the Schwann cell sheath (teloglia) except on those surfaces of the terminal expansions of each branch which lie partly embedded in shallow troughs on the surface of the motor endplate of the muscle fibre from which it is separated by a cleft of about 500 Å width. In mammals the terminal branches are normally grouped closely together in a "plaque" applied to a discrete region of a muscle fibre. The neurilemma
which lies outside the terminal Schwann sheath merges into the sarcolemma of the muscle fibre at the margin of the endplate zone and takes no actual part in the junction. The synaptic cleft is apparently filled with a fluid or thin gel which extends into the junctional folds in the endplate (Palay, 1958). At the edge of the neural contact the synaptic cleft opens into the extracellular space.

![Graph](image)

**Fig. 10.** Myasthenia gravis. Supramaximal stimulation of R. ulnar nerve at 4 and 50 sec showed delayed myasthenic response and post-tetanic facilitation of abductor dig. min. Recording is continuous apart from brief breaks of duration indicated on trace. (From Simpson, 1966a.)

The cytoplasm of the axon terminal contains many mitochondria and a number of ovoid particles which can be identified with the electron microscope (Lehrer and Ornstein, 1959). These are generally believed to be vesicular structures containing acetylcholine or its precursor. Birks (1966) casts doubt on this interpretation. It is possible that the vesicles are artefacts formed from a tubular system better seen more proximally in the axolemma. There is much indirect evidence that the vesicles, if such they be, represent packets of the transmitter substance. Certain zones of the presynaptic membrane show increased thickening and density on electron microscopy.
At these zones some vesicles are considered to discharge spontaneously into the synaptic cleft, liberating a quantum of acetylcholine, but the majority are released only by some unknown change accompanying the depolarization of the terminal knob by the occurrence of an action potential at its surface membrane which liberates many quanta of transmitter substance. The number of quanta released by a single presynaptic action potential is only about 1/100th part of the amount readily available so that the store of preformed acetylcholine (ACh) is not exhausted by a single impulse though it falls progressively with repeated stimulation. Regeneration by choline-acetylase may also be accelerated by stimulation, and release of pre-formed acetylcholine at nerve terminals is potentiated (Hutter, 1952; Liley and North, 1953). In the superior cervical ganglion of the cat, accurate measurements by Birks and MacIntosh (1961) of the acetylcholine content and output over a wide range of frequencies indicate that presynaptic fibres contain a small amount of acetylcholine in an inactive form (“stationary-ACh”) possibly formed in the soma and transferred distally by flow of axoplasm. This compartment cannot be depleted by prolonged stimulation. In the presynaptic terminals Birks and MacIntosh (1961) found evidence of “depot-ACh”, probably in the synaptic vesicles, and “surplus-ACh” in the extravesicular space (Fig. 11). Only the “depot-ACh” is available for release by nerve impulses and it disperses into the extravesicular “surplus-ACh” for hydrolysis by cholinesterase and recycling except for a small quantity of “readily releasable fraction” which escapes into the synaptic cleft. Activation by presynaptic volleys depletes the depot-ACh and so increases the rate of synthesis of ACh by an amount equivalent to the facilitated release from the readily releasable fraction. A similar mechanism may exist in motor nerves to skeletal muscle. In ganglionic presynaptic fibres hemicholinium-3 (HC-3) inhibits ACh synthesis by choline acetylase. At the neuromuscular junction HC-3 reduces the quantal size of ACh (Elmqvist and Quastel, 1965).

The presence of extracellular calcium is essential for the liberation of ACh, and changes in its concentration affect the number of quanta liberated by a nerve impulse (del Castillo and Katz, 1954a). Magnesium, normally present intracellularly, depresses acetylcholine release if it is applied extracellularly at the neuromuscular synapse (del Castillo and Engbaek, 1954). It is possible that the coupling between nerve terminal action potential and release of synaptic vesicles requires calcium ions (Hubbard, 1961). The rate of spontaneous release of ACh quanta, as estimated from miniature endplate potentials, is controlled presynaptically—and only presynaptically—by the membrane potential of the nerve endings (Katz, 1962). The most important factor depolarizing the normal presynaptic membrane is the nerve action potential, but it is possible that an intense efflux of potassium ions from a stimulated muscle fibre could accumulate around the nerve endings and depolarize them sufficiently to increase the rate of ACh release (Katz, 1962). Under the influence of anticholinesterase drugs the large muscle spike potential may cause generation of impulses at motor
nerve terminals and ACh in the synaptic cleft may depolarize the presynaptic membrane (Werner, 1960, 1961) where there are some cholinceptive sites (Feng and Li, 1941). Eccles (1964) considers this a more probable cause of the presynaptic effect of anticholinesterase substances than the potassium theory. When the presynaptic terminal is depolarized, normally by an action potential, the probability of secretion of acetylcholine quanta increases by 100 000 times or more (Katz, 1958).

![Diagram](image)

**Fig. 11.** Diagrammatic representation of the acetylcholine metabolism of the cat superior cervical ganglion. (From Birks and MacIntosh, 1961.)

Acetylcholine liberated into the synaptic cleft reacts with the postsynaptic membrane of the motor endplate and, as just described, with the presynaptic membrane of the nerve terminal. It is widely accepted, though there is no direct proof, that there is one or more proteins on the membrane with a molecular structure complementary to the shape of the acetylcholine molecule. This acts as a “receptor” surface to which ACh or similarly formed molecules readily bind themselves. The receptor protein probably has anionic and esteratic sites. When the cationic head of ACh is attached to the anionic site of the receptor protein it in some way increases the permeability of the endplate zone to ions in a relatively non-specific way. As a consequence of this an ionic current flows across the postsynaptic membrane with a direction and intensity which depends solely upon the ionic gradients and potential drop across the membrane. Unlike the muscle action potential, it has no regenerative link. Other things being equal, the endplate current increases in proportion to the number of quanta of ACh reaching the reactive zones of the endplate. Its duration is brief (about 2 msec) but the
resulting endplate potential (EPP) declines more slowly due to a passively decaying electrotonus. The endplate current may be prolonged by eserine, suggesting that its duration is a measure of the occupation of receptor sites by ACh (Takeuchi and Takeuchi, 1959). The spontaneously released quanta of ACh give rise to miniature endplate potentials (MEPPs) which spread electrotonically, with exponential decrement, to the neighbouring membrane of the muscle. The frequency of the miniature potentials is controlled entirely by the conditions of the presynaptic membrane, while their amplitude is controlled by the properties of the postsynaptic element (Katz, 1962). A possible exception to this occurs during prolonged tetanization of muscles treated with hemicholinium-3 (HC-3) when there is a progressive and severe diminution of the MEPPs, virtually to extinction, and a parallel diminution of the EPPs (Elmqvist and Quastel, 1965). This is attributed to reduction of the quantal size of ACh but the possibility of receptor block cannot be excluded (Martin and Orkand, 1961; Thies and Brooks, 1961).

As the MEPP is the sign of a spontaneously released quantum of ACh linking with a receptor site, so the endplate potential resulting from a nerve impulse is a measure of the number of quanta reacting with the endplate receptors. If the endplate current reaches a critical level it causes sufficient flow through the adjacent resting muscle membrane to generate an action current in it. This has a regenerative property so that the full action current of the muscle fibre results and is propagated along it, activating the contractile substance. Thus if all fibres in a muscle are depolarized simultaneously the compound action potential is more or less directly related to the accompanying twitch tension. It is because of this relationship that measurement of the synchronized action potential of the muscle is now used as a measure of muscular response in the Harvey-Masland test (1941b) in place of tension as in the original test of Jolly (1895). (Staircase phenomena are usually neglected.) It should be noted that although the amplitude of the EPP is a measure of the ACh released by a nerve impulse, the amplitude of the compound action potential of muscle to a synchronized nerve volley is not, because the full action potential is triggered at a critical threshold. Further increase in the EPP does not increase muscular activity but constitutes a "safety factor" which will be discussed below.

The muscle cell becomes repolarized by the outward passage of potassium ions and almost immediately by the expulsion of excess sodium ions by a metabolically controlled "pump". This does not normally lead to regenerative activity even though receptors may still have ACh molecules attached. There appears to be some mechanism causing temporary refractoriness but its nature is not understood (Axelsson and Thesleff, 1958).

Acetylcholine diffuses rapidly from the synaptic cleft into the extracellular space. At the receptor sites (pre- and postjunctional) it is hydrolysed at a very high rate by acetylcholinesterase (and other cholinesterases) so that the concentration of acetylcholine there falls very rapidly, permitting the muscle to respond to a rapid series of nerve impulses. It is the failure of hydrolysis which causes a brief period of stimulation to be followed by
prolonged “depolarization block” when the closely related quaternary ammonium compound decamethonium is given to the normal subject. Acetylcholine will have a similar effect if its hydrolysis is prevented by complete inhibition of cholinesterase by neostigmine and similar substances, giving rise to the paralysis described clinically as “cholinergic crisis”. The term, imprecise as it is, has some merits since the persistent block described above may be due to the insensitive state described by Axelsson and Thesleff (1958) rather than to prolonged depolarization as suggested by the early work of Burns and Paton (1951). A transmission block of this type is increased by injecting acetylcholine or an anticholinesterase drug which prolongs the absence of hydrolysis and which is unable to displace the blocking substance from receptor sites. Closely related substances, exemplified by d-tubocurarine, are capable of attaching themselves to receptor sites but do not depolarize the endplate membrane and are easily displaced by a high concentration of acetylcholine. These substances interfere with neuromuscular transmission by competing for receptors (“competitive block”) but do not stimulate contraction and transmission block is relieved by injecting acetylcholine or anticholinesterase substances. The distinction between “competitive block” and “depolarization block” is not absolute. In the monkey, dog, or rabbit administration of decamethonium causes increased motor activity suggestive of initial depolarization, which is followed by transmission block which is reversible by acetylcholine (Zaimis, 1953). This is not entirely a species difference since in the cat decamethonium produces a “depolarization block” in the tibialis and gracilis muscles, while in the soleus it appears to be initially depolarizing and then produces “competitive block”. The differing effects may be due to the former being pale muscles and the latter a red muscle (Jewell and Zaimis, 1954). In the present state of knowledge, the only difference between the endplates of red and white muscle is geometrical. For example, the soleus muscle of rabbit and monkey contains motor endplates that have a mean size significantly greater than the endplates in the gastrocnemius internus and tibialis anterior which are chiefly white muscles (Coers, 1955).

Similar geometrical differences probably account for functional differences between amphibian and mammalian muscle. Couteaux (1961) stresses the importance of the depth, separation and orientation of the subneural grooves and the distribution of acetylcholinesterase. With repetitive activation of the curarized amphibian neuromuscular junction there is an initial phase of potentiation that passes off after several volleys (Eccles and MacFarlane, 1949). If the calcium is low or magnesium high at the junction the endplate potential produced by a single impulse is greatly depressed and the potentiation by a train of stimuli is greatly increased or prolonged (del Castillo and Katz, 1954a). In the curarized mammalian neuromuscular junction the successive endplate potentials evoked by a train of nerve stimuli show progressive decrement at first, then slight recovery as potentiated release of acetylcholine partially compensates for decreasing stores (Liley and North, 1953). Under conditions where the release of ACh is
greatly depressed, as with deficient calcium or excess magnesium in the external medium, the mammalian preparation also shows the initial potentiation shown by the amphibian. Meanwhile the sensitivity of the postjunctional receptors is unchanged (Otsuka et al., 1962). It appears that both types of junction show phenomena of transmitter exhaustion and release facilitation but that in the mammalian junction a single impulse produces a maximal response and there is much less storage of ACh than in the amphibian. If the excess of acetylcholine released by a nerve impulse over that required to stimulate the muscle fibre is considered as the "safety factor" it follows that the safety factor is initially greater in the mammal but the backing reserve is lower. This suggests that the nerve impulse invades the nerve terminal and liberates transmitter more efficiently in the mammalian type of neuromuscular junction as the postsynaptic membrane apparently responds in the same way in each (Otsuka et al., 1962).

An attempt at numerical assessment of the population of synaptic vesicles in motor nerve terminals and their probable rate of discharge during repetitive stimulation is made by Eccles (1964). Taking the total length of the long finger-like nerve terminals of an amphibian neuromuscular junction as at least 500 μ and the discharge of acetylcholine due to a nerve impulse as about 200 quanta, Eccles makes an estimate of one quantum for 3 μ length of nerve terminal or 10 μ² of synaptic contact. In the rat, a similar quantal liberation occurs over an area of little more than 1 μ². In normal human muscle the mean diameter of motor endplates is 32.2 μ ± 10.5 μ with a range from 10–80 μ (Coers, 1955). Most endplates are of the plate type. The cluster type found in other mammals is rare in man but may occur in extraocular muscles (Kupfer, 1960). The linear type which is found in the frog does not normally occur in human muscle. Eccles and Jaeger (1958) have examined the factors determining efficiency of synaptic transmission. Although the amphibian neuromuscular junction is taken as representative of the motor end-organ of vertebrates it is clear that the efficiency of a synapse is largely determined by its geometry. The linear type of ending is much less efficient than the terminal knob type as the concentration of transmitter is lower and its removal by diffusion more rapid. The efficiency of the knob terminal is severely reduced if its diameter is increased. All junctions require a synaptic cleft which must be as narrow as possible compatible with removal of transmitter by diffusion. The subsynaptic membrane is also important since del Castillo and Katz (1956) have shown that individual packets of acetylcholine have an extremely localized action on it. The number of receptor areas available to a nerve ending is greatly increased without widening the synaptic cleft if the subsynaptic membrane is thrown into folds as in the vertebrate neuromuscular junction (provided the folds are not too deep). In short, the most efficient junction has a terminal knob which is not too broad, separated by a narrow synaptic cleft from a relatively large but folded subsynaptic membrane. In all of these respects the mammalian neuromuscular junction is superior to the amphibian, and this difference is sufficient to account for the functional
differences described above which, it will be noted, include differences in response to quaternary ammonium compounds as well as in response to nerve impulses. The probable importance of the density of synaptic contact in myasthenia gravis will be referred to later after an examination of the pharmacological properties of the myasthenic neuromuscular junction.

E. Reactivity of the subsynaptic membrane

Early workers, including Oppenheim (1887) and Jolly (1895), recognized a resemblance between myasthenia gravis and curare poisoning and suggested using physostigmine, the antidote to curare, in its treatment. Apparently it was used but abandoned as ineffective until reintroduced by Remen (1932) and Walker (1934). The effective use of neostigmine by Walker (1935) coincided with Dale and Feldberg's (1934) confirmation of the role of acetylcholine in neuromuscular transmission and turned all thinking on the pathogenesis of myasthenia towards the concept of a biochemical lesion causing a block of transmission at the neuromuscular junction.

The concentration of cholinesterase in the subneural apparatus is normal when estimated histochemically (Cohen and Zacks, 1959). The prompt improvement of myasthenic “fatigue” after injection of a cholinesterase inhibitor is adequate evidence that the enzyme is functionally normal and that the subsynaptic membrane is capable of maximal response to acetylcholine, but it is possible that a displaceable competitive substance with properties resembling curare could be present. The curarizing dose for myasthenic patients is only 5-10% of that of most normal people (Bennett and Cash, 1943) but Dillon and Sabawala (1959) showed that the hypersensitivity to D-tubocurarine could still be demonstrated in biopsied muscle and the effect persisted after repeated washing.

Grob et al. (1956a, b) investigated the effect of acetylcholine and choline injected directly into the arterial supply of limb muscles in normal subjects and in patients with myasthenia gravis. In normal subjects ACh produced transient stimulation of motor activity followed by a brief period of depression of neuromuscular transmission. After recovery from this “prompt” depressant effect there was a “late” and more prolonged reduction in function (Fig. 12). The “prompt” depression was increased and prolonged by the prior administration of neostigmine, and this appeared to be due to persistence of the initial depolarization caused by the injected ACh. In contrast the “late” depression was not increased. Its time course and the degree of block resembled that produced by intra-arterial injection of choline, suggesting to the authors that the “late” depression might be due to choline released by hydrolysis of ACh (Grob et al., 1956a). The “late” depression due to ACh was not reversed by further injection of ACh or by neostigmine. In normal subjects the type of transmission block produced by choline varied with the degree of block. When moderate it had the properties of the depolarization type (p. 364), resembling the “late” depression of
ACh, but when it was marked it had many of the properties of the competitive type of block (p. 364), as judged by the effect of added ACh or neostigmine and the presence of post-tetanic facilitation.

In myasthenic patients Grob et al. (1956b) found that the "prompt" depression after intra-arterial ACh was reduced and a transient improvement in transmission was interposed between the "prompt" and "late" phases of depression. Myasthenic patients also differed from the normal in that the "late" phase of depression was reversed by neostigmine or by further injection of ACh (Fig. 13). The time course of the depressant effect of choline in the myasthenic patients differed significantly from that observed in normal subjects. The latency of the depressant effect was greatly increased and the block was always of "competitive" type. The depressant effect of choline on clinically unaffected muscles was less than normal but in some of these patients the type of block was intermediate between that observed in normal subjects and clinically affected muscles in myasthenic patients. (Other pointers to a progressive series of changes from fully normal to fully myasthenic will be omitted, but they are important in indicating that the transmission at the "myasthenic" neuromuscular junction differs quantitatively rather than qualitatively from normal.) Grob et al. (1956b) consider that their results indicate the presence of "competitive block" in myasthenia. They suggest that the time-course and other properties of the "late" depressant effect of ACh in myasthenic patients indicate that it may be produced by choline released following the
hydrolysis of ACh, but they draw attention to an anomalous feature. The "late depression" caused by acetylcholine was three times as much as that caused by an equivalent mass of choline, whereas calculation of the amount of choline yielded by hydrolysis would indicate that acetylcholine should be less active. This could be accounted for by postulating that choline released by acetylcholine hydrolysis would be in closer proximity to the endplate receptors than would choline injected intra-arterially, but the possibility that the receptor substance is altered in myasthenia requires further scrutiny.

An increased sensitivity of the myasthenic patient to quinine and curare was demonstrated by Harvey and Whitehill (1937) and Bennett and Cash (1943). In contrast Paton and Zaimis (1950) suggested from a consideration of the different modes of action that a depolarizing blocking substance such as decamethonium might prove less effective in myasthenic patients than in normal subjects. This was soon confirmed by Sellick (1950) though
Dundee and Gray (1951) thought that the response was within the limits of normality. Systematic studies by Churchill-Davidson and Richardson (1952) indicated a generalized tolerance of myasthenic muscle to decamethonium iodide, particularly in those patients in whom the only clinical evidence of myasthenia was ptosis and diplopia. Indeed decamethonium sometimes increased the strength of myasthenic muscles. Grob et al. (1956c) were unable to confirm that the clinically spared muscles were unusually tolerant when decamethonium was injected intra-arterially. The tolerance described by Churchill-Davidson and Richardson was more difficult to demonstrate when the muscular "fatigue" was widespread as it was less marked in those muscles affected by the disease.

This implies that when the drug is injected the paralysis caused by it is first seen in the clinically affected muscles. Churchill-Davidson and Richardson (1952, 1953) found that the weakness so induced was rapidly and completely reversed by anticholinesterase substances and that tetanic rates of stimulation of the muscle were not so well sustained as a twitch. This was later explained by the finding of Grob et al. (1956c) that the first response to stimulation ("twitch") was more resistant than later ones ("tetanus"). Their results have been confirmed by Grob et al. (1956c) and by the writer who, however, has noted transitional states between the "normal" and the ClO resistance type of response in myasthenic patients and in other muscular disorders (Fig. 14). Decamethonium, normally a "depolarizing" blocking agent in the human, appears to act in the clinically affected muscles as a "competitive" blocking substance after a fleeting depolarization, but there is no sharp distinction between normal and abnormal. Churchill-Davidson and Richardson (1953) describe this as a "dual block", a term coined by Zaimis to describe similar actions on certain muscles in some animal species (p. 364).

As the urinary excretion rate of decamethonium is unchanged in myasthenics, Churchill-Davidson and Richardson (1952) concluded that the different action could not be accounted for by an alteration in the composition of the drug. They postulated a change in the characteristics of the subsynaptic membrane and suggested that this could be responsible for the myasthenic reaction by altering the response to ACh or one of its breakdown products. As ACh, choline, and decamethonium all act in similar ways in the myasthenic patient it is undesirable to explain the abnormal findings of Grob et al. (1956b) as being due to choline or to the production of a toxic product during nervous activity since the same change of function at the motor endplates could explain both sets of findings.

The position would be clarified by a study of the sensitivity of the endplate to ACh. The literature is conflicting in this respect. Lanari (1937) was the first to inject ACh into the brachial artery of a myasthenic patient. His opinion that the myasthenic muscle was abnormally sensitive to ACh was confirmed by Harvey and Lilienthal (1941). These authors injected 20–40 mg into the brachial artery distal to a cuff obstructing the venous return. The violent motor response which they obtained was much greater than they had
found in normal subjects and the grip-strength seemed unchanged when the spasm subsided. They concluded that the myasthenic muscle fibres retain the capacity for vigorous contraction when adequately stimulated and that they were hypersensitive to ACh applied in this way. They were drawn to this conclusion although it conflicted with their belief that there was a close analogy between the myasthenic state and partial curarization.

Acheson et al. (1948) made similar studies using 0.3–3.0 mg of ACh intra-
arterially and concluded that the sensitivity of myasthenic muscle to this substance was normal or slightly depressed. Wilson and Stoner (1917) and Engbaek (1951) concluded that the myasthenic endplate showed diminished sensitivity to ACh. Engbaek (1951) reconciled the conflicting opinions by showing that the result depended on the dose of ACh. The normal threshold dose was 100–350 μg. She found that 0.2–0.3 mg caused a motor response in most normal subjects but was insufficient to do so in myasthenic patients who required far greater doses, indicating a raised threshold. The apparent hypersensitivity reported previously was due to stimulation by an overdose which would have caused depolarization block in normal muscle. Grob et al. (1956a, b) later changed their views in agreement with Engbaek and showed that a suitable dose of intra-arterial ACh will cause rapid depolarization block of normal muscle but facilitation of activity at the myasthenic neuromuscular junction.

These studies on ACh, choline and decamethonium indicating a raised threshold to depolarizing agents could account in part for the well known tolerance of myasthenic patients to doses of anticholinesterase drugs which would be toxic in normal individuals. The writer has proposed a theory of drug action which accounts for the reported observations (Fig. 15) (Simpson, 1960). It involves a “concentration and rate theory” stressing the density of ionic charges attached to the receptor substance of the subsynaptic membrane. Thus an applied quaternary ammonium substance will be effective in depolarizing the subsynaptic membrane only if the ionic charge of the substance is adequate (Riker, 1953) and only if there is an adequate charge per unit of receptor area. If this limiting charge density is not reached (as with tubocurarine) no depolarization occurs and the receptors are blocked (“competitive block”). If an adequate charge density is built up within a limited period of time the membrane permeability is altered and a local response occurs. Should the charge density reach a critical level (lower horizontal line in Fig. 15) depolarization becomes sufficient to generate a propagating action potential and the muscle fibre twitches (p. 362). And should the charge density rise well above the critical level (upper horizontal line in Fig. 15) or should the stimulating substance be resistant to hydrolysis, depolarization persists causing a “depolarization block”.

Grob et al. (1956c) considered it to be anomalous that decamethonium does not inhibit “prompt” depolarizing action of ACh in myasthenic subjects even though its later block is of competitive type. This finding is predictable from the present hypothesis. As the concentration of depolarizing substance falls, ionic charge is lost from the receptors and the subsynaptic membrane is allowed to repolarize by the normal sodium pump mechanism, but further stimulation is prevented by “desensitization” of the membrane (p. 364). If, however, escape or destruction of the active substance is delayed and some ionic charge remains within the “critical zone” after membrane sensitivity has recovered, there will be a brief spell of facilitation as reported by Grob et al. (1956a, b). When the charge density falls below the critical level it enters the zone of
"competitive block" (shaded in Fig. 15). Muscular response to neural stimulation or to application of further depolarizing substances will be blocked until the "sticky molecules" are slowly detached. The two latter phases would be more prominent with large doses and with compounds resistant to hydrolysis. This sequence of events would account for the "dual block" of Zaimis and can be made to accommodate all the phenomena associated with the action of drugs on the endplate.

This model removes the necessity to postulate abnormal breakdown products of ACh or decamethonium in myasthenia gravis. It may now be used to study some of the possible mechanisms involved in the transmission of neural signals at the motor endplate.
failure. If the ionic charge density (not necessarily the total charge) is decreased, the "critical level" is raised [Fig. 15 (b)]. Acetylcholine produced by nerve activity or drugs injected into the artery supplying the muscle will accumulate charge on the receptor site too rapidly to permit detection of the early competitive zone, but the presence of a raised threshold would be detected (Engbaek, 1951). A higher dose which in the normal would stimulate and then cause depolarization block ("prompt depression" of Grob et al.) would cause only stimulation in the myasthenic (Grob et al., 1956b) whereas a massive dose would still cause strong contraction which may pass into a brief depolarization block (Harvey and Lilienthal, 1941). This would be followed by a brief period of facilitation and then by a phase of "competitive block" (Grob et al., 1956b) with a duration depending on the decay constants of the particular substance. Where the latter phase is marked it may be recognized as the "dual response" of Zaimis (1953). Smaller doses which merely enter the stimulation zone and then drop rapidly through the competitive zone give rise to such phenomena as "decamethonium resistance" (Churchill-Davidson and Richardson, 1952).

The model can also be used to examine the various theories for the abnormal transmission in myasthenia. These can be resolved into the factors determining the position of the "critical level" of ionic charge density for depolarization which is inversely related to the "safety factor" of transmission. From the earlier discussion it was concluded that this depends mainly on geometrical factors and attention was drawn to evidence that these could account for local and species differences in response to depolarizing drugs. From the analysis on p. 365 it is clear that a structural change of the endplate will alter the factors governing release of transmitter from the nerve terminals and the response of the subsynaptic membrane. The properties of the myasthenic junction show resemblances with the neuromuscular junction of amphibia. In this situation it is meaningless to argue whether the differences between one synapse and another are pre-or postjunctional. Unresolvable arguments of this type have bedevilled an appreciation of the transmission defect in myasthenia gravis because they are invariably based on the false presumption that the geometry of the endplate is normal.

III. Possible Causes of Lowered "Safety Factor" in Myasthenia

The raised threshold of the myasthenic postjunctional membrane could not be due to unusually high concentration of cholinesterase in the subneural apparatus as this would decrease the rate of rise of the model curve, lower the crest, and steepen the falling phase. The results which can be deduced do not fit the observed facts. By histochemical methods the concentration of cholinesterase in the subneural apparatus appears normal (Cohen and Zacks, 1959) and there is no increase in the venous blood (Stedman and Russell, 1937; Wilson et al., 1951). The raised threshold could be due to alteration of the properties of the endplate receptors, requiring a
greater charge density for activation, to simple geometrical considerations, or to occupation of receptors by molecules with negligible charge ("curare-like substance" or "myasthenic toxin").

Curare blocks receptors without stimulating. The spontaneous MEPPs retain their normal frequency but are reduced in amplitude and the EPPs following stimulation of the motor nerve are reduced in amplitude, thus lowering the "safety factor" of transmission (del Castillo and Katz, 1957). The natural facilitation-depression events of nerve stimulation thus cause a similar cycle of changes in the action potential and tension curves of the stimulated muscle. Space does not permit a review of the numerous attempts to demonstrate curare-type activity in the serum of myasthenic patients, but none of these have been convincing. The writer is equally sceptical of the Walker response (which purports to demonstrate a remote blocking effect after exercising a limb). The report of clinical improvement after haemodialysis (Stricker et al., 1960) is capable of a different interpretation. The strongest evidence for a "myasthenic toxin" transmissible through the placenta is the neonatal myasthenia sometimes found in babies born to myasthenic women. This condition lasts for 2–12 weeks, which is too long to be accounted for by transmission of a substance with molecular resemblance to tubocurarine. An alternative immunological explanation has been proffered (Simpson, 1960). Desmedt (1957, 1958) has compared the time course of muscular response to repeated supramaximal nerve stimulation in human myasthenia and in the curarized cat. The reduced safety factor in the curarized animal discloses the balance of exhaustion of readily available transmitter, facilitated release, and post-tetanic potentiation of the normal nerve terminal. Desmedt underlines the rapid restoration of the store of readily available transmitter when tetanization stops, as shown by the return of the single twitch response, and compares this with the relatively prolonged "post-activation exhaustion" commonly found in myasthenic muscle (p. 357). He interprets this as evidence of defective synthesis of depot-acetylcholine. An alternative explanation would be defective mobilization of "readily releasable fraction" from depot-ACh (p. 361). It is most unlikely that the defect is at the presynaptic membrane as a disorder here would interfere with tetanic and post-tetanic facilitation, which are normal in myasthenia gravis, and the effect of neostigmine would be negligible. In these respects there is a major difference from botulism (Brooks, 1954) and from the myasthenic reaction sometimes seen in peripheral neuropathy (Simpson, 1966a). In support of his interpretation Desmedt (1957, 1958) has shown that post-activation exhaustion of similar time-course is seen in the cat injected with hemicholinium-3. No information has been published about the responsiveness of the block to neostigmine. It is assumed by analogy with the action of hemicholinium on the sympathetic ganglion that the drug arrests synthesis of acetylcholine. At that synapse the interference is increased by physostigmine and antagonized by choline (MacIntosh et al., 1956). Some intracellular recordings from motor endplates show mainly postjunctural action of hemicholinium at neuromuscular synapses.
If the post-activation exhaustion is due to the same mechanism as the
tetanic "fatigue" the two curves should be continuous if facilitation effects
are subtracted. But they are not. It is therefore still necessary to consider
another mechanism reducing the safety factor of transmission. Desmedt
has made a valuable contribution in drawing attention to the clinical impor-
tance of post-activation exhaustion, but the tetanic decrement can not be
ignored. There are some other differences between myasthenia gravis
and hemicholinium poisoning which demand further investigation. It is,
for example, strange that if synthesis of acetylcholine is inhibited by iodo-
acetic acid the time course of recovery of neuromuscular transmission (in
the frog) is unchanged (Takeuchi, 1958). Surprisingly, there is no informa-
tion about the effect of neostigmine on hemicholinium block. If the mecha-
nism is a failure of synthesis of acetylcholine it is surprising that spon-
taneous fibrillation is not more common, as after poisoning with botulinus
toxin. By the same analogy, the entire muscle membrane would be
expected to become sensitive to applied acetylcholine (Hofmann et al.,
1964). Furthermore, the mechanism suggested must also account for the
not infrequent finding of muscles in myasthenic patients which show gross
facilitation and little "fatigue" (p. 355). These considerations prejudice
the writer against accepting Desmedt's theory of defective synthesis of
transmitter in myasthenia gravis (though it may have validity in other types
of myasthenia described by Simpson, 1966a), but it may be accepted that
post-activation exhaustion is prima facie evidence of a presynaptic disorder.

More direct evidence should be obtainable from microelectrode studies.
Elmqvist and Quastel (1965) showed that hemicholinium-3 caused pro-
gressive reduction in amplitude of MEPPs and EPPs (in the rat diaphragm)
only after nerve stimulation. As endplate responsiveness to bath-applied
carbachol was unchanged, they interpreted this as indicating a presynaptic
action leading to reduction in quantum content of ACh as presynaptic
stores became depleted. The same authors compared these findings with
microelectrode studies on intercostal muscle removed from normal and
myasthenic humans. Their first report on the latter (Dahlbäck et al.,
1961) stated that MEPPs were absent or, if found, infrequent but of normal
amplitude. They could not be evoked in the normal manner by raising the
potassium concentration. The responses to iontophoretically applied ACh
were normal and the area of chemosensitivity of normal and myasthenic
muscle fibres were the same (0.1-0.2 mm). They concluded that the
myasthenics showed failure of release of ACh. On tetanizing the myasthenic
preparation no decrementing response occurred (perhaps not surprisingly
as their cases did not have a respiratory muscle weakness) but high frequency
stimulation (100/sec) caused a lasting block of transmission with slight or
short-lasting post-tetanic facilitation. A later paper (Elmqvist et al., 1964)
contradicted the earlier report. Spontaneous junctional activity was again
absent at most endplates, but when found the frequency was normal (mea
0.22 sec). The amplitude was only one-fifth that of normal MEPPs and could be increased by neostigmine. Furthermore, indirect tetanic stimulation or raising the external potassium ion concentration did increase their frequency. The discrepancies are attributed to improvement of the recording technique. It is a general principle of interpretation of MEPP studies that the frequency of the miniature potentials is controlled entirely by the conditions of the presynaptic membrane, while their amplitude is controlled by the properties of the postsynaptic element (Katz, 1962). These results should therefore point to a postsynaptic disorder in myasthenia, but the Swedish workers prefer to interpret them as indicating lowered quantum content of ACh by analogy with their hemicholinium findings supported by studies of the depolarization of endplate regions of normal and myasthenic muscles by decamethonium and carbachol. These were both applications and not local threshold studies. In their second paper the authors admit to being unable to rule out the possibility of a postsynaptic defect in addition to a presynaptic failure. Decreased receptor density with enlargement of the chemosensitive area could account for their findings (Axelsson and Thesleff, 1959). Elmqvist (1965) considers this possibility but discounts it in view of the normal response to iontophoretic micro-application of ACh. He also states that an abnormally wide synaptic cleft or interposition of a diffusion barrier between the nerve terminals and the motor endplate would increase the rise time of the postsynaptic response, and this was not found.

The nature of the disagreement between the two papers should be noted. In the first paper the MEPPs were infrequent but of normal size; in the second they were frequent, and usually less than normal in amplitude. It is postulated that the small MEPPs were true observations missed in the first paper because of inferior recording technique. The normal-sized MEPPs actually recorded are explained away as synchronous quanta released from a damaged nerve terminal. An alternative explanation would be that normal-sized MEPPs are in fact evoked at receptor sites which are more widely separated than normal. The small potentials found in the second paper would then be MEPPs detected electrotonically by a microelectrode placed a little distance from a receptor site (Katz and Thesleff, 1957). The micro-electrode findings differed markedly from those in hemicholinium poisoning in that no changes of quantum size occurred either with prolonged stimulation or with rest. As Elmqvist (1965) points out, this would make unlikely the possibility that the defect in myasthenia is due to the inability of the acetylcholine synthesizing mechanism to keep up with release as a result of lack of substrate. The Swedish workers are aware that their interpretation is debatable and that both pre- and postsynaptic disorders may be present in myasthenia. Their quandary is only meaningful if it is assumed (as do all physiologists contributing to this field) that the structure of the endplates is normal. As suggested above, the antithesis is meaningless if the normal and myasthenic endplates differ geometrically (Simpson, 1960).

It is now possible to summarize the disorders of function at the neuromuscular junction as follows. (i) There is evidence that release of transmitter
is normal (p. 351). (ii) The disorder of function is a measure of a decreased "safety factor" which can be accounted for by altered geometry of the end-plate towards the morphology of the amphibian junction. (iii) Altered responses to depolarization and to blocking drugs can be similarly explained. (iv) The small MEPPs can be accounted for in the same way. If therefore there is evidence of such a change in the structure of the endplate, it is then suggested that no other "explanation" of the decreased safety factor is required.

IV. Histopathologic Considerations

A. Histopathology of neuromuscular junction

It was generally accepted that there is no histological abnormality of the neuromuscular junction in myasthenia gravis until the use of intravital staining with methylene blue was applied to the problem. Mott and Barrada (1923) used this method in one patient without detecting any abnormality. Either they were unlucky or, as suggested by contemporary critics, the diagnosis was erroneous, but it was by a similar method supplemented by staining for cholinesterase in the subneural apparatus that Coers and Woolf (1954, 1959) showed florid morphological changes of the intramuscular nerve endings. These changes are of two types (Fig. 16). In one, the "dystrophic" type, there is increased branching of the terminal arborization and the terminal knobs are distributed over a wider area of the muscle fibre than normal. This type is probably reactive as the related muscle fibre is usually (though not invariably) abnormal, and the same type of endplate has been found in other neuromuscular disorders. In the other type, the "dysplastic", there are few terminal knobs, and these are arranged serially along a scanty number of terminal branches ending on a long endplate region running parallel to the muscle fibre. These changes are present in 1-30\%, of endings examined in biopsy specimens from myasthenic patients and in less than 1\% in normal subjects and other neuromuscular diseases, including carcinomatous myasthenia (Coers et al., 1966). Woolf et al. (1956) and Bickerstaff and Woolf (1960) consider that the elongated terminals do not represent the primary cause of myasthenia gravis, but more likely indicate degeneration and repair of a type calculated to restore conduction. Nevertheless, the reader who has followed the argument to this point will recognize that the morphology of the "dysplastic" endings has shifted towards that of normal amphibia and so could account for all the disorders of function so far described.

Bickerstaff and Woolf (1960) and MacDermot (1960) described a remarkable degree of sprouting of the terminal axons after their emergence from the nerve bundle, resembling the collateral reinnervation which accompanies primary degeneration of the lower motor neurone, but which is also seen in dermatomyositis. Axonal sprouting was most prominent in the vicinity of a lymphorrhage, but could occur with muscle fibres which were apparently normal. MacDermot (1960) noted that regenerative sprouting frequently
Fig. 16. Methylene blue preparations of human motor nerve terminals. (a) Normal endplate. (b) Elongated endplate from a case of myasthenia gravis. (c) Motor endplate with shrunken terminal expansions from a case of myasthenia gravis resistant to neostigmine. (d) Axonal sprout with diminutive ending in case of myasthenia gravis with prominent lymphorrhages – myositic response, previously termed "dystrophic". (By courtesy of Dr A. L. Wooff.)
arose from endplate knobs (Fig. 17). These prolongations may terminate as an endplate on the same or another muscle fibre, and at this site a similar process may again be seen, giving rise to a chain of endplates connected by a single fine nerve fibre. The final elongated endplates may give off large numbers of very fine ultra-terminal sprouts. The extended endplate zone so formed may cover a distance of several hundred microns along the length of the muscle fibres involved. MacDermot (1960) and Bickerstaff and Woolf (1960) put less emphasis on the two types of response and appear to suggest

![Fig. 17. Drawings of methylene-blue stained motor endplates in human muscle.](image)
(a) Normal innervation pattern. An intramuscular nerve bundle breaking up into distal nerve fibres each supplying one motor endplate. (b) Myasthenia gravis. Abnormal swellings of the axons and myelin. Very small endplates. Tortuosity of nerves. (c) Myasthenia gravis. Abnormal swellings of the axons and myelin. Tortuosity and distal branching of nerves. Endplates elongated or small showing ultra-terminal branches. (d) Myasthenia gravis. Elongated endplates and ultra-terminal fibres. Abnormally fine beaded fibres running alone. (From MacDermot, 1960.)
that they are different aspects of the same process. They confirm the importance of the elongated endplate in myasthenia. Ultraterminal fibres have also been described in various types of neurogenic disorder in animals and man when reinnervation of muscle is taking place (Gutmann and Young, 1944; Coers and Woolf, 1959).

The neuromuscular junction has been further studied by electron-microscopy. Bickerstaff et al. (1960) were unable to detect any feature which differed from other mammalian endplates (they had no normal human controls) and in particular they remarked on the presence of numerous round or oval profiles 300–500 Å in diameter in the axoplasm of the terminal expansion of the axon. Circular structures were described in the terminal expansions of some cases, possibly replacing mitochondria. Their significance is unknown.

Zacks et al. (1962) studied muscle biopsies from five patients with myasthenia gravis and noted two types of abnormalities of the ultrastructure of the neuromuscular junction. The subsynaptic membrane within secondary synaptic clefts showed focal decrease of electron density in two specimens described as being from “chronic moderately severe myasthenia gravis”, though one was a child aged 2½ years with a somewhat uncertain diagnosis of congenital myasthenia. The second type of abnormality, noted in three patients with “rapidly progressive myasthenia or acute exacerbations of chronic myasthenia gravis”, were major degenerative changes in axon branches and subsynaptic apparatus. These authors support the concept of the workers previously quoted using the methylene blue technique that degeneration of endplates and subsequent repair may occur during the course of exacerbations and remissions of the myasthenic syndrome but, unlike them, Zacks et al. (1962) stress the importance of subsynaptic damage. A later case with improved fixation technique showed nearly complete absence of secondary synaptic clefts (Zacks, 1964). Woolf (1966) states that the number of synaptic vesicles is not reduced in the nerve terminal. He now considers that the elongated endplate is not specific but is a compensatory reaction and draws attention to shrunken endplates in muscle of myasthenics who have ceased to respond to neostigmine. Nevertheless, a combination of elongated nerve terminals and disorganization of secondary synaptic clefts would provide a satisfactory morphological explanation for the functional defects described previously (p. 365). Further advances in this field may be expected but interpretation will be impossible until more normal human material has been studied.

B. Histopathology of muscle fibres

A detailed account of the histopathology of muscle in myasthenia gravis is unnecessary for the present purpose. There is no consistent abnormality but common findings are the presence of lymphorrhages and three types of fibre atrophy, all of which are common to a number of diseases (Russell, 1953). Simpson (1960) has suggested a common antigen-antibody reaction
in each of these and interpreted them as focal myositis. More recently stress has been laid on the presence of grouped fibre atrophy (Fenichel and Shy, 1963) and atrophy of Type II fibres. Engel and McFarlin (1966) found “denervation atrophy” in 63% and “Type II fibre atrophy” in 50% of 30 patients. The diagnosis of “denervation” depends on a personal interpretation of the significance of certain histochemical appearances. It would be compatible with the more severe endplate abnormalities described above but the possibility that the latter may be secondary to a primary “myositis” cannot be excluded. The probability of defective function of contractile substance of muscle has already been referred to (p. 347). In any event, the common occurrence of abnormal muscle fibres should be better known as this accounts for the fact, unknown to clinicians of limited experience, that myasthenic weakness is not always fully reversible by anticholinesterase drugs. “Myasthenic” weakness can pass to “cholinergic” weakness with increasing dosage without passing through a phase of normal power.

V. Further Problems

Before accepting the morphological explanation two questions must be posed. First, are there other human conditions with morphologically similar endplates? Second, how does an explanation based on geometrical considerations account for the clinical fluctuations of myasthenia gravis?

At birth human motor endplates are immature, many consisting of terminal clubs and a terminal arborization, if present, is simple (Tello, 1917). Many immature endplates are seen up to the age of two years (Coers and Woolf, 1959). These geometrical features may be correlated with the functional studies of Churchill-Davidson and Wise (1963). In children under the age of six months they found that successive muscle responses to repetitive stimulation of the motor nerve showed a decrement of amplitude followed by post-tetanic facilitation. Furthermore, such infants were remarkably resistant to high doses of depolarizing drugs such as decamethonium.

The relevance of Miledi’s work on regenerating neuromuscular junctions in the frog (Miledi, 1960) will be obvious. The spontaneous MEPPs were decreased in frequency. When EPPs appeared at the regenerated endplates as the result of nerve stimulation or of iontophoretic application of ACh they were always adequate to generate spike potentials. On repetitive stimulation the progressive decrease in amplitude of the EPPs was greater than normal and this was increased by post-tetanic potentiation. Some fibres showed abrupt failure (cf. p. 348). These features bear a striking resemblance to the phenomena of the myasthenic junction as reported by Elmqvist (1965). On the other hand, Thomson et al. (1950) found that the decrementing twitch response of muscle stimulated repetitively by a regenerating nerve was made worse by ACh. This might depend on the
duration of denervation since cholinesterase may regenerate slowly. Obvi¬
ously further work is required on mammalian preparations though it is
necessary to make the reservation that experiments on healthy animals may
not reproduce the effects of degeneration and regeneration of motor nerve
terminals associated with, and perhaps secondary to, a disorder of the
muscle fibre.

With regard to the second question, the relapsing and remitting nature of
myasthenia gravis, it is immediately apparent that this could be accounted
for if the myasthenic defect is due to a balance between degeneration and
regeneration which can vary from time to time, possibly regulated by
humoral factors (Simpson, 1964b; Zacks, 1964). Some evidence of retrograde
degeneration among the terminal branches of motor axons innervating
normal muscle with regenerative sprouting is reported in the cat, rabbit and
rat (Barker and Ip, 1966). These authors suggest that normal motor endings
may have a limited life-span and be periodically replaced in normal muscle
by collateral regeneration.

The investigations of Barker and Ip (1966) also throw light on the
peculiarities of the distribution of myasthenic weakness, which is more
likely to affect ocular or bulbar muscles than those of the limbs or trunk
(Simpson, 1960). Regional differences in the morphology of endplates have
not been investigated, though Kupfer (1960) has suggested that cluster types
of endings may be seen in human extraocular muscles and the endplates of
"fast" and "slow" muscles may differ in their morphology (p. 364). Unlike
the hindlimb muscles, the extraocular muscles of the cat receive a multiple
motor innervation (Feindel et al., 1952) and preliminary observations by
Barker and Ip (1966) suggest that these may show a greater degree of axonal
sprouting. The size of the innervation ratio may be another factor affecting
the degree of sprouting, the rate of turnover being quicker for motor axons
bearing only a few endplates.

VI. Concluding Remarks

In the introduction it was pointed out that "causation" of a disease could
be discussed at five levels. This is not the place, nor is there sufficient evi¬
dence, to discuss in detail all that is known about the underlying causes
leading to myasthenia gravis, but it must be underlined that the account in
this essay has been limited to the first two levels listed on p. 346. A full
review of the pathophysiology has been followed by a briefer review of the
path-anatomical features which are shown to account for them. These aspects
of the problem are the subject of this chapter, but it would be less than complete to close
without reference to a more fundamental level of causation — what causes the suggested
degeneration/regeneration imbalance of motor endplates?

An association of pathological changes in the thymus gland with myas¬
thenia gravis has been recognized since Weigert (1901) described a patient
with a thymic tumour. Many years later it was established that operative
removal of the thymus was beneficial if carried out within a limited period from the onset of symptoms (Simpson, 1958). The relationship between thymus and muscle remained baffling but was generally thought to indicate that the thymus produced a "myasthenic toxin" with curare-like properties. Claims to extract a neuromuscular blocking substance from human thymus gland removed from myasthenic patients and normal infants (Wilson et al., 1953) or from foetal whales (Nowell et al., 1959) have not proved acceptable. Numerous claims to isolate a blocking substance from the serum of myasthenic patients have not stood up to critical scrutiny (for references see Bergh, 1953, and Nastuk et al., 1959). The most recent "positive" results by Parkes and McKinna (1966) used plasma, serum, or serum globulin prepared from patients who were under treatment with anticholinesterase drugs, and the type of block induced was a "dual block". They used the rat as a test animal and acknowledge that this animal may show a dual response to drugs which are depolarizing in the human.

A new approach to the problem was suggested by Simpson (1960). Presenting personal observations and a review of the literature, the writer suggested that myasthenia gravis was a multisystem disease with clinical and pathological evidence of a breakdown of immunological tolerance. He suggested that the thymus – at that time considered to be an endocrine gland – was concerned with the control of antibody production. As the suggestion was highly unorthodox it was shown how the concept would fit known facts and for this purpose the possibility was suggested that an antibody was produced against receptor proteins of the neuromuscular junction. This hypothesis offered an explanation for the "personal" nature of any humoral blocking substance while accounting for neonatal myasthenia occurring in infants born to some myasthenic women. Working independently from an observation that myasthenic serum could cause cytolysis of frog muscle in an organ bath (Nastuk et al., 1959) which suggested an immunological reaction, Strauss et al. (1960) found evidence of a complement fixing globulin in myasthenic serum which would bind to muscle fibres. This antibody, later confirmed by the writer and others, did not have the predicted properties but was attached to the A-band of skeletal muscle fibres and to the myoid cells of the thymus. Subsequent studies (reviewed by Simpson, 1964b, 1966b) leave no room for doubt that there is a disorder of immunological tolerance involving thyroid, stomach, and joints as well as muscle in myasthenia gravis, but the relationship between this disturbance and the abnormal endplate structure remains obscure. It is most unlikely that the Strauss antibody is the cause of the neuromuscular disorder, and current techniques have not been successful in detecting the presence of antibody fixed to the endplates (McFarlin et al., 1966).

In the light of the present review it is clear that it is not necessary to postulate a humoral blocking substance, but an immunological disorder could readily account for the degenerated endplates with abnormal regeneration, as well as for the myositic features seen in the histology. It should be remembered that current techniques are not capable of detecting humoral
or cellular antibodies in allergic neuropathies in the human or in the experimental animal.

Since Miller (1961) confirmed the importance of the thymus in immunology, the concept of "auto-immune disease" has become fashionable. That there is a breakdown of immunological tolerance seems beyond question, but it is by no means certain that "self-destructive" antibodies are responsible for the disorder of various organs. For example, the wasting syndrome after thymectomy in one-day old mice resembles "runt disease" in being associated with the absence of immunological reactivity. It is also difficult to understand how "auto-immune disease" could remit—it should escalate.

Simpson and Anderson (1956, unpublished) were unsuccessful in producing a myasthenic syndrome by immunizing mice with homologous muscle. Goldstein and Whittingham (1966) appear to have been successful in producing something very like myasthenia in guinea pigs immunized with either calf thymus or muscle (no original EMG records are reproduced). A proportion of these animals showed "experimental auto-immune thymitis". These authors consider that the thymus is affected by an immunological disorder but does not cause it. They postulate release of a neuromuscular blocking substance from the damaged thymus. This variation of the "auto-immune hypothesis" is still less than completely satisfying.

In the original presentation of the hypothesis, Simpson (1960) suggested that the thymus, under pituitary control, might play a role in tissue differentiation during embryogenesis of which the control of blood cells and plasma proteins may be a fraction which survives after birth, thus harmonizing the immunological role with earlier work on growth and differentiation. Burnet (1962) later proposed a similar theory. Szent-Györgyi et al. (1962) claim to have isolated a growth-promoting hormone (promine) and a growth-inhibiting factor (rcrine) from calf thymus. If the concept has any validity it might be extended to the regulation of breakdown and repair of organs which show a regular "turn over" of cells, with the immunological mechanism acting as a regulator or detector of tissue breakdown. Disturbance of this mechanism at motor nerve terminals may be the fundamental defect in myasthenia gravis.

References

11. DEFECT IN MYASTHENIA GRAVIS

DEFECT II: MYASTHENIA GRAVIS

EXPERIMENTAL MYASTHENIA GRAVIS

J. M. Vetters  J. A. Simpson

A. Folkaede

University Department of Pathology, Western Infirmary, Glasgow W.1, and University Department of Neurology, Institute of Neurological Sciences, Glasgow

Summary

Guineapigs were immunised with antigen prepared from fresh calf thymus or muscle mixed with equal parts of complete Freund's adjuvant (C.F.A.). When compared with control animals inoculated with C.F.A. alone there was no significant difference in neuromuscular transmission, or response to neostigmine, or in the histological appearance of thymic medulla and skeletal muscle. These findings constitute a failure to confirm an immunological hypothesis for myasthenia gravis put forward by other workers in 1966.

Introduction

In 1960 one of us presented a hypothesis on the nature of myasthenia gravis. From clinical and pathological considerations it was proposed that there was a breakdown of immunological tolerance in which the thymus was implicated. An earlier attempt at simulating the disease in the mouse by inoculation with homologous muscle and Freund's adjuvant had been unsuccessful. Goldstein and Whittingham described production of a myasthenic state in guineapigs inoculated with thymic tissue or muscle in complete Freund's adjuvant. Immunisation with both tissues evoked lymphocytic collections around Hassall's corpuscles of the guineapig thymic medulla which Goldstein and Whittingham provisionally interpreted as "experimental autoimmune thymitis". They suggested that an autoimmune reaction in the thymus liberates a humoral substance which causes the characteristic neuromuscular block. The essence
of their findings are given in two papers by Goldstein and Whittingham.\textsuperscript{3,4} Our findings do not support the claims of the Australian workers.

**Materials and Methods**

**Animals**
Outbred adult Dunkin-Hartley guineapigs weighing 500-700 g were used.

**Immunisation Procedures**
Homogenates of fresh calf thymus and muscle were prepared using the method of Goldstein and Whittingham.\textsuperscript{3} Sixteen animals were given 0.1 ml of a mixture of equal quantities of complete Freund’s adjuvant (C.F.A.) and thymus antigen into the pad of each hind foot. Twelve were given 0.1 ml of muscle extract in C.F.A. into each hind foot pad and fifteen were given 0.1 ml of C.F.A. alone into each hind foot pad. The guineapigs were numbered so that subsequent tests could be made without knowledge of the antigen given.

**Electromyography**
The median nerve was stimulated beneath the axillary fold by needle electrodes 3 mm. apart connected via a 1:1 isolation transformer (Muirhead type D-39-E) to a valve stimulator producing square-wave pulses of 0.25 millisecond duration. The electromyographic response (E.M.G.) of the flexor digitorum muscle was recorded by two needle electrodes inserted into the belly and tendon of the muscle and connected through a preamplifier of input impedance 10 MΩ, 50 pF, to a 'Tektronix 564' storage oscilloscope and to an ultraviolet recording oscillograph (Consolidated Electrodynamics type 5-127, with a 5 KHz galvanometer).

The stimulator and oscilloscope time-base were triggered from a 'Digitimer' programmer (Devices Ltd.) to produce trains of stimuli at 1, 5, 10, and 50 per second. The E.M.G. response was recorded on ultraviolet recording paper moving at 2 cm. per second and simultaneously displayed on the tektronix oscilloscope with a 10 millisecond sweep, so that the waveform of each response could be monitored. Measurements were made and normalised directly from the oscillograph records with a Gerber variable scale set to read 100 for the peak-to-peak amplitude of the first evoked muscle potential.

14 days after immunisation each animal was anaesthetised with pentobarbitone sodium 40 mg per kg body-weight by intraperitoneal injection and fixed by its limbs to a board; the electrodes were inserted. Increasing single shocks were applied to the stimulating electrode at intervals of more than 5 seconds until the maximum E.M.G. response was
obtained. The stimulus strength was then increased by 50%, and trains of stimuli lasting 3–4 seconds were applied at frequencies of 1, 5, 10, and 50 per second. Neostigmine bromide 0.2 mg. per kg. was then injected intraperitoneally; after 10 minutes the sequence of stimulation was repeated. The amplitude of the first response after neostigmine was expressed as a percentage of the first response to supramaximal stimulation before injection of neostigmine.

Histological Methods

Immediately after the E.M.G. the animals were killed and necropsy specimen of thymus and of pectoral and diaphragmatic muscles were obtained. These were fixed in 10% formal saline, processed conventionally, and embedded in paraffin wax. 6 μ sections from each thymic block were stained with hematoxylin and eosin, periodic-acid/Schiff, Masson, Heidenhain’s iron haematoxylin, and Fontana stains. Sections from muscle blocks were routinely stained by haematoxylin and eosin and other stains as indicated.

Results

Neuromuscular Transmission

The amplitude of the response to a single supramaximal stimulus did not differ significantly in the three groups of animals (“controls” and “muscle” and “thymus” immunised). The mean change in amplitude of such single responses 10 minutes after neostigmine was +2% for “controls”, −8.3% for “muscle”, and −5.5% for “thymus” immunised animals.* These results were not significantly different from the pre-neostigmine values (p > 0.05) by Student’s t test.

On stimulation at 5 and 10 per second there was no consistent decrease in successive responses in any animal. On stimulating at 50 per second some animals showed no change in the amplitude of the muscular action potential. Some animals showed progressive increment of the muscular response to the first 10–15 stimuli and the amplitude was then maintained. In a number of animals the response progressively decreased but the half-time of the decrement and the proportion of animals showing this response did not differ in the three groups. In none of these cases did the action potential decrease by 30% by the tenth stimulus, and the rate of decrement was not altered after neostigmine (fig. 1).

In a few cases in each group there was a rapid drop in the amplitude of the muscle action potential within the first ten stimuli, but if further shortening of the

* Numerical data may be obtained from J. A. S.
muscle was prevented the action potential returned by the twentieth stimulus to its former level and the further course with continued tetanisation was by one of the types of response just described. Comparing control and test groups using the Mann and Whitney test there was no significant difference in the response to tetanisation. We noted that rapid decrement in the first 10 responses was always accompanied by obvious movement of the limb and could be prevented by improving the splintage. A decrement of this nature was never reversed by neostigmine; indeed the most striking examples were recorded after neostigmine (fig. 2). Post-activation exhaustion was never observed.

**Histological Examination**

All sections of the thymus were examined and graded without knowledge of the group to which the animals belonged. Despite careful application of the
criteria described by Goldstein and Whittingham no clear division of the appearances into thymitis and non-thymitis could be made because almost all glands showed some degree of clustering of lymphocytes around the Hassall’s corpuscles (fig. 3). This is also shown in figs. 1a and 1b of Goldstein and Whittingham which showed normal guineapig thymus. To try to overcome this difficulty we classified the sections twice, using different densities of lymphocyte grouping round the Hassall’s corpuscles to fulfil the minimum requirements for inclusion in the “thymitis” group. The results from both assessments were the same; no statistical difference between the control and test groups could be demonstrated. Using the criteria most favourable to the Goldstein and Whittingham hypothesis the distribution was: control group ten out of fifteen “positive”; muscle group eight out of twelve “positive”; thymus group eight out of sixteen “positive”.

Stains for reticulin were not helpful since the glands showed a variable pattern of reticulin deposition. The “reticulin barrier” (Goldstein and Whittingham) could be seen in control animals and even when it was present in test animals it was not present throughout the gland.

Fig. 3—Normal guineapig thymus.

The densely cellular outer layer is the cortex inside which lies the medulla; this contains a cystic Hassall’s corpuscle (H) with lymphocytes clustered round it. (Hematoxylin and eosin; reduced to 1/4 of x 120).
Muscle sections from test and control groups were essentially normal.

**Discussion**

Goldstein and Whittingham's findings are important because, if substantiated, they would provide direct support for an immunological hypothesis of myasthenia gravis and offer a possibility of studying this interesting disease in an experimental preparation. Their suggestion that the basic lesion is a thymitis which causes release of a neuromuscular-blocking humoral substance is, however, materially different from the hypothesis of Simpson and others.

We have not confirmed their two main findings. We found no significant difference between controls and immunised animals in muscular response to supramaximal neural stimulation. In our opinion the first 10 responses to a fast (50 per second) train of stimuli are not suitable for comparative measurement unless it can be guaranteed that the muscle contraction is isometric. Goldstein and Whittingham used a bipolar coaxial needle electrode for recording the muscular response. But electrodes of this type have directional properties causing preferential recording of the action-potential field sensed by the bevel of the needle tip. When a motor nerve is maximally stimulated it is most unlikely that the coaxial electrodes will remain spatially related to the same muscle fibres as when the muscle is at rest. Since this type of recording is highly selective it is not suitable for estimating changes in the number of muscle fibres responding to a supramaximal nerve stimulus. For these reasons the coaxial needle electrode is not used in man for the diagnosis of myasthenia gravis. For preference there should be one electrode over or in the belly of the muscle and a reference electrode over inactive tendon, and we have used an electrode system of this type. Kaufman et al. using bipolar electrodes in a needle cannula, a system with many of the disadvantages of the coaxial electrodes, also conclude that the changes observed in immunised animals do not differ from those in controls and that they are due to movement artefacts. They agree with us that neostigmine has no significant effect and that there is no evidence for a myasthenic response in the muscles of animals immunised with thymus or muscle antigen.

With regard to the histological studies, our results show no significant difference between controls and
test groups with respect to lymphocytic clustering around Hassall's corpuscles of the thymus. Careful histological examination forces us to conclude that there is a wide spectrum of intensity of lymphocytic infiltration of the thymic medulla of guineapigs and this is without apparent pathological significance. This is in agreement with the findings of Webb.

The view that histological appearances of immunological reactivity in the thymus are not necessarily abnormal is supported by studies of human thymus gland. Middleton found structures which he identified as germinal centres in the thymus gland of 70% of young people dying suddenly. Vettes and Barclay examined thymus-gland biopsy specimens of children and young adults who had had a thoracotomy for congenital heart-disease and found germinal centres in a third of cases and rounded lymphoid aggregates in another third. These patients had no obvious disease other than the congenital cardiac lesion. We conclude that thymic changes by themselves cannot be regarded as abnormal.

We agree with Goldstein and Whittingham that the histological appearances of muscle were essentially normal in all groups. These findings differ from those of Webb who found that a significant proportion of guineapigs immunised with muscle in complete Freund's adjuvant had cellular infiltrates in skeletal muscles. Webb, however, used a more prolonged immunisation schedule and this may explain the difference between his findings and those of ourselves and Goldstein and Whittingham.

REFERENCES
Myasthenia gravis

J. A. SIMPSON

Department of Neurology, University of Glasgow, and Institute of Neurological Sciences, Glasgow, United Kingdom

The first case of myasthenia gravis was described by Thomas Willis in 1672, but it was not defined as a clinical syndrome until two centuries later with the classical papers of Erb, Goldflam and Jolly. In 1900 Campbell and Bramwell reviewed all the case-reports up to that date and since that time the disorder has become more familiar.

CLINICAL PICTURE

The characteristic feature from which its name derives is a severe weakness of muscles which comes on after exercise and which may disappear after a short rest. Weakness may affect any muscle, but most commonly the eyelids, extraocular muscles, bulbar muscles, neck, and the proximal muscles of the upper limbs. The hand, lower limb and trunk muscles are usually involved later, but in some cases they are first to be affected. The early descriptions, still appearing in textbooks, emphasised the absence of atrophy, but this is not absolute. Permanent weakness with moderate wasting occurs eventually in 10% of female and about 20% of male cases, the incidence being slightly higher if there is a thymic tumour (Simpson, 1958). This is most common in the extraocular muscles, triceps brachii, and quadriceps femoris. A small number of patients show a characteristic type of atrophy of the tongue which gives rise to three longitudinal furrows (Buzzard, 1905). Tendon reflexes are usually present and may be so brisk that clonus is produced. If a reflex is elicited repetitively, the jerk may decrease progressively until it disappears. Persistent absence of many reflexes should suggest that the weakness is due to carcinomatous myasthenia rather than myasthenia gravis.

PRECIPITATING FACTORS

The diagnosis is simple if the clinician considers myasthenia gravis from the history of variable weakness with fatigability (though it is remarkable how many patients fail to notice this aspect unless directly questioned). Most cases are missed in the early stages, being wrongly diagnosed as multiple sclerosis or hysteria. The diagnosis of hysteria is particularly common as there may be no objective signs when the patient reaches the doctor's consulting room, and because the most common precipitating factor is emotional disturbance. Other factors which appear to bring on the first attack or relapses are infection, allergy and pregnancy. Once the disease is established, weakness is greater just before menstruation, in exciting or embarrassing situations, or with extremes of heat or cold. Time does not permit me to spend longer on these points, but I wish to emphasise the rôle of emotional disturbance and conversely the beneficial effect of hope and peace of mind.

14

Reprinted from
Excerpta Medica International Congress Series No. 199
MUSCLE DISEASES
Proceedings of an International Congress, Milan, May 1969
When myasthenia is suspected, the diagnosis is established by demonstrating abnormal fatigability of muscle using simple bedside tests or an ergograph; and by restoring power to normal by an anticholinesterase drug such as edrophonium or neostigmine. Electromyography is used to demonstrate failure of neuromuscular transmission.

**NATURAL HISTORY**

Now I want to pass on to some less familiar aspects of the natural history of the disease which can only be appreciated by those who have seen a large number of cases. It occurs in every race. Estimates of prevalence range from 1 in 50,000 to 1 in 10,000 of the population. I see about 15 new cases in a year. It affects both sexes, women twice as frequently as men. In young people the ratio is 4.5 females to 1 male, but myasthenia starting in later life is commoner in males. The most common age of onset for both sexes is about 20 years. About 10% of cases have a thymic tumour. This occurs in an older group of patients (modal age 45 years). It is rarely seen under the age of 30, but is present in 30% of cases starting over the age of 40.

The characteristic course is one of relapses and remissions, but the long-term remissions are not so common as many believe. Fewer than half of the cases have a remission of a month or more and long remissions rarely occur more than once. The first 7 years appear to be the ‘active’ stage of the disease (Fig. 1). Most of the deaths directly attributable to myasthenia gravis occur during the first 7 years, particularly during the first year (Simpson, 1958). After 10 years, death from myasthenia per se rarely occurs, though the patient may be constantly at risk of asphyxiation from inhaled foreign bodies because of the diminished

![Diagram](image-url)
respiratory reserve. On the other hand, most of the significant remissions occur during this period and it is also the time when thymectomy is most profitable (Simpson, 1958). In the next stage the response to surgery is less impressive, but further progression is unlikely. Finally there may be a 'burned-out' stage in which the response to anticholinesterase drugs disappears. At that stage the clinical picture closely resembles polymyositis and has been termed 'myasthenic myopathy'.

The clinical course may be benign and the weakness may remain limited to a few muscles. If subjective symptoms are limited to the extraocular muscles and remain so for 2 years, the prognosis is good. On the other hand, if a thymic tumour can be demonstrated radiologically, the myasthenia is often difficult to control by drugs or by thymectomy.

DISORDERS OF OTHER ORGANS

A relationship between myasthenia gravis and disorders of the thyroid gland has been recognised for many years. Millikan and Haines (1953) found that 5% of cases had hyperthyroidism which did not necessarily coincide in time with the myasthenia. In my experience about 9% of males and 18% of females with myasthenia gravis show signs of a thyroid disorder at some time during their life, but the disorder need not be thyrotoxicosis. Thyrotoxic symptoms may be subsiding when myasthenia appears, and vice versa. If the period of observation is limited, this may lead to the mistaken conclusion that the two disorders have a 'see-saw' relationship to each other (Simpson, 1968).

Non-toxic goitre and, more rarely, primary myxoedema may be found and indeed most of the early reports stress the presence of non-toxic nodular goitre, and the histological appearance of lymphadenoid goitre (Ringertz, 1951). It is now recognised that many of these cases have Hashimoto's thyroiditis (Simpson, 1960, 1964, 1966a; Becker et al., 1964). Routine tests of thyroid function in myasthenic patients show that only a minority are outside normal

TABEL 1  Associated disorders in series of cases of myasthenia gravis

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma (non-thymic)</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>'Rheumatoid' arthritis</td>
<td>20</td>
<td>15</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(±1?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nephritis</td>
<td>2</td>
<td>1</td>
<td>1(?)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Raynaud's syndrome</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Psychosis</td>
<td>11</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy, etc.</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pemphigus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

...
limits and as many of these are hypothyroid as hyperthyroid (Simpson, 19666, 1968). Obviously the association is not a causative one. In my experience the incidence of thyroid disease in the families of myasthenic patients is unusually high, suggesting that the two diseases are linked by a common genetic factor (Simpson, 1958, 1960).

The incidence of diabetes mellitus in myasthenics and their relatives may also be a little higher than normal. Other endocrinopathies have been reported in myasthenic patients, but so rarely that they are probably coincidental.

It is well known that aplastic anaemia is correlated with tumours of the thymus and hence with myasthenia gravis. In fact, in my experience, pernicious anaemia is a much more common blood disorder in myasthenics. Haemolytic anaemia, lymphadenopathy, and splenomegaly are occasional associates, suggesting that the reticuloendothelial system may be abnormal. Other disorders occurring in a personal and in other series are shown in Table I. An arthropathy resembling rheumatoid arthritis is not uncommon. It is often transient and may precede or follow the myasthenic illness. Acrocyanosis is also common, resembling polymyositis in this respect. Other disorders such as Sjögren’s disease, pemphigus and ulcerative colitis are rare and do not individually approach 'statistical significance', but it may be considered meaningful that the group of disorders listed occurs in several series although these are very small samples of the general population. There is increasing evidence that the factor common to these disorders is a disturbance of immunological tolerance.

AUTOIMMUNE HYPOTHESIS

The factors which led me to propose the autoimmune hypothesis for myasthenia gravis (Simpson, 1960) were as follows:

1. The age and sex incidence, and the natural history of myasthenia gravis were strikingly similar to systemic lupus erythematosus as described by Harvey et al. (1954).
2. There was some reason to consider that myasthenia is a multisystem disease.
3. The thymus gland is usually abnormal. There has been a lot of misunderstanding about this. Some textbooks describe ‘thymic hypertrophy’ and others ‘failure of atrophy’ such as a normal gland is assumed to undergo. Both descriptions are wrong. The glands removed from myasthenic patients are rarely larger than normal and the change of size with age is within normal limits. The characteristic change is in the presence of ‘germinal centres’ in the cortex and medulla (Castleman and Norris, 1949). The epithelial cells are not proliferated unless there is a tumour. Even if there is a thymoma, the surrounding lymphoid tissue commonly shows the germinal centres which are typical of the disease. In 1960 the thymus was generally accepted as an endocrine gland, but it seemed to me to have the appearance of an active lymph organ associated with immunological reactions.
4. The muscles commonly showed the lymphocytic infiltrations described by Weigert (1901) and named ‘lymphorrhages’ by Buzzard (1905). The latter author also described degenerative changes of muscle fibres. These were classified into three types by Russell (1953) who considered that they were non-specific on account of similar appearances occurring in the muscles in certain rheumatic-type diseases and endocrine myopathies. Simpson (1960) suggested that the lymphorrhage was very suggestive of an immunological reaction and that an autoimmune mechanism could be present in each of the diseases listed by Russell.
5. The concept of an autoimmune disease would account for one of the most curious facts about the disease. If a myasthenic woman has a child, the baby may have myasthenia at birth in about one in seven live births, but if the affected child survives it recovers completely in 1-12 weeks without later relapse. This strongly suggests that some ‘myasthenic toxin’ can pass the placenta, but all attempts to transmit the defect to another adult by cross-transfusion have failed. This could be accounted for if the toxic substance was an antibody against some
tissue — muscle or nerve — with common antigens in mother and child. The duration of neonatal myasthenia would fit this concept admirably.

6. While I was investigating this hypothesis, Nastuk et al. (1959) reported that, in common with most earlier experimenters, they had not succeeded in demonstrating a curariform substance in myasthenic serum, but in the course of their experiments they had noticed that the serum caused lysis of frog muscle cells, a reaction later shown to be associated with fixation of complement.

7. Finally, it had been known for a number of years that administration of cortisone to myasthenic patients may cause remission after a temporary deterioration.

These seven points seemed to me to justify publication of the autoimmune hypothesis in 1960, although the concept required that the thymus should have an immunological rather than a hormonal function. Almost simultaneously Strauss et al. (1960) published their further studies on the cytolytic effect of myasthenic serum on skeletal muscle. Using a fluorescent technique, they were able to demonstrate that this was associated with the presence in the serum of an antibody against muscle. My colleagues and I had been searching for an antibody of this type for the previous 4 years, but without success. In the light of later work it is now clear that we were unfortunate in not using serum from patients with a thymoma, who have higher titres of antibody. It is impossible to review the many contributions to the subject in the time available. It appears to us in Glasgow that many workers do not distinguish between A band fluorescence, which Vetters (1965) considers is virtually confined to cases with a thymoma, and 1 band fluorescence which is not confined to myasthenia gravis. We were also able to show, in common with other workers, that many myasthenic patients have abnormal serum antibodies against thyroid and gastric mucosa, and antinuclear factor is commonly present in their serum (Simpson, 1964, 1966a). Gammaglobin is commonly increased in the blood and, less commonly, in the cerebrospinal fluid (Simpson, 1966b). These findings, which would not be deduced from the Nastuk-Strauss experiments, are strong support for the concept of a breakdown of immunological tolerance involving many organs. When Miller published his well-known studies on the immunological function of the thymus in 1961, the concept became less heretical. For the first time a logical connection had been established between the thymus and the muscular disturbance of myasthenia gravis.

The manner in which the neuromuscular junction is involved remains unknown. In the original hypothesis I proposed that an antibody against the protein of the receptor substance of the muscle end plate could function as a very individual competitive blocking substance, but there is no evidence that this is the mechanism (McFarlin et al., 1966). It seems unlikely that the antibody which fixes on the A band of muscle is responsible for the transmission defect, though it may be responsible for the myositic type of abnormality which is probably more common in association with a thymoma. Goldstein and Whittingham (1966) claim to have produced thymitis in guinea pigs immunized with thymus or muscle and postulate that the abnormal gland produces a substance which blocks neuromuscular transmission. We have not been able to confirm their findings (Vetters et al., 1960).

NEUROMUSCULAR TRANSMISSION

In 1895 Jolly showed that the pathological fatigability of myasthenia gravis could be reproduced by faradic stimulation of motor nerve while the ‘fatigued’ muscle would still respond fully to locally applied galvanism.

If electromyography is used to record the muscular response, it is seen that there are two effects of tetanic stimulation: firstly a progressive decrement of response, and secondly a facilitation which may temporarily restore transmission, especially with rapid rates of stimulation. Desmedt (1957) has analysed the late phase of 'post-tetanic exhaustion' which persists
for a long period after any temporary facilitation has passed off. He considers that it is the cause of the clinical weakness and that it differs from the block caused in normal neuromuscular transmission by curare. In his opinion the type of block is best explained by a failure of acetylcholine production at the nerve terminals.

On the other hand, Churchill-Davidson and Richardson (1952) showed that the neuromuscular blocking drug decamethonium did not have its normal depolarising action in myasthenia. Instead it produced a curare-type competitive block, sometimes preceded by a brief depolarisation block ('dual response'). Grob et al. (1956) showed that the action of acetylcholine and of choline was altered in a similar manner. These results pointed to a postsynaptic change, probably involving receptor sites.

Elmqvist (1965) carried out a series of microelectrode studies on an isolated nerve-muscle preparation obtained by biopsy of myasthenic patients. He considered that miniature end-plate potentials were reduced in size, indicating that there was a reduced quantum content of acetylcholine at the motor nerve endings. The present trend of opinion is therefore in favour of a postsynaptic abnormality. In the pharmacological studies just described, I have briefly indicated previously (Simpson, 1960), I consider that there are both pre- and postsynaptic changes in myasthenia gravis and that all the known findings can be explained on structural grounds. There is only time for a summary of these, but I have published a detailed analysis elsewhere (Simpson, 1969).

PATHOLOGY OF THE NEUROMUSCULAR JUNCTION

Although it had been known since the beginning of this century that the thymus was often abnormal and that lymphorrhages frequently occurred in the muscles, it was usual to classify myasthenia as a 'functional' disorder of synaptic transmission, because in the thirty years following the demonstration of the therapeutic value of physostigmine and its analogues most writers considered that the block must be due to a circulating substance like curare, and therefore the histological changes must be non-specific. But as we have seen, they are exactly the sort of pathology one would expect in an autoimmune disease. Further, it is incorrect to assume that there is no anatomical defect of the neuromuscular junction since Coers and Woolf (1959) have shown by special supravital staining techniques that the motor nerve endings are usually abnormal. They describe two types. The 'dystrophic type' has sprouting of subterminal axons and terminal knobs which are shrunken and distributed over a wider area of the muscle fibre than normal. It is probably secondary to changes in the muscle fibre and is often related to a lymphorrhage. The 'dysplastic type' has few terminal knobs and these are arranged serially along a scanty number of terminal branches ending in a remarkably elongated end-plate region. It has not yet been established that these changes are specific, but I consider that they can account for the known facts about transmission in the myasthenic (Simpson, 1969). Electron microscopy has not added significantly to our knowledge at the time of writing.

TREATMENT

The first thymectomy for myasthenia gravis was performed by Sauerbruch in 1912, but there was little interest in the operation until Blalock et al. (1941) reported a series. The value of the operation was hotly debated for the next 17 years. Later American reports were discouraging, although Keynes (1946, 1955) found it a valuable procedure. An independent study by Simpson (1958) confirmed the claims of Keynes that the operation was valuable if no thymoma was present and review of the various American statistics showed that they were consistent with this. Eaton and Clagett (1955) acknowledged that their earlier unfavourable
opinion had been due to failure to make this differentiation. Simpson (1958) showed that thyromectomy was most beneficial in women with a myasthenic history not exceeding 7 years, though some patients were improved by later operation. Male patients showed the same trend, though the numbers were too few to reach statistical significance. Some patients with a thymoma were also strikingly improved, but the prognosis remained poorer if the patient had a thymoma. Keynes (1955) advised radiotherapy before operation for a thymoma, but this is not necessary in the absence of a tumour.

Radiotherapy alone or carotid sinus denervation are inferior methods of causing atrophy of the thymus and I do not recommend them. Radiotherapy may cause temporary but dangerous increase of myasthenic symptoms and the same is true of steroid therapy, possibly due to thymolysis.

There is often an immediate improvement after thyromectomy. If the physician is not alert to this possibility the patient may be plunged into a cholinergic crisis by a dose of anticholinesterase drug which had previously been ineffective. This is particularly dangerous about 48 hours after operation. Some patients maintain this improvement and may be cured of their myasthenia or require a greatly reduced dose. More often, the dose requirement increases again to about the preoperative level. After a few months the patient may be disappointed with the result of thyromectomy. However, if the same patient is questioned two or three years later he will usually state that 'the tide turned' at the time of the operation. Deterioration stopped, the myasthenia stabilised and then improvement occurred slowly but progressively. It is for this reason that I have advised thyromectomy for all cases of less than 7 years' duration, and for later cases showing deterioration or unsatisfactory response to drug therapy.

My reason for this advice has been that the patient has nothing to lose if he is in skilled hands. Working closely with two thoracic surgeons (in Edinburgh and later in Glasgow) there have been no post-operative deaths in patients under my care during the last 13 years. On the other hand it is only fair to point out that there have been striking improvements in the medical care of the myasthenic patient since 1956 when I compared the results of thyromectomy with medical treatment (Simpson, 1958). Since that date there has been wider use of pyridostigmine, better recognition of the difference between myasthenic and cholinergic crisis, greater familiarity with the use of edrophonium to evaluate the cause of weakness, and tremendous advances in the management of ventilatory failure. It might well be that these advances have restored the balance in favour of drug therapy. Unfortunately I do not have time to describe these advances today. I still advise operation because it seems to me to provide the best chance of cure without the necessity for continuous medication, but the time has certainly come to re-evaluate the relative merits of thyromectomy and medical care.

REFERENCES


Desmedt, J.E. (1957): Nature of the defect of neuromuscular transmission in myasthenic patients. 'Post-tetanic exhaustion'. Nature (Lond.), 179, 156.


J. A. SIMPSON


The Drug Treatment of Myasthenia Gravis

J. A. Simpson, MD, FRCP (Lond., Ed. & Glas.),
FRS Ed,
Institute of Neurological Sciences,
University of Glasgow.

Myasthenia gravis is a disease of unknown aetiology characterized by impairment of transmission at the motor nerve junctions in skeletal muscle. There are two main lines of treatment (a) anti-cholinesterase drugs, which form the subject of this article, and (b) thymectomy which can be dramatically effective in some cases, usually those of less than 6 years' duration.

The Pharmacological Basis of Drug Treatment

Acetylcholine, the chemical transmitter at the motor nerve end-plate in skeletal muscle, is destroyed by cholinesterase which is present in the vicinity of the neuromuscular junction. If cholinesterase is inhibited the activity of the released acetylcholine is increased and prolonged, and this is the pharmacological action which is made use of in the treatment of myasthenia gravis.

There are several factors that limit the efficacy of this treatment. If too much acetylcholine is present there is over-depolarization of the neuromuscular junction, which results in blockade of transmission of further nerve impulses, i.e., the so-called "depolarization block" or "cholinergic crisis". This state is sometimes reached without 9
passing through a phase of normal muscle power. In severe myasthenia gravis it is not possible to restore muscular power to normal with anti-cholinesterase drugs and the degree of transmission failure varies from one muscle to another. Certain muscles, such as those concerned in respiration, may be in "depolarization block" while others (for instance the eye muscles) remain under-dosed or have reached a stage where no drug will improve junctional transmission. Failure to recognize these points leads to over-dosage in an attempt to overcome supposed "neostigmine resistance".

Choice of Anti-Cholinesterase Drugs

Neostigmine: This drug is often used as an intravenous injection by anaesthetists, but should only be used by this route for the treatment of myasthenia gravis in an emergency. Intravenous administration is dangerous. Intramuscular or subcutaneous injection of neostigmine may be useful in the initial stages of treatment as a guide to the likely effective oral dosage. Crushed neostigmine tablets can be given by gastric tube if dysphagia prevents normal administration by mouth. The drug is incompletely and irregularly absorbed from the gut. Following a dose of the tablets there is a surge of muscular power for 30-60 minutes followed by continued activity at a lower level for 2-6 hours, after which strength is lost rapidly. This makes it difficult to adjust timing of dosage for a smooth control, but if the tablets are given half-an-hour before a meal, or in anticipation of a special effort, the "boost" is valuable. The usual dose is 15 mg orally.

Pyridostigmine ('Mestinon'): The duration of activity of this drug is not substantially longer than that of
neostigmine, but it does not have the early peak effect and its activity wanes more slowly, thus allowing a sustained level of activity by judiciously timed dosage. This is the drug preferred by most patients. The usual dose is 60 mg. Some patients who require the “boost” provided by neostigmine are best treated with a combination of the two.

Ambenonium Chloride ("Mytelase"): The duration of action of the 25 mg tablet of this drug is slightly longer than that of 60 mg pyridostigmine. This increases the risk of accumulation, which may be difficult to detect as the side effects are less prominent than with pyridostigmine. Ambenonium chloride may be a valuable alternative for patients who would otherwise have to be roused from sleep for medication. It is available in the United Kingdom as 10 mg tablets (25 mg tablets are available overseas).

Adjustment of Dose
All of these drugs are active in inhibiting cholinesterase at other sites in the body and they cause side effects due to over-activity of the parasympathetic nervous system. These include abdominal cramps, diarrhoea and excessive salivation. Atropine can ameliorate these symptoms, but should not be used routinely.

Whichever drug is selected the duration of activity of a single dose must first be established, because this varies from about 2 up to about 8 hours in individual patients. The duration of action determines the frequency of dosing, which must then be fitted to the day’s programme for meal times and other activity. The amount of each dose should then be
adjusted to give optimal strength about one hour after administration. Some day-to-day fluctuation is common, but I do not advise frequent changes of dosage or leaving it to the patient’s choice, a way which leads inevitably to “cholinergic crisis” due to the patient’s reliance on taking too many tablets too often.

A patient with disease of average severity requires 8-10 tablets per day of one of the anti-cholinesterase drugs. At a level of 18-20 tablets daily there is a danger of cholinergic block, particularly with the longer-acting drugs. As these are slowly cumulative the signs of over-dosage may become apparent after 3-4 weeks of unchanged dosage. The onset of a “cholinergic crisis” may be concealed if atropine is used routinely because this drug blocks the parasympathomimetic action of anti-cholinesterase drugs without affecting neuromuscular activity. “Neostigmine resistance” does exist in the later stages of myasthenia, but there is no doubt that many cases so termed have a cholinergic block. One suspects that myasthenic patients may suffer more from over-dosage than from under-dosage.

Experience is required to obtain optimal control. In an early stage of the illness an appropriate level of anti-cholinesterase medication will restore strength to normal; but later it is not possible to obtain normal power and an increase in dose may cause further deterioration as “depolarization block” develops. One must be careful not to overdose the bulbar and respiratory muscles while attempting to improve the extra-ocular ones.

The pupil size is a valuable guide. To be on the safe side the pupils should not be smaller than 3 mm.
in diameter in normal room lighting. This valuable sign is lost if atropine is used.

Assessment of Weakness Not Responding to Treatment

First consider the daily dosage and the timing of deterioration. If the dosage of the drugs described above is not greater than 15 tablets daily, and if the pupil diameters exceed 3 mm, it is unlikely that the weakness is cholinergic. Next enquire carefully about the effect of the last few doses. Weakness which increased 2 or more hours after a dose, and which was temporarily arrested by the next dose, is probably myasthenic. On the other hand, weakness which was marked about one hour after a dose and was not significantly improved by the next dose is almost certainly cholinergic. Despite careful supervision it is inevitable that some patients will be over-dosed. The usual causes are (1) the patient altering the dose; (2) cumulation of long-acting drugs; (3) miscalculation of equivalent dosage when substituting parenteral for oral medication; (4) increase in medication after achieving optimal though still sub-normal power; (5) sudden decrease of drug requirements, notably in the 48 hours following thymectomy.

Management of Anti-Cholinesterase Overdose

No further anti-cholinesterase medication should be given until there is clinical evidence of a return to a myasthenic state. Respiration should be safeguarded by a cuffed tube intubation or tracheostomy, with positive pressure ventilation if required. If signs of parasympathetic over-activity are marked, atropine sulphate 2 mg should be injected intravenously every 60 minutes until the patient is
obviously fully atropinized or is improving. (Oxime drugs such as Pralidoxime-P2S are of no practical value as antidotes in this situation.)

When the patient appears to have returned to a myasthenic state the diagnosis should be confirmed with a test dose of edrophonium ('Tensilon'). The patient should then resume anti-cholinesterase treatment at the same intervals as before, but with half the previous dose. A new level of dosage is then gradually re-established, as for a new case.

The main risk facing a myasthenic patient is respiratory failure due to weakness of the respiratory muscles. This may occur because of a severe myasthenic state or during a "cholinergic crisis", also when either of these conditions is complicated by respiratory infection. Few cases of myasthenia die if respiratory function is adequately safeguarded. All are at risk if it is not.

Contraindications
In my opinion no myasthenic patient should receive an enema as this may cause sudden death.

Drugs which potentiate neuromuscular weakness should be avoided. These include streptomycin, neomycin, quinine, quinidine and chlorpromazine. If sedation or analgesia is necessary, it is important to avoid such drugs as morphine which may depress respiration.

Further Reading
PATHIOGENESIS OF MYASTHENIA GRAVIS


Sun.—As your unsigned leading articles tend to acquire the authority of holy writ I hope you will permit me to comment on your first leading article (3 April, p. 1) to correct some misunderstandings which have appeared elsewhere. You kindly refer to my paper giving the first fully formulated autoimmune hypothesis but infer that this was confirmation of earlier work by demonstrating clinical relationships with other autoimmune diseases. In 1960 this was hardly possible (and Miller's paper on the thymus was still a year off). It showed that myasthenia gravis was probably a multisystem disease with some resemblances to the natural history of systemic lupus erythematosus. An autoimmune hypothesis was presented to account for this. In the next decade many of the associated conditions have subsequently been recognized as autoimmune.

Reference to the original paper will show that it reported the conclusions of five years of clinical and experimental work, freely discussed in teaching and research seminars. The passing reference of Smithers, a few months before final publication, to the possible implication of autoimmunity in the thymic changes of myasthenia gravis was referred to, but that paper offered no concept of the nature of the neuromuscular disorder.

It is not possible to decide whether Smithers considered that the thymic pathology was the cause or the result of autoimmune disease. This is a critical point in current thinking about myasthenia. It is unfortunate that your leading article ignored the valuable symposium at the New York Academy of Sciences in December 1970. It quotes the only group of workers supporting Goldstein's experimental work. To the longer list of negative findings will be added a paper (in press) from an Australian group which includes Goldstein's original collaborator. My original hypothesis was that a breakdown of immunological tolerance resulted from a thymic disorder (genetic or acquired). The Goldstein hypothesis considers that the thymus is damaged immunologically without indicating where the primary immunological disorder may occur and the theory requires a neuromuscular blocking substance, which is against all the evidence. The theory lacks the heuristic value of the original hypothesis, for which no incompatible evidence has yet been produced, as it does not predict involvement of other tissues—the very reason for conceiving an autoimmune theory—I am, etc,

J. A. SIMPSON

Southern General Hospital, Glasgow, S.W.1

2 Smithers, W. V., Journal of the Faculty of Radiologists, 1969, 10, 5.
A MORPHOLOGICAL EXPLANATION OF THE TRANSMISSION DEFECT IN MYASTHENIA GRAVIS*

John A. Simpson

Department of Neurology, University of Glasgow
Institute of Neurological Sciences, Southern General Hospital
Glasgow, Scotland

Since the cholinergic mechanism of neuromuscular transmission was accepted before the second World War, it has been the custom to introduce a paper on the nature of the transmission failure in myasthenia gravis with an account of the normal mechanism and the sites at which transmission can be blocked by a number of pharmacological agents. Further argument is then by analogy, and the author of each paper stresses that aspect of experimental pharmacology that most closely resembles his findings in myasthenia gravis. In this way, earlier workers were impressed with some resemblances between the myasthenic phenomena and curarization. The search for a "myasthenic toxin" possessing curare-like properties has been long and fruitless. The hypersensitivity of biopsied myasthenic muscle to D-tubocurarine persists after repeated washing.

A variant of the circulating toxin theme is that the transmitter substance may be abnormal. Thus, Grob and coworkers postulated that choline or a toxic product of nerve activity might be responsible for certain disturbances of function on the grounds that these could be mimicked by close-arterial injection of choline into myasthenic muscle. The abnormalities noted were apparent anomalies of the immediate and delayed depression of muscle responsiveness caused by acetylcholine (ACh). However, these anomalies were qualitatively similar to the altered responses found with other depolarizing quaternary ammonium substances. Churchill-Davidson and Richardson considered that the different action of decamethonium in the myasthenic subject could best be accounted for by a change in the subsynaptic membrane. On the other hand, Desmedt found that the postactivation exhaustion which he studied in myasthenics was closely comparable to the effect of hemicholinium-3, which has its principal activity in blocking synthesis of acetylcholine.

This is not an exhaustive review, but it is sufficient to indicate that three main theories have emerged: (1) the circulating curare-like substance; (2) the postsynaptic defect; (3) the presynaptic defect. Each of these has been constructed from a pharmacological analogy. It is assumed that all mammalian neuromuscular junctions show the same pharmacological reactions. (Indeed, there would be no reason to think otherwise if one consulted the three recent major monographs on synaptic transmission.)

In fact, with the possible exception of postactivation exhaustion, the phenomena of myasthenia gravis only indicate reduction of the "safety factor" for transmission that could be accounted for by either postsynaptic or presynaptic mechanisms. (In my opinion, the circulating curare-like substance is a myth.) However, the argument is meaningless if the geometry of the endplate is altered, since changes are both pre- and postsynaptic. Furthermore, we must find an explanation that will take account of the relapsing and remitting nature of myasthenia gravis and the peculiarities of its asymmetrical distribution in the skeletal musculature.

* Supported by a grant from the Muscular Dystrophy Group of Great Britain.
Using the technique of intravitral staining with methylene blue, Coers and Woolf showed two types of florid morphological changes of the intramuscular nerve endings. In the "dystrophic" type there is increased branching of the terminal arborization, and the terminal knobs are distributed over a wider area of the muscle fiber than normal. The other type, the "dysplastic," has few terminal knobs, and these are arranged serially along a scantly number of terminal branches ending on a long end-plate region running parallel to the muscle fiber. Coers and coworkers found these changes in 1-30% of endings examined in biopsy specimens from myasthenic subjects. In normal subjects and in other neuromuscular diseases the elongated end plates occurred in less than 1% of biopsies. The dystrophic type is, however, found in other disorders and is considered to be reactive, since the related muscle fiber is usually abnormal in these diseases and in myasthenia gravis. MacDermot and Bickerstaff and Woolf infer that the two types of response are different aspects of one process, which is reparative, in order to restore conduction. They described a remarkable degree of sprouting of the terminal axons after emergence from the nerve bundle and also from the end-plate knobs. These prolongations may terminate as an end-plate on the same or on another muscle fiber, and at this site a similar process may again be seen, giving rise to a chain of end plates connected by a single, fine nerve fiber. Very fine ultraterminal sprouts may be present at the final elongated end plate.

Similar ultraterminal sprouting occurs in various types of neurogenic disorder in animals and in man when reinnervation of muscle is taking place. It also occurs during recovery from poisoning with botulinum toxin. Botulinum intoxication is generally considered to block release of acetylcholine at nerve endings. Much that has been written about it requires reconsideration in view of the structural changes recently discovered at the end plates.

Changes of ultrastructure of the neuromuscular junction in myasthenia gravis are also reported but will not be reviewed, since they are based on few cases that disagree in regard to findings. The light-microscopy findings, however, show three important features. (1) The extended end-plate zone, which may cover a distance of several hundred microns along the length of the muscle fibers involved. (2) Active axonal regeneration is common and is of a type associated with disorders of the motor neuron. (3) Muscle fiber atrophy of different types is common. It may be grouped as denervation atrophy.

These findings indicate structural changes on both sides of the neuromuscular junction. Functional conclusions based on the pharmacology of the normal junction are not necessarily relevant in this situation. Is it possible that the geometrical changes of end-plate structure could alone account for the myasthenic phenomena?

The Effect of Synaptic Geometry on Function

The most compelling argument for the postsynaptic theory of myasthenia has been the abnormal response of the myasthenic subject to quaternary ammonium compounds such as acetylcholine and choline and decamethonium. In the normal human subject, decamethonium causes potentiation of the maximal twitch, followed by transmission failure which is nondecremental and is potentiated by anticholinesterase agents (caused depolarization block, although the mechanism is debatable). In some muscles of many mammals, including the monkey, dog, rabbit, hare and guinea pig, the action of decamethonium changes during the blocking process into that of a substance competing with ACh, such as curare.
This mode of action was described as a dual response by Zaimis. In the competitive phase a tetanus, though not well sustained, antagonizes the block and so does neostigmine. Muscles showing the dual response are relatively insensitive to decamethonium (and suxamethonium). This is not entirely a species difference since, in the cat, decamethonium causes a depolarization block in the tibialis and gracilis muscles, whereas in the soleus it appears to be initially depolarizing and then produces competitive block. Jewell and Zaimis suggested that the different effects may be due to the first group being pale muscles and the second a red muscle. Simpson pointed out that this also correlated with a difference of end-plate structure as described by Coers, and suggested that similar geometrical features of the immature end plate could account for the myasthenic response and resistance to depolarizing drugs shown by the muscles of human infants. A recent study leads to the conclusion that the development of a differential response to succinylcholine in the fast and slow twitch muscle of the kitten depends on morphological differences in end plates, including surface area.

Similar geometric differences probably account for functional differences between amphibian and mammalian muscle, including the different response of end-plate potentials in the curarized preparation. It appears that transmitter is liberated more efficiently by the mammalian than by the amphibian motor nerve terminal. The mammal has a greater initial safety factor but a lower backing reserve.

**Efficiency of Synaptic Transmission**

The factors determining efficiency of synaptic transmission were analyzed by Eccles and Jaeger, who found that the linear type of ending, represented by the amphibian neuromuscular junction, is much less efficient than the terminal knob type since the concentration of transmitter is lower and its removal by diffusion more rapid. Above a certain optimal size, increasing the diameter of the knob terminal severely reduces its efficiency.

The size of the prejunctional surface is not the only factor which is important in the geometry of the neuromuscular junction. The width of the synaptic cleft must be as narrow as possible, and yet sufficiently wide to allow transmitter substance to escape by diffusion.

With regard to the subsynaptic membrane, this should provide many receptor areas close to the site of ACh release. This implies a folded subsynaptic membrane, but the folds must not be too deep or too shallow. More information about the secondary folds in myasthenic end plates is essential, since this could be the most critical factor.

In all of these respects the terminal knob motor ending of the normal mammal is superior to the elongated ending of the amphibian. It allows a greater charge-density to gather at receptor sites.

Eccles has calculated that in the amphibian end plate, one nerve impulse liberates about one quantum of ACh for 3μ length of nerve terminal or 10μ of synaptic contact compared with 1μ in the rat.

In the human, most neuromuscular junctions are of the plate type with diameter ranging from 10–80μ. The cluster type found in other mammals is rare in man, but may occur in extraocular muscles. I am seeking evidence that local differences in end plate structure may account for the distribution of myasthenic weakness and the selective vulnerability of certain muscles to curare-like substances, but I do not have results to report at this time. The linear type of ending does not seem to occur in the normal human. It is, however, characteristic of
myasthenia gravis and this would account for the loss of safety factor of transmission, with assumption of characteristics resembling the amphibian junction.7

The Diminished Quantum Theory

There has been a noticeable omission in the presentation up to this point. Elmqvist24 reported that the miniature end-plate potentials (MEPPs) of human myasthenic muscle (biopsy of intercostal muscle) are abnormally small and that this must be due to a smaller content of ACh in each quantum liberated spontaneously, since the postsynaptic membrane responds normally. The first report of the Swedish group25 stated that MEPPs were either absent or infrequent, but of normal amplitude. They could not be evoked in the normal manner by raising the potassium concentration. In a later paper,26 spontaneous junctional activity was again reported absent at most end plates but, when present, the frequency was normal and the amplitude was only one-fifth that of normal MEPPs. Amplitude could be increased by neostigmine. Normal frequency with lowered amplitude of MEPPs normally indicates a postsynaptic disorder.27 This conclusion is rejected by Elmqvist because he found normal depolarization of end-plate regions by decamethonium and carbachol in bath applications and normal response to iontophoretic microapplication of ACh.

The normal sized MEPPs recorded by Dahlbäck and coworkers25 were subsequently attributed to synchronous quanta released from a damaged nerve terminal and the small MEPPs were accepted as true observations. An alternative explanation (FIGURE 1) might be that normal sized MEPPs are evoked at receptor sites which are more widely separated than normal and are then con-

![Diagram](image-url)

**Figure 1.** Diagram to represent spontaneous release of quanta of ACh at motor nerve terminals. The ACh is presumed to be stored in vesicles grouped at elective release sites. Receptors are presumed to be more evenly distributed on the subsynaptic membrane. The wider spacing of receptors indicated in the author's hypothesis is not an essential part. It is introduced to account for the altered reactivity of the subsynaptic membrane to depolarizing drugs.
ducted electrotonically to the recording microelectrode. Elmqvist\(^2\) believes that the small MEPPs indicate small quanta of ACh as in hemicholinium poisoning of nerve but, unlike the latter, they are not altered in size by prolonged stimulation or with rest. This would make it unlikely that the defect in myasthenia is due to the inability of the ACh synthesizing mechanism to keep up with release because of lack of substrate. The release of ACh at nerve terminals appears to be normal, including the facilitation by a tetanus. The interpretation in terms of small quanta is unnecessary if the geometrical abnormality of the end plate is recognized. The onus of proof rests with the proposer of disorder of function, which would be unique in natural pathology.

**Regeneration of End Plates and the Role of the Thymus**

There are few studies on the function of regenerating neuromuscular junctions, but those of Miledi\(^2\) on the frog closely resemble the findings of Elmqvist\(^2\) on myasthenic muscle, except that small MEPPs were not noted. Barker and Ip\(^3\) found some evidence of retrograde degeneration among the terminal branches of motor axons innervating normal muscle in the cat, rabbit and rat. They also found regenerative sprouting at end plates which, they suggested, might indicate that normal motor endings may have a limited life-span and be periodically replaced by collateral regeneration. They found a greater degree of axonal sprouting in muscles receiving a multiple motor innervation, such as the extracocular muscles of the cat.

This evidence that the motor nerve terminals are included with the many other organs which show cycles of breakdown and regeneration requires confirmation. It is interesting to note that there is a possible role for the thymus. Szent Györgyi and associates\(^3\) claim to have isolated a growth-promoting hormone (promine) and a growth-inhibiting factor (retine) from calf thymus that could play a part in the regulation of breakdown and repair of organs which show a regular turnover of cells. It is possible that the immunological mechanism acts as a regulator or detector of tissue breakdown, as one side of a homeostatic mechanism controlled in young animals by the thymus.\(^6,33\) Presumably, these substances can cross the placental barrier. If this control is disordered by disease of the thymus, it might be anticipated that in addition to immunological disorders of other organs the motor terminals would show excessive breakdown and faulty regeneration.\(^5,7\) This could be the proximate basis of myasthenia gravis.

**Summary**

The rival theories of the disorder of neuromuscular transmission in myasthenia gravis are derived by analogy from pharmacological experiments on normal muscle. The geometry of the end plates in myasthenia is not normal. Theoretical considerations suggest that the abnormal end plates would have a greatly reduced safety factor for transmission. The geometrical changes would account for the altered response to depolarizing substances and possibly for the phenomena recorded by microelectrodes at myasthenic end plates.

The changes may represent a disturbance of a breakdown regeneration cycle in which the thymus is implicated. This would account for variability of localization of myasthenic weakness and the fluctuating clinical course.
References


Myasthenia
J A SIMPSON

In the English-speaking countries the term 'myasthenia' is reserved for a weakness of muscles which comes on after exercise and which may disappear after a short rest, and which is associated with a failure of neuromuscular transmission. The conductivity and excitability of the lower motor neurones and muscle fibres are normal in myasthenia gravis. In some other causes of the myasthenic syndrome they may be abnormal but the progressive loss of power with sustained or repeated muscular activity in these symptomatic myasthenias is likewise due to failure of transmission at the neuromuscular junction. When a motor nerve is supramaximally stimulated there is normally a sustained power of muscular response. The evoked compound muscle action potential at first sustains its amplitude and then gradually decrements. The period of sustained response depends on the frequency of stimulation. Response is sustained in face of a falling output of transmitter substance because there is normally a large 'safety factor'. When this safety-factor is reduced, regardless of mechanism, the tension and action potential of the responding muscle show a decrement even at quite slow rates of stimulation such as 3/second which is abolished or postponed by anticholinesterase drugs (neostigmine, edrophonium, etc.). This is the myasthenic reaction and it may have many causes (Simpson, 1966).

SYMPTOMATIC MYASTHENIA

A myasthenic reaction, responsive to neostigmine, may be found in dermatomyositis, polymyositis and systemic lupus erythematosus. It is usually present for a short period (one or two weeks) early in the disease and the response to anticholinesterase drugs is less impressive than in myasthenia gravis. It may also be present in the myasthenic syndrome associated with carcinomatosis. In the latter case there is an excellent recovery of function on administration of guanidine.

In all of these acquired myopathies, stimulation faster than 10/second may produce an incrementing muscular response instead of the decrementing response seen at lower rates of stimulation. The incrementing response is
most striking in the myasthenic syndrome associated with carcinomatosis.

Detailed analysis by Elmqvist and Lambert (1968) of a case not yet associated with carcinoma has shown that nerve and muscle excitabilities are normal, including the endplate sensitivity to acetylcholine. In most respects the abnormality of function resembles that caused in normal mammals by an excess of magnesium at the endplates, the only difference being that the mean frequency of spontaneous miniature endplate potentials (MEPPs) is increased. These studies indicate that the lowered safety-factor is, in this instance, due to deficient release of acetylcholine at nerve terminals. Recent ultrastructural studies showing post junctional changes (Engel & Santa, 1971) have yet to be evaluated.

In most hereditary muscular dystrophies the safety-factor for transmission appears to be normal but there may be progressive failure of the muscle response (post junctionally) in metabolic myopathies (chronic hypoxia or hypokalaemia) and in myotonic disorders.

Myasthenic reaction is a rare finding in disorders of the lower motor neurone associated with 'dying back'. Examples are poliomyelitis, motor neurone disease, syringomyelia and various types of peripheral neuropathies. The reduction in the safety-factor for transmission is probably due to failure of synthesis or release of acetylcholine and is seen shortly before the transmission fails completely.

Almost certainly this mechanism accounts for most of the symptomatic myasthenias but the possible significance of altered geometrical structure of the endplate has not been assessed.

MYASTHENIA GRAVIS

The symptoms and signs of the myasthenic disorder will not be enumerated. It is necessary, however, to underline certain aspects of the natural history which have to be accounted for when discussing the pathogenesis.

PATHOLOGY

About 10% of myasthenic patients have a lymphoepithelial tumour of the thymus. All, including the tumour group, have a thymus containing more than the normal number of germinal centres. This is the only sense in which the term 'hypertrophy' is justified. The gland is no larger than normal. Lymphorrhages are common in the muscles, especially in relationship to venules and degenerated muscle fibres. Motor endplates of muscle have an abnormal structure (vide infra). It is, therefore, scarcely credible that for many years this was considered to be disease without morbid anatomy.

AGE AND SEX

The disease affects people of all ages and races. If there is no thymoma the
modal age of onset is about 20 years at which time female cases outnumber males by 4.5 to 1. Myasthenia starting in later life is commoner in males. Thymoma is rarely seen under the age of 30 but is present in 30% of cases starting over the age of 40.

MULTISYSTEM DISEASE

In 1955 I began to examine a large series of myasthenics with a view to establishing the value of thymectomy as a treatment (Simpson, 1958). Certain disorders recurred in the case histories with sufficient frequency to suggest the possibility that myasthenia gravis might be a multisystem disease. The age and sex incidence, the relapsing history, the multisystem pathology and the lymphorrhages suggested to me that this was an immunological disorder related to systemic lupus erythematosus. At the time of publishing this hypothesis (Simpson, 1960) the thymus was still considered to be an endocrine gland though I thought it looked more like an active lymph gland. Also, the incidence of each related disorder, with the exception of thyroid disease, and possibly arthropathy, was 'not statistically significant'. It is necessary to be clear about this term. It means that chance cannot be excluded, but does not mean that correlation must be fortuitous. Since 1960 one after the other of all the disorders listed in Table I have been associated with immunological abnormality, and the Table (bringing my own series up to date) shows that these comparatively rare disorders occur in other myasthenic series. Quite soon the immunological function of the thymus became accepted dogma (Miller, 1961)

Table I. Diseases occurring in patients with myasthenia gravis, other than thyroid disorders

<table>
<thead>
<tr>
<th>Simpson</th>
<th>Oslerman</th>
<th>Storm-Mathisen</th>
<th>Oosterhuis</th>
<th>Dowees et al</th>
<th>Wolf et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>650</td>
<td>320</td>
<td>100</td>
<td>180</td>
<td>74</td>
</tr>
<tr>
<td>Carcinoma (non-thymic)</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>'Rheumatoid' arthritis</td>
<td>20</td>
<td>15</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>2(17)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nephritis</td>
<td>2</td>
<td>1</td>
<td>17</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Raynaud's syndrome</td>
<td>10</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Psychosis</td>
<td>11</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy, etc.</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
| Pemphigus | 1  | 0  | 0  | 0  | 1  | 143
At this point it is necessary to examine the role of the thyroid gland about which there have been many speculations on its relation to myasthenia gravis. Millikan and Haines (1953) found that 5% of myasthenic patients had hyperthyroidism which did not necessarily coincide in time with the myasthenia. This figure is supported by my own experience and by most of the literature (Simpson, 1968) and yet in a clinic for thyrotoxicosis it is rare to find myasthenia unless the metabolic rate was allowed to rise. Routine tests of thyroid function in myasthenic patients show that only a minority are outside normal limits and as many of these are hypothyroid as hyperthyroid. Indeed myasthenia gravis is associated with a raised incidence of all non-malignant thyroid disorders, including Hashimoto’s thyroiditis (Simpson, 1968). Obviously thyroid hormone does not cause myasthenia gravis. It is possible that they have a common immunological link. My experience indicates a high incidence of thyroid disease in the families of myasthenic patients.

A GENETIC FACTOR

It may well be that there is a common genetic factor between thyroid disease and myasthenia gravis, as in other ‘autoimmune disorders’. Namba and Grob (1970) have recently shown a similar link with rheumatoid arthritis. One member of a family may have thyroid disease or rheumatoid arthritis while another has myasthenia; occasionally they occur in the same individual. Two cases of myasthenia gravis occasionally occur in the same family and I have records of two related patients (of different generations). Familial myasthenia of late onset is certainly rare. Familial thyroid or autoimmune disease of other tissues is more common. If there is a genetic factor it is only one. A linkage of this type would account for the rarity of myasthenia gravis in identical twins.

CONGENITAL MYASTHENIA

This occurs in siblings and usually manifests at an earlier age than sporadic myasthenia gravis. It has little tendency to remissions. The identity of the two conditions remains in doubt. It is not the same disorder as Neonatal Myasthenia Gravis.

NEONATAL MYASTHENIA GRAVIS

This is a myasthenic syndrome which appears in 1 in 7 live children born to myasthenic mothers. There is a classical myasthenic syndrome which persists for only 1 to 8 weeks with a mean duration 18 days (Namba et al,
This indicates that some 'myasthenic toxin' can pass the placental barrier and yet all attempts to transmit the myasthenic 'block' to another adult by cross-transfusion or to block an isolated nerve-muscle preparation have failed (Nastuk et al, 1959). The duration of neonatal myasthenia is much too long for a conventional curare-like substance, but it is compatible with an antibody which might be presumed to block only antigenically related endplates (Simpson, 1960).

IMMUNOLOGICAL STUDIES

These clinical and pathological considerations led, in 1956-1959, to a search for antibody against muscle end-plates and an attempt to produce autoimmune myasthenia in mice. Both approaches were unsuccessful. An entirely different consideration led Strauss to the detection of an antimuscle antibody. While demonstrating the absence of a curare-like substance in myasthenic serum, Nastuk et al, (1959) noticed that the serum lysed muscle cells of the frog, a reaction later shown to be associated with fixation of complement. Later studies published almost simultaneously with my own autoimmune hypothesis demonstrated the presence in myasthenic serum of a globulin which would bind to muscle (Strauss et al, 1960). Their success was due to the use of sera pooled from a number of patients, always including one with a thymoma in which it is now known that the titre of antibody is much higher. But the antibody was not the predicted anti-endplate one. The Strauss antibodies react against antigens in the muscle fibre. There are probably various antibodies: one causing I-band fluorescence is common but not confined to myasthenia gravis; A-band fluorescence, on the other hand, is almost confined to patients with a thymoma (Vetters, 1965). Some antibodies react with skeletal muscle only ('S' type), others with both skeletal and cardiac muscle ('SH' type) (Beutner et al, 1962). The skeletal muscle antibody also reacts with a common antigen in myoid cells which are present in the thymus (van der Geld et al, 1964).

Antinuclear factor, rheumatoid factor and antibodies against thyroid and gastric parietal cells are also more common in myasthenic patients. Thus the evidence for a breakdown of immunological tolerance is considerable although the concept of an endplate-blocking antibody has had to be abandoned. What we have learned about immunological disease in the last 10 years makes it more likely that myasthenia is a disorder of cellular rather than humoral immunity.

Housley and Oppenheim (1967) found no abnormality of lymphocyte transformation. Delayed hypersensitivity induced by 1-chloro-2, 4-dinitrobenzene, which is mediated by lymphocytes, has been reported to be either reduced (Adner et al, 1964) or normal (Kornfeld et al, 1965). Lymphocytes from a myasthenic patient injected into rat muscle are stated to cause greater graft-
versus-host reaction locally than do lymphocytes from control subjects, including alterations in the structure of the motor endplates (Namba et al., 1969).

This seems to me to be a more promising approach than other attempts to produce an animal model. Goldstein and Whittingham (1966) claim to have produced myasthenia gravis in guinea pigs immunised with thymus or muscle and they postulate that these antigens produced an autoimmune thymitis. The damaged thymus is then considered to release a neuromuscular blocking substance, 'thymin'. The role postulated for the thymus is quite different from the Simpson hypothesis and would not predict a breakdown of immunological tolerance to other antigens with associated multisystem disease. Goldstein (1971) now has very impressive evidence for the validity of his animal model and claims to have isolated 'thymin' in small quantities. His work is supported by Kalden et al (1970) but cannot be confirmed by Vetters et al (1969) and other workers including Jones et al (1971) who had the benefit of collaboration with Goldstein's original collaborator Miss Whittingham. Unless these negative results are due to strain differences in the experimental animals it is possible that the Goldstein model resembles the myasthenia of human polymyositis described above rather than myasthenia gravis.

PHARMACOLOGICAL MODELS

The present position may be summarised as follows. There is clinical, pathological and immunological evidence for a breakdown of immunological tolerance in myasthenia gravis but how this causes neuromuscular block remains obscure. I have recently presented a detailed argument in favour of the following possible mechanism (Simpson, 1969; 1971). In brief, the electrophysiological and pharmacological changes of the myasthenic muscle indicate a reduction of the safety-factor for transmission at the neuromuscular junction. It is not possible to account for everything by a purely pre-junctional or post-junctional disorder of function.

We are all children of our age and adopt the conceptual models which are appropriate to our time. In problems of neuromuscular block most people argue by analogy from pharmacological experiments. Thus the workers of the late 19th and early 20th centuries looked for a 'myasthenic toxin' with curare-like properties. In the 1950's, the era of competitive and 'depolarising' neuromuscular blockers, the concepts were of altered transmitter substance or a change in the subsynaptic membrane. Later the favoured analogue was hemicholinium - 3 which has its principal activity in blocking the synthesis of acetylcholine. All of these pharmacological analogues assume the presence of normal endplate structure and it is implicit that all mammalian neuromuscular junctions show the same pharmacological reactions. But the endplates are not normal in structure in myasthenia
HISTOPATHOLOGY OF NEUROMUSCULAR JUNCTION

Coers and Woolf (1959) showed two types of florid morphological changes of intramuscular nerve endings in myasthenia gravis.

1. a 'dystrophic' type associated with myositis and lymphorrhages.
2. a 'dysplastic' type which is apparently unique to myasthenia gravis.

Later studies by the same authors and others have shown that the most characteristic change is that the motor nerve ending has a reduced number of branches. These terminate in greatly elongated endplates which sometimes show a remarkable degree of ultraterminal sprouting with development of a chain of endplates connected by a single fine nerve fibre. The appearance indicates that degeneration and regeneration are both taking place, features which could account for the variability of the clinical picture with its characteristic relapses and remissions.

These changes, demonstrated by supravital staining, are associated with ultrastructural changes. The endplate zone is extended although according to Engel and Santa (1971) each terminal area is reduced. The synaptic cleft is widened and the subsynaptic membrane has shallow, relatively wide, folds and clefts. The secondary clefts may be absent (Engel & Santa, 1971; Bergman et al., 1971). The concentration of vesicles per unit of presynaptic area is normal. The appearances are similar to immature developing neuromuscular junctions and suggest a progressive remodelling of endplates and abandoning of old, as proposed by Simpson, (1969). Bergman et al (1971) have recently drawn attention to the capillaries in the muscle of myasthenia gravis. The endothelium appears damaged and the basement membrane is grossly thickened. A tentative immunological explanation was preferred. Similar findings are present in dermatomyositis but further assessment is required as thickening of the basement membrane of muscle capillaries is also reported in other myopathies, especially in dystrophia myotonica (Monticone et al, 1970).

MECHANISM OF TRANSMISSION FAILURE

These structural changes are sufficient to account for all known electrophysiological and pharmacological phenomena of myasthenia. The argument about presynaptic or postsynaptic lesion is irrelevant when both are involved and there is no need to postulate a 'chemical lesion' (Simpson, 1960, 1969, 1971). The transmissible factor related to the thymus must cause a geometrical change of the endplate rather than a curare-like block. This may be an exaggeration of a normal process of remodelling of endplates for which there is some evidence in normal mammals (Barker and Ip, 1966).
A review of earlier experimental studies on the thymus indicates that the differentiation of the immunologically competent cells and antibodies may be a survival of a wider action on tissue differentiation in the foetus (Simpson, 1960). Szent-Gyorgi et al, (1962) claimed to have isolated a growth-promoting hormone (promine) and a growth-inhibiting factor (retine) from calf thymus which could play a part in the regulation of breakdown and repair of organs showing a regular turnover of cells. It is possible that the immunological mechanism acts as a regulator or detector of tissue breakdown, as one side of a homeostatic mechanism, controlled in young animals by the thymus (Simpson, 1960; Burnet, 1962). Presumably these substances can cross the placental barrier. Unfortunately Szent-Gyorgi has discontinued his studies. It would be anticipated, if his findings are confirmed, that disease of the thymus would result in excessive breakdown and faulty regeneration of motor terminals in addition to immunological disorders of other organs. This could be the basis of myasthenia gravis (Simpson, 1969).

SUMMARY
This is a long and complicated chain of evidence. It involves some unconfirmed links, but one is encouraged to present these as the original hypothesis proved to have heuristic value and successive steps in the argument have been validated in the last decade. It is proposed (Figure I) that a genetic factor, which commonly manifests as thyrotoxicosis, is sometimes associated with disordered thymic function. (The precipitating role of emotional disturbance and the effects of pregnancy and the menstrual cycle suggest that they are linked via the hypothalamus and pituitary gland.) Abnormal thymic function

---

**Figure 1. Myasthenia**
disturbs the immunological mechanisms causing autoimmune disorders of humoral and cellular type. The latter includes breakdown of motor nerve terminals but there is active remodelling. The regenerative phase may be adrenal or thymic-dependant. This is an exaggeration of a normal process. It results in dysplastic endplates with ultraterminal sprouting. Endplates of this type have a reduced safety-factor for transmission which leads to the clinical manifestations of myasthenia gravis.

The possibility of similar morphological explanations should be investigated in the symptomatic myasthenias associated with lower motor neurone degeneration and acquired myopathies.

REFERENCES


Burnet, F. M. (1962) British Medical Journal, 2, 807


Engel, A. G. (1961) Archives of Neurology (Chicago), 4, 663


Kalden, J. R., Williamson, W. G. and Irvine, W. J. (1970) Clinical and Experimental Immunology, 6, 519

Korenfeld, P., Siegal, S., Weiner, L. B. and Osserman, K. E. (1965) Annals of Internal Medicine, 63, 416


Namba, T. and Grob, D. (1970) Archives of Internal Medicine, 125, 1056

Nastuk, W. L., Strauss, A. J. L. and Osserman, K. E. (1959) American Journal of Medicine, 26, 394

Oosterveld, H. J. G. H. (1964) Journal of Neurological Sciences, 1, 512


Simpson, J. A. (1958) Brain, 81, 112
Vetters, J. M. (1965) Immunology, 9, 93
Myasthenia gravis is known to be influenced by menstruation and pregnancy but the effects are unpredictable in individual patients and even in different pregnancies of one patient. Schrire (1959) reported subnormal excretion of pregnandiol in untreated myasthenic patients, including men and post-menopausal women. We have not confirmed these findings. When the response to different stages of pregnancy is plotted on a balance chart a general trend is evident but there are too many exceptions to justify correlation with hormonal levels. Close interrogation of a few patients suggests that there is a better correlation with the emotional response to pregnancy or menstruation.
Pemphigus vulgaris and myasthenia gravis

J.M. VETTERS, N.K. SAIKIA, JANE WOOD AND J.A. SIMPSON

University departments of Dermatology and Pathology, Western Infirmary, Glasgow and the Institute for Neurological Sciences, Glasgow

Accepted for publication 11 October 1972

SUMMARY

This paper describes the occurrence of pemphigus vulgaris in a 46-year-old female with a thymic tumour. The myasthenia gravis went into remission within a few months of thymectomy, but the patient continued to need steroids for pemphigus vulgaris for a further 5 years. The histological appearances of the thymic tumour and skin and the serological findings are described.

Myasthenia gravis (Simpson, 1960) and skin diseases of the pemphigus/pemphigoid group (Beutner & Jordan, 1964; Beutner et al., 1965) are both considered to have an autoimmune basis. It is known that individuals with one autoimmune disease are more likely to develop other autoimmune diseases (Anderson et al., 1964). Although immunological and clinical studies have been performed on large numbers of patients with myasthenia gravis, the simultaneous occurrence of myasthenia gravis and autoimmune skin disease has rarely been described. The literature in English and French contains only three reports (Wolf et al., 1965; Beutner et al., 1968; Peck et al., 1968). No details are given of Wolf's case. The subject described by Beutner et al. (1968) was a 45-year-old male with a thymic tumour who developed myasthenia gravis 1 year before he was diagnosed as having pemphigus erythematosus. Peck et al. (1968) described four cases of myasthenia gravis associated with pemphigus vulgaris; one of these was known to have a thymic tumour.

In addition to these patients with both pemphigus and myasthenia gravis, other reports have described the detection of skin antibodies in a small proportion of myasthenic patients who had no detectable skin lesions (Thivolet, Bejum & Andre, 1970; Whittingham & MacKay, 1971).

We wish to describe in detail a further case in which both myasthenia gravis and pemphigus vulgaris were present (this case was previously listed by Simpson (1970, 1971).

METHODS

(a) Immunological
The tests for epidermal antibody were performed according to the method of Beutner et al. (1968, 1970). Human vaginal epithelium was used as a substrate.

Tests for other autoantibodies were performed as previously described (Vetters, 1967).

(b) Histological
Tissue for microscopic examination was fixed in 10% formol saline and embedded in paraffin wax. Sections were cut 6 µm thick and stained with a variety of conventional stains.
CLINICAL DETAILS

The patient, a 46-year-old Jewish female, developed painful mouth ulceration and nodular thickening on the dorsum of her hands in February, 1966. Four months later she developed dysphagia and subsequently noted slurring of speech and ptosis. After some delay she sought medical help and was admitted to hospital in December, 1966. She was diagnosed as having a thymic tumour and myasthenia gravis and in January 1967 underwent thymectomy. Shortly after operation she developed a generalized bullous eruption which was particularly severe round the thoracotomy scar. The eruption was biopsied (see below).

Post-operatively her myasthenia gravis rapidly remitted and since July, 1967 she has not taken anticholinergic drugs. Her pemphigus vulgaris did not improve so dramatically and it was not until July 1972 that it was possible to discontinue steroids. She continues to have oral ulceration due to monilial infection.

BIOPSIES

(a) Thymoma

This measured $10 \times 8 \times 7.5$ cm. Microscopic examination showed that the predominant cell type was the spindle shaped epithelial cell. In places, especially close to small blood vessels, the tumour cells were more ovoid in shape. Sparse numbers of lymphocytes were present. The appearances are illustrated in Fig. 1.

![Thymic Tumour](image)

**FIGURE 1.** Thymic tumour. The predominant cell type is the spindle shaped epithelial cell. Relatively small numbers of lymphocytes are scattered amongst the epithelial cells. It is more common for the epithelial cells of thymic tumours to be plump when thymomas are associated with myasthenia gravis (H & E, x 250).

(b) Skin biopsy

One of the skin lesions was biopsied before thymectomy. Histological examination detected suprabasal clefts in the epidermis, dyskeratosis and giant cell formation. A mild leucocytic infiltrate was present in the underlying dermis. The appearances were found difficult to interpret and it was not until a
Pemphigus vulgaris and myasthenia gravis

439

Figure 2. Skin biopsy. This section shows the characteristic suprabasal intra-epidermal cleft formation of pemphigus vulgaris. Notice that the clefting also affects a hair follicle (H & E, x 100).

Table 1. Autoantibody test results pre-operatively

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal antibody</td>
<td>Positive; 'intercellular' antibody</td>
<td>1/50</td>
</tr>
<tr>
<td>Muscle antibody</td>
<td>Positive; I band antibody</td>
<td>1/60</td>
</tr>
<tr>
<td>Nuclear antibody</td>
<td>Positive</td>
<td>1/64</td>
</tr>
<tr>
<td>Gastric parietal cell antibody</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Thyroid microsomal antibody</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Thyroglobulin antibody</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Alterations in titre of intercellular antibody

<table>
<thead>
<tr>
<th>Date</th>
<th>Titre</th>
<th>Steroid dosage (mg prednisolone/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 1967</td>
<td>1/80</td>
<td>15</td>
</tr>
<tr>
<td>May 1968</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td>March 1969</td>
<td>1/40</td>
<td>10·8</td>
</tr>
<tr>
<td>March 1970</td>
<td>1/10</td>
<td>3·5</td>
</tr>
<tr>
<td>March 1971</td>
<td>1/10</td>
<td>2·5</td>
</tr>
<tr>
<td>March 1972</td>
<td>&lt;1/10</td>
<td>2·5</td>
</tr>
<tr>
<td>July 1972</td>
<td>&lt;1/10</td>
<td>Nil</td>
</tr>
</tbody>
</table>
second biopsy was performed, after the post-operative development of the generalized eruption, that a confident diagnosis of pemphigus vulgaris could be made (see Fig. 2).

IMMUNOLOGICAL STUDIES

The results of the pre-operative and post-operative tests are shown in Tables 1 and 2 respectively. The demonstration of intercellular antibody by immunofluorescence is illustrated in Fig. 3.

![Figure 3. Intercellular antibody. The characteristic staining pattern caused by binding of antibody to cell membranes of the granular cell layer is illustrated (Guinea-pig lip, x 250).](image)

DISCUSSION

It is recognized that the occurrence of one autoimmune disease increases the likelihood of developing other autoimmune diseases (Anderson et al., 1964). Autoimmune disorders fall into two groups, the organ-specific, e.g. autoimmune thyroiditis, idiopathic Addison's disease and pernicious anaemia, and the non-organ-specific such as systemic lupus erythematosus, progressive systemic sclerosis and rheumatoid arthritis. A patient with an organ-specific autoimmune disorder stands an increased chance of developing another specific autoimmune disease. Similarly, subjects with non-organ-specific autoimmune diseases are more likely to develop other diseases of this group.

The position of both pemphigus vulgaris and myasthenia gravis with regard to these groups is somewhat anomalous. Pemphigus vulgaris is an organ-specific autoimmune disease, but the autoimmune disease most frequently associated with it is systemic lupus erythematosus. Myasthenia gravis appears to occupy an intermediate position between the organ-specific and the non-organ-specific groups. Patients with myasthenia gravis have an increased incidence of organ-specific autoimmune diseases, e.g. primary myxoedema and thyrotoxicosis (Feinberg, Underdahl & Eaton, 1957; Simpson, 1964) pernicious anaemia (Simpson, 1960; Howard, Silverstein & Mulder, 1965) and also of systemic lupus erythematosus (Wolf, 1966). It is therefore reasonable to expect that cases of pemphigus vulgaris and myasthenia gravis should co-exist in the one individual. What is surprising is the very
small number of patients who have both disorders. As stated above, the present case is only the seventh reported in the English language. Of these, three have been associated with a thymic tumour. Unfortunately no details of the histological appearances are given in the other two reports and it is therefore not possible to determine whether any particular type of thymoma is associated with the simultaneous occurrence of myasthenia gravis and pemphigus vulgaris. It should be noted, however, that the predominantly spindle celled epithelial thymoma encountered in the present case is not the type usually found in myasthenic patients. When thymic tumours are present in myasthenic patients they usually have plump epithelial cells (Castleman, 1966). However, the thymic tumour associated with systemic lupus erythematosus and pemphigus vulgaris described by Kough & Barnes (1964) was similar to that in our case. It is therefore possible that spindle celled thymic tumours have a special relationship to pemphigus vulgaris.

REFERENCES


41. Myasthenia gravis and myasthenic syndromes

J.A. SIMPSON, Glasgow, United Kingdom

Myasthenia (decrementing muscular response to repetitive nerve impulses) indicates lowered safety factor of n-m transmission. (1) Lower motor neurone disorders (central or peripheral) preceding final failure of ACh synthesis. (2) Myasthenic syndrome of carcinomatosis, due to defective ACh release. Lower limbs, often painful and areflexic, are mainly involved, rarely bulbar or extraocular muscles. Muscle response decrements with slow and increments strikingly with rapid indirect tetanisation. Anticholinesterases are of little value; guanidine is effective. (3) Acquired myopathies associated with collagen diseases may have myasthenia with partial response to anticholinesterases at early stage, soon becoming unresponsive. Incrementing response is common. Mechanisms similar to (2) and (4) are postulated. (4) Myasthenia gravis is a clinical entity, 10% have thymoma, usually over 30 years of age. At 65 years, 60% have thymoma. Clinical course shows active stage (5–10 years) with remissions, major mortality and response to thymectomy. Inactive stage (5–20 years) with no remissions, low mortality, poor response to thymectomy but continuing response to anticholinesterases. 'Burned-out' stage—residual weakness (myopathic? neurogenic atrophy) with little response to drugs but low mortality. Transient neonatal MG in 1 in 7 live babies of myasthenic mothers. Clinical links with disorders of other organs indicate autoimmune disorder, supported by serology. MG attributed to altered endplate structure due to defect of normal remodelling involving an immunological role of thymus. Therapeutic conclusions.

259. Histological assessment of the myasthenic thymus gland and the response to thymectomy

J.A. SIMPSON and J.M. VETTERS, Glasgow, United Kingdom

For 30 years germinal centres have been recognised as the characteristic features of non-neoplastic thymus glands in patients with myasthenia gravis. Despite this it is uncertain whether the histological changes in the thymus are related to the response to thymectomy. Relatively few studies have been made on this relationship. Three differing conclusions have been reached: (a) There is no correlation (Castleman and Noms, Medicine, 1949, 28, 27). (b) The more germinal centres the better the prognosis (McKay et al., Australas. Ann. Med., 1968, 17, 1). (c) The fewer germinal centres the better the prognosis (Alpert et al., Arch. Path., 1971, 91, 55).

The present study involved more than 50 patients who had been thymectomised for myasthenia gravis; their operations were performed between 18 and 1½ years before. The thymus glands were examined using precise quantitative histological techniques. The response to thymectomy was assessed without knowledge of the histological findings. The authors’ data are in broad agreement with that of Alpert et al. (1971) but the results as yet do not reach statistical significance.
Comparison of thymic histology with response to thymectomy in myasthenia gravis

J. M. VETTERS1 AND J. A. SIMPSON

From the Department of Pathology, Western Infirmary and Institute of Neurological Sciences, Southern General Hospital, Glasgow

SYNOPSIS Fifty-four thymus glands removed surgically from patients with myasthenia gravis were examined using an accurate morphometric technique and the data compared with the response to thymectomy. There is a tendency for patients with relatively unreactive thymus glands to obtain a better result from thymectomy but this is not statistically significant.

Germinal centres were first reported in the thymus glands of patients with myasthenia gravis by Barton and Branch (1937) and Miller (1940). They made only passing reference to them; at that time it was believed that the characteristic histological features of the myasthenic thymus was 'hyperplasia'. However, Sloan (1943) recognized that germinal centre formation was the characteristic feature of myasthenic thymus glands. Subsequently, the studies of Keynes (1946, 1949) showed that thymectomy caused amelioration of the patients' clinical status. Despite this, only five groups have studied whether there was any correlation between the histological changes in the thymus and the response to thymectomy.

No uniform opinion has emerged from these studies. Castleman and Norris (1949), in a study of 23 cases, failed to find any relationship between the thymic changes and the response to thymectomy. Mackay et al. (1968) studied 10 patients and concluded that the patients who did best were a group of young females whose thymus glands contained numerous germinal centres. Alpert et al. (1971) studied 79 myasthenic patients, assessing the thymic histology semiquantitatively, and noted that the most favourable response to thymectomy was obtained in subjects whose thymus gland contained few or no germinal centres. Seybold et al. (1971) in a study of 102 patients with juvenile myas-

thenia gravis could detect no correlation between 'thymic hyperplasia' and the response to thymectomy. Finally, Reinglass and Brickel (1973) studied 12 patients and failed to detect any correlation between the histological appearances and the response to thymectomy.

The present study using strict histological quantitation methods was initiated to determine the following:

1. If the percentages of thymus glands occupied by cortex and medulla in subjects with myasthenia gravis differs from the data obtained by Hammar (1926) in a study of normal subjects who died suddenly—that is, do they show stress involution or other changes in the amounts of cortex and medulla present?

2. The relationship between the density of germinal centres and the following: (a) response to thymectomy; (b) age at thymectomy; (c) duration of symptoms preoperatively.

3. Relationship between germinal centre size and the postoperative response.

METHODS

THYMIC QUANTITATION All available blocks were studied. The area of each section and the percentage of it occupied by cortex, medulla, and interstitial tissue were determined by methods previously described (Vetters and Barclay, 1973).

As previously discussed (Vetters and Barclay, 1973), the human thymus may contain structures which have all the classical features of germinal centres (GC)—that is, lymphocyte cuff, pale centre

---

1 Present address: Division of Pathology, The University of Calgary, Calgary, Alberta T2N 1N4, Canada.
containing reticulum cells, macrophages, and mitotic figures. However, a second 
structure is frequently present—we have named them 'rounded lymphoid clusters' (RLC). These show a 
range of appearances. (1) Dense aggregate of lymphocytes with the peripheral lymphocytes 
arranged concentrically. (2) Lymphocyte cuff with a pale centre; no tangible bodies or macrophages 
present. (3) Lymphocyte cuff with pale centre; either tangible bodies or mitoses present. 

Evidence was produced to show that the RLC are usually obliquely sectioned germinal centres. They 
are enumerated separately here because some authors—for example, Goldstein and Mackay (1967)
—disregard structures other than classical germinal centres. The number of germinal centres and RLC in each section was recorded. Their size was determined by measuring each structure in two axes at right angles and calculating the mean.

From the data obtained, it was possible to calculate the percentage of each gland occupied by cortex and medulla, the density of germinal centres/cm² medulla and to obtain a ratio of the number of germinal centres to the area of cortex present. These figures were analysed statistically using a Hewlett-Packard 9100B calculator.

CLINICAL EVALUATION The clinical assessment was made by J.A.S. from his clinical notes using the same grading system as Alpert et al. (1971). The assessment was performed without knowledge of the results of the quantitation studies.

DETAILS OF GROUP STUDIED A total of 54 patients was studied. Of these, six had thymic tumours; their ages were 32, 41, 43, 49, 59, and 65 years. Excluding two subjects with juvenile myasthenia gravis (both 2 year old females), the average age of the remaining subjects was 27.8 ± 10.9 years. The age distribution of the subjects without thymic tumours is shown in Fig. 1.

The duration of symptoms before surgery is shown in Fig. 2. The average duration of symptoms preoperatively was 3.0 years; the standard error of the mean was 3.7. The rather long preoperative period is largely due to the inclusion of early Glasgow thymectomy cases and patients referred from other centres; both of these groups were given prolonged medical treatment before surgery was undertaken. When circumstances permit, we prefer to operate at an earlier time.

RESULTS

COMPARISON WITH HAMMAR’S DATA FOR SUBJECTS WHO DIED SUDDENLY It is known from the experimental and clinical studies (de Sousa and Fachet, 1972; Hammar, 1926, 1929) that disease or stress causes rapid involution of the thymus gland. For this reason, Hammar’s (1926) data obtained by study of thymus glands removed from previously healthy subjects who died suddenly remains the best information on the variations of cortex and medulla found in normal subjects. The data of groups of thymus glands were compared with Hammar’s figures.

Juvenile myasthenia gravis Definitions of juven-

tile myasthenia vary. Subjects of 12 years and over have been excluded from this group. Only two subjects hadtemp unusual form. Both were 2 year old females. Neither thymus contained germinal centres. The percentages of thymus occupied by cortex were 52.5 and 59.6 respectively and the percentages occupied by medulla were 18.2 and 25.2. The mean values given by Hammar’s data on 28 children ranging up to 5 years old are 56.8 ± 1.7 and 21.8 ± 0.9 for cortex and medulla respectively. It is therefore clear that these two subjects do not show a consistent alteration from the figures given by Hammar (1926).

Adult myasthenia gravis not associated with thymic neoplasm There were 46 cases in this group. Of those, 41 fell within the range 12–40 years. The results of comparison of these data with Hammar (1926) are shown in Table 1. It

| TABLE 1 |
| COMPARISON WITH HAMMAR’S DATA: PATIENTS WITH NO THYMIC NEOPLASM |

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Hammar (No.) %</th>
<th>Present series (No.) %</th>
<th>Student’s t test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>11–20</td>
<td>45 23.8 ± 14</td>
<td>24 27.9 ± 2 3.7</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>26 6.6 ± 22</td>
<td>27 28.8 ± 2 2.600</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>21 1.6 ± 19</td>
<td>24 28.8 ± 2 0.266</td>
<td>0.356</td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>14 21.6 ± 19</td>
<td>24 28.8 ± 2 1.488</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>20 9.9 ± 24</td>
<td>24 28.8 ± 2 0.262</td>
<td>0.398</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>11 7.7 ± 12</td>
<td>20 28.5 ± 2 2.943</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

will be noted that the percentage of thymus occupied by medulla is significantly increased in the second and fourth decades and that the percentage cortex is considerably decreased in the second decade.

Adult myasthenia gravis associated with thymic tumour Only six subjects with thymic tumour had detectable residual or non-neoplastic thymic tissue. Of these, four subjects were between 40 and 60 years (the others were 32 years and 65
years of age). Comparison with Hammar's data (Table 2) shows increase in the proportion of medulla and decrease in the proportion of cortex but these are not statistically significant.

DENSITY OF GERMINAL CENTRES AND ROUNDED LYMPHOID CLUSTERS

Response to thymectomy

No relationship was detectable between response to operation and density of reactive lymphoid clusters—that is, germinal centres and RLC—expressed as ratios of medulla or cortex (Table 3). Exclusion of the tumour group did not affect this.

Age at operation

No correlation was found between age and numbers of reactive lymphoid structures (Table 4). Exclusion of subjects with thymomata did not affect the result.

Duration of symptoms

The duration of symptoms before operation bore no relationship to the density of germinal centres and RLC (Table 5).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Hammar (C%)</th>
<th>Present series (No.)</th>
<th>Student's t test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>7/8</td>
<td>7/0</td>
<td>0·204</td>
<td>0·42</td>
</tr>
<tr>
<td>Medulla</td>
<td>6/7</td>
<td>10/5</td>
<td>0·107</td>
<td>0·15</td>
</tr>
</tbody>
</table>

* Two subjects aged 32 years and 65 years are omitted.

| TABLE 3 |
| RESPONSE TO THYMECTOMY |
|---|---|---|
| Response/GC per cm² cortex | −0·147 | −0·983 | 0·160 |
| Response/GC + RLC per cm² cortex | −0·149 | −1·001 | 0·197 |
| Response/GC per cm² medulla | −0·129 | −0·861 | 0·320 |
| Response/GC + RLC per cm² medulla | −0·071 | −0·471 | 0·655 |

Non-neoplastic cases

Response/GC per cm² cortex | −0·186 | −1·640 | 0·055 |
Response/GC + RLC per cm² cortex | −0·190 | −1·190 | 0·121 |
Response/GC per cm² medulla | −0·189 | −1·125 | 0·134 |
Response/GC + RLC per cm² medulla | −0·137 | −0·854 | 0·199 |

| TABLE 4 |
| RESPONSE TO THYMECTOMY |
|---|---|---|
| All cases |
| Age/GC per cm² cortex | −0·142 | 0·955 | 0·173 |
| Age/GC + RLC per cm² cortex | −0·143 | 0·957 | 0·172 |
| Age/GC per cm² medulla | 0·083 | 0·553 | 0·292 |
| Age/GC + RLC per cm² medulla | 0·027 | 1·122 | 0·452 |

Non-neoplastic cases |
| Age/GC per cm² cortex | 0·219 | 1·386 | 0·087 |
| Age/GC + RLC per cm² cortex | 0·221 | 1·399 | 0·085 |
| Age/GC per cm² medulla | 0·060 | −0·408 | 0·343 |
| Age/GC + RLC per cm² medulla | −0·020 | −0·122 | 0·452 |

| TABLE 5 |
| DURATION OF SYMPTOMS |
|---|---|---|
| All cases |
| Duration/GC per cm² cortex | −0·013 | −0·868 | 0·466 |
| Duration/GC + RLC per cm² cortex | −0·016 | −1·069 | 0·499 |
| Duration/GC per cm² medulla | −0·198 | −1·342 | 0·093 |
| Duration/GC + RLC per cm² medulla | −0·231 | −1·574 | 0·061 |

Non-neoplastic cases |
| Duration/GC per cm² cortex | 0·016 | 0·156 | 0·438 |
| Duration/GC + RLC per cm² cortex | 0·000 | 0·002 | 0·499 |
| Duration/GC per cm² medulla | −0·173 | −1·048 | 0·142 |
| Duration/GC + RLC per cm² medulla | −0·181 | −1·132 | 0·132 |

CORRELATION BETWEEN REACTIVE LYMPHOID STRUCTURE SIZE AND RESPONSE TO THYMECTOMY

No correlation was detectable.

| TABLE 6 |
| RESPONSE TO THYMECTOMY |
|---|---|
| Favourable | Unfavourable |
| Average diameter of GC and RLC | 0·385 ± 0·012 |
| Student's t test | 0·866 |
| Probability | 0·193 |

**DISCUSSION**

**COMPARISON WITH HAMMAR'S DATA**

There is a relative decrease in the percentage area of thymus occupied by cortex and a relative increase in the amount of medulla compared to Hammar's data for subjects who died suddenly. This is highly significant statistically for the alterations in
medulla in the 11–20 year and 31–40 year groups and for the altered cortical values in the 11–20 year group.

Hammar's data, obtained with meticulous attention to detail, represent the best available data on the values of normal thymic tissue. Two explanations are possible for the apparent increase in the medulla in myasthenia gravis: (1) this is genuine medullary hyperplasia; (2) the changes are due to stress.

It would be possible to resolve this difficulty if the precise weight of each thymus had been determined. It would then have been possible to calculate the mass of each tissue component. Unfortunately, it was not possible to determine the weight of each gland with sufficient precision in many cases. However, the alterations in the relative proportions of cortex and medulla are similar to those detected in individuals who are stressed by illness for several days before death (Hammar, 1929). It is therefore reasonable to conclude that, while the data may indicate a genuine increase in medullary tissue—that is, true medullary hyperplasia—it is equally likely that the changes are due to stress. Moreover, the data of Castleman and Norris (1949) show that it is more common for myasthenic thymus glands to weigh less than average, compared with normal subjects of the same age. The term 'thymic hyperplasia' is therefore misleading and should be avoided.

**Comparison of thymic histology with response to thymectomy in myasthenia gravis**

Correlation of histological features with response to surgery

Previous studies into a possible relationship between the histological appearances of thymus glands removed surgically for myasthenia gravis and the postoperative response have fallen into three groups: (1) no relationship (Castleman and Norris, 1949; Seybold et al., 1971; Reinglass and Brickel, 1973); (2) favourable response correlates with presence of numerous germinal centres (Mackay et al., 1968); (3) favourable response more likely in thymus glands with few germinal centres (Alpert et al., 1971).

No previous authors have used quantitative methods. The only previous studies to use semiquantitative methods were those of Alpert et al. and Reinglass and Brickel. The study of Mackay et al. (1968) used subjective assessment of the histological changes. The period of follow-up was short—a maximum of 30 months. In addition, the group studied by Mackay was small and contained an unusually high proportion of subjects with thymic tumours. It is therefore reasonable to regard these results as being anomalous because of the small number of cases studied.

The study of Alpert et al. (1971) also included a relatively high proportion of patients with thymic tumours (27 out of 79 cases) but the period of follow-up was much longer and the histological assessment was made in a semiquantitative manner. Alpert and his associates concluded that the patients with the least reactive thymus glands tend to experience more rapid benefit from thymectomy.

Reinglass and Brickel (1973) studied 12 patients, most of whom were young females; none of their subjects had thymic tumours. They used semiquantitative methods and were unable to detect any correlation between the result of thymectomy and the histological appearances of the thymus. The seven patients who benefited from thymectomy did so within one year of surgery.

The present study shows a tendency for better results to be obtained in subjects with relatively non-reactive thymus glands, but the results are not statistically significant. They are therefore not in disagreement with the conclusions of Castleman and Norris (1949), Seybold et al. (1971), and Reinglass and Brickel (1973) who failed to find any connection between the postoperative response and the histological appearances of the resected thymus glands.

A study of the data presented by Alpert and his associates also shows a tendency for patients with relatively non-reactive thymus glands to respond favourably. However, the difference in incidence of thymic germinal centres between those who respond favourably and those who do not experience benefit from thymectomy is not statistically significant. Additionally, statistical analysis of the data given in Alpert et al.'s Fig. 3 shows that there is no statistical difference in the incidence of germinal centres between the group of patients who developed remission within one year and those who developed a clinical remission after two years. Although both the present group and that of Alpert et al. show a tendency to obtain better results in subjects with relatively non-reactive thymus glands, neither
groups' data achieve statistical significance. Unfortunately, because of differences in the quantitation methods used, it is not possible to pool the results in an attempt to achieve statistical significance.

The hypothesis advanced by Alpert et al. (1971) that the myasthenic thymus produces lymphocytes which in turn induce muscular damage is an attractive one. In view of the well-known long life span of thymic derived lymphocytes it would be reasonable to assume that the more active the thymus is in producing lymphocytes which can induce muscular damage, the longer it would take for a patient to undergo remission.

An alternative hypothesis, advanced by Gideon Goldstein (Goldstein, 1966) that the thymus in myasthenia gravis is damaged by an autoimmune reaction 'thymitis' and releases excessive amounts of a postulated neuromuscular transmission inhibiting hormone 'thymin' has several flaws. Firstly, the presence of lymphoid follicles in human thymus which Mackay et al. (1968) term 'thymitis' is not specific for myasthenia gravis. Lymphoid follicles are readily detectable in many young subjects who die suddenly (Middleton, 1967), or who have operations to correct congenital heart disease (Henry, 1968; Vettes and Barclay, 1973). Further, lymphoid follicles are seen frequently in other diseases now believed to have an autoimmune basis—indeed, Sloan (1943) observed lymphoid follicle formation in subjects with Addison's disease, acromegaly, and thyrotoxicosis. It would therefore be necessary to postulate that only one disease with thymic germinal centre formation causes excessive release of 'thymin' while others, histologically identical, do not. Secondly, if 'thymin' does exist and is a hormone, its site of production in the thymus has not been elicited. It is true that ultrastructural evidence of hormone production by the thymus (in Hassall's corpuscles) has been produced by Vettes and Macadam (1973), but we do not believe this substance is 'thymin' but 'thymosin', a hormone isolated by Allan Goldstein (Goldstein et al, 1972) and detected in peripheral blood by Bach (1973). This conclusion is supported by the fact that, in some patients with a congenital deficiency of T cell function, the Hassall's corpuscles are absent. Further, to date no observations have been made of specific abnormalities in the structure or form of Hassall's corpuscles in myasthenia gravis.

Careful histological assessment of myasthenic thymus glands has failed to detect a statistically significant relationship between germinal centre formation and the response to thymectomy. It remains to be determined whether any historical features will correlate better.

REFERENCES


Letter to the Editor

reprinted from THE LANCET, November 3, 1973, p. 1033

IMMUNE RESPONSE GENES IN MYASTHENIA GRAVIS

Sir,—The cause of myasthenia gravis is unknown, but there is increasing evidence that it may be immunological.1,2 The incidence of autoimmune diseases is increased in patients with myasthenia.1 The thymus is abnormal histologically in 80% of cases, a thymoma being present in approximately 10%.3 Anti-muscle and other humoral antibodies are found in significant titre in 84% of cases,4 and recent reports have demonstrated cell-mediated immunity to muscle, thymic, and brain antigens in 71% of myasthenic patients.4 The significance of these findings is still undetermined. Genetic factors have also

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Associated illnesses</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>26</td>
<td>Atopic illnesses</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>37</td>
<td>Multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>68</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>39</td>
<td>Atopic illnesses</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>Raynaud’s phenomena; rheumatic fever; pemphigus; candida; ulceration of tongue; keratotic skin nodules</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>41</td>
<td>Hayfever, atopic illnesses</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>22</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>36</td>
<td>Thyrotoxicosis</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>21</td>
<td>Pruritus after hot baths</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>27</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>39</td>
<td>Thyrotoxicosis</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>41</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>22</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>16</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>7</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>41</td>
<td>Rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>29</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>37</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>35</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>53</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>26</td>
<td>Sarcoidosis, atopic illnesses</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>31</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>42</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>15</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>48</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>41</td>
<td>Nil</td>
<td></td>
</tr>
</tbody>
</table>

Identical twin

Congenital myasthenia

Congenital myasthenia

Congenital myasthenia

Sisters

Congenital myasthenia

Congenital myasthenia

Congenital myasthenia

Congenital myasthenia

Sarcoidosis, atopic illnesses
been suspected to be operative in the disease, since there is an increased familial incidence and myasthenia has occurred in twins. During the past few years, studies in humans and laboratory animals have demonstrated a relationship between histocompatibility antigens, immune responsiveness, and certain autoimmune and neoplastic disorders. We therefore decided to study the histocompatibility antigens in patients with myasthenia gravis and in their families.

The patients are listed in Table 1. HL-A typing was performed using a modification of the technique of Kissmeyer-Nielsen and Kjerbye as employed by this laboratory in the 5th Histocompatibility Workshop. A total of 88 sera were employed and the following specificities were sought: At the first locus, HL-A1, HL-A2, HL-A3, HL-A9, HL-A10 (W25, W26), W28, W29, W30, W31, W32; at the second locus, HL-A5, HL-A7, HL-A8, HL-A12, HL-A13, W10, W14, W15, W16, W17, W18, W22, and W27.

The results of the histocompatibility testing are shown in Table II. Histocompatibility antigens HL-A1 and HL-A8 occur with disproportionate frequency in patients with myasthenia and to a lesser degree in their families. HL-A1 occurs in 84.6% of myasthenics ($\chi^2 = 32.57$) and 62.06% of their families, as opposed to a random frequency in the population of Scotland of 30.8%. HL-A8 is present in 65.38% ($\chi^2 = 13.28$) of myasthenics, in 58.72% of their families, but in only 31.5% of the Scottish general population. These figures are highly significant, denoting a true association between antigens HL-A1 and HL-A8, and myasthenia. Two preliminary reports have shown similar findings, but in both an increased frequency of HL-A8 was present only in female myasthenic patients. In 8 of our cases where both parents were examined, 4 inherited the HL-A1-8 haplotype from the mother and 4 inherited it from the father.

The possession of HL-A1 and HL-A8 antigens can be only one factor in the determination of susceptibility to the development of myasthenia, since, of the identical twins studied, only 1 had the disease. Also, in our 2 cases from the

<table>
<thead>
<tr>
<th>Locus</th>
<th>Random</th>
<th>Myasthenia patients</th>
<th>Myasthenia families</th>
</tr>
</thead>
<tbody>
<tr>
<td>I HL-A1</td>
<td>597 30.8</td>
<td>26.4 ($^2 = 32.57$)</td>
<td>29.6</td>
</tr>
<tr>
<td>II HL-A8</td>
<td>597 31.5</td>
<td>26.3 ($^2 = 13.28$)</td>
<td>29.6</td>
</tr>
</tbody>
</table>
same family of congenital myasthenia, both parents had HL-A1 and 8 antigens but the child who was homozygous had a milder clinical course than her heterozygous sister.

It is interesting to note that in coeliac disease, which is also considered to be an autoimmune disorder, HL-A1 antigen is present in 78% of cases and HL-A8 in 88%.11 Similar findings occur in autoimmune hepatitis12 and childhood asthma.13

The pattern of inheritance of HL-A haplotypes within the family showed some imbalance on a preliminary analysis. This finding clearly justifies further investigation into this and immunological parameters.

This work was supported by the Muscular Dystrophy Group of Great Britain.

Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF.
Tissue Typing Laboratory, Department of Bacteriology, Royal Infirmary, Glasgow.

PETER O. BEHAN
J. A. SIMPSON.
HEATHER DICK.

THE INHERITANCE OF HL-A ANTIGENS IN MYASTHENIA GRAVIS

HEATHER M. DICK, P. O. BEHAN, J. A. SIMPSON AND W. F. DURWARD

*Tissue Typing/Clinical Immunology Laboratory, Department of Bacteriology, Royal Infirmary, Glasgow and Institute of Neurological Sciences, Southern General Hospital, Glasgow

(Received 3 October 1974)

SUMMARY

Thirty-one patients with myasthenia gravis were studied for their HL-A antigen expression. Eighteen patients had family members available for HL-A typing, and full genotyping for HL-A antigens was possible. The inheritance of HL-A8 was studied in detail, and it was noted that this did not correlate exactly with the development of myasthenia gravis. The multifactorial nature of the genetic component of this disease was confirmed by these studies.

In the first paper to present a comprehensive account of myasthenia gravis with a postulated immunological pathogenesis due to thymic disease, one of us (Simpson, 1960) commented briefly on the rarity of familial myasthenia but drew attention, for the first time, to a familial linkage between myasthenia gravis and thyrotoxicosis and possibly diabetes mellitus. It was suggested that a genetic factor may express itself as either thyroid disease or myasthenia gravis, with occasional presentation of both disorders in the one individual. The correlations were elaborated in more detail by Simpson in 1968. The link between genes, thymus, thyroid and neuromuscular function was considered to be convergent rather than linear, with a disorder of immunological tolerance in common. The original autoimmune hypothesis, independently supported on serological grounds by Strauss et al. (1960) was immediately supported by demonstration of the immunological role of the thymus (Miller, 1961) and clinical and serological evidence of autoimmune disorders in myasthenia patients (Simpson, 1966; Vetters, 1967; Kott & Rule, 1973). There is now general acceptance that myasthenia gravis is associated with immunological abnormality. Current research is concerned with establishing the nature of this association and the mechanism of the neuromuscular abnormality.

A genetic study by Jacob et al. (1968) found no secondary cases of myasthenia gravis in 448 relatives of seventy myasthenia patients and no association was found between myasthenia

Correspondence: Dr H.M. Dick, Tissue Typing/Clinical Immunology Laboratory, Department of Bacteriology, Royal Infirmary, Glasgow G4 0SF.
antigens and systems as clear-cut associations

In an instance, system is of immune aspects have circulatory models, where the al. 1969) report antigens (HL-A) histocompatibility. There have been (clinical) expression limited findings of different manifestations the Feltkamp 1972; Nantba & Grob noted a arthritis and rheumatoid in first-degree relatives of the early onset type but one myasthenic child had a mother with rheumatoid arthritis and a father with pernicious anaemia. In the later onset type, however, 6% of first-degree relatives had a thyroid abnormality, 17% had an allergic disease and there were single cases of connective tissue disorders.

Possible familial linkage with thyroid disease is confirmed by Oosterhuis (1964) who also noted a raised incidence of rheumatoid arthritis in mothers of patients with myasthenia gravis. Namba & Grob (1970) reported a familial concurrence of myasthenia gravis and rheumatoid arthritis. We also have records of this association and also of pernicious anaemia in close relatives of myasthenic patients (Simpson, unpublished). These reports, supporting the original suggestion of Simpson (1960) that genetic expression may be variable with different manifestations of immunological abnormality, would nullify the negative genetic findings of Jacobs et al. (1968) and Herrmann (1971). The survey of Bundey (1972) gives limited support to the concept of alternative expression. Genetic surveys based on recognized (clinical) expression of gene function must necessarily be incomplete. We have, therefore, been interested in surveying the human histocompatibility antigens (HL-A) in patients with myasthenia gravis and their relatives and, in particular, those related to immune response genes. There are reports of striking alterations in the frequency of at least one of the human histocompatibility antigens (HL-A) in patients with myasthenia gravis (Pirskanen et al., 1972; Feltkamp et al., 1974; Fritze, 1973; Behan et al., 1973; Fritze et al., 1974). All the studies report an increased frequency of the second locus antigen HL-A8, with some increase in HL-A1 and in one series (Fritze et al., 1973) of HL-AC, both first locus antigens. Fritze et al. (1974) report that the presence of HL-A8 correlated with the presence of thymic follicular hyperplasia and with early onset in females.

These disease association studies have been prompted by experimental work with murine models, where the genes defining the major histocompatibility system, H-2, have been shown to have a close genetic relationship with immune response genes (Lilly, 1968; McDevitt & Benacerraf, 1969). These immune response (Ir) genes are demonstrably responsible for many aspects of immune responsiveness in the mouse including quantitation of antibody production, susceptibility to some leukemogenic viruses, and delayed hypersensitivity. The H-2 system is believed to be the homologue of the HL-A system in man (Klein & Shreffler, 1971). In an outbred population like man, however, it is relatively more difficult to demonstrate clear-cut associations between a disease process or immunological response and iso-antigen systems as polymorphic as the HL-A antigens. Family studies of the inheritance of HL-A antigens and the occurrence of a particular disease may help to clarify the genetic associations.
The inheritance of HL-A antigens in myasthenia gravis

currently postulated for the HL-A genes and possible human Ir genes (McDevitt & Bodmer, 1972; Bodmer, 1972).

We wish to present data on such family studies in a group of patients with myasthenia gravis, some of which we have reported previously in relation to HL-A frequency (Behan et al., 1973).

MATERIALS AND METHODS

This report involves thirty-one patients with myasthenia gravis, six males and twenty-five females. For eighteen of these patients we were able to obtain material for HL-A typing from family members. Two patients were sisters, so that the family data are for a total of seventeen families. Six of these were families where the parent had the disease and there were children of the marriage. In seven families, the patients were studied as offspring, in some cases without sibships. The distribution of sibships, etc. is outlined in Tables 1 and 2. There

<table>
<thead>
<tr>
<th>Table 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Without family data</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>With family data</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Families studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8 (a)</td>
</tr>
<tr>
<td>(b)</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>17</td>
</tr>
</tbody>
</table>
DISCUSSION

Myasthenia gravis is clearly multifactorial in its origin: although familial cases do occur, the genetic factors are apparently not overriding. We have twin sisters, one with and one without the disease (previously described by Simpson, 1965) and many sibships with only one case. It is clear that despite the frequent association of the HL-A8 antigen and myasthenia gravis,
<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>1st locus</th>
<th>2nd locus</th>
<th>Deduced haplotype*</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td>Patient</td>
<td>Female</td>
<td>1</td>
<td>8</td>
<td>W16</td>
</tr>
<tr>
<td></td>
<td>Husband</td>
<td>2</td>
<td>W29</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Son</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Family 2</td>
<td>Patient</td>
<td>Female</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Brother 1</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Brother 2</td>
<td>1</td>
<td>8</td>
<td>W14</td>
<td>1,8</td>
</tr>
<tr>
<td></td>
<td>Sister</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Family 3</td>
<td>Patient</td>
<td>Male</td>
<td>2</td>
<td>8</td>
<td>W16</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>1</td>
<td>2</td>
<td>W16</td>
<td>W17</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 4</td>
<td>Patient</td>
<td>Female</td>
<td>2</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Husband</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>W15</td>
</tr>
<tr>
<td></td>
<td>Son 1</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Son 2</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>W15</td>
</tr>
</tbody>
</table>

*Comment:
- No evidence of MG
- Same 1, 8 haplotype as patient
- HL-A identical with patient
- The patient could have inherited either haplotype
- Homozygous 8
- 8 from father
<table>
<thead>
<tr>
<th>Family 6</th>
<th>Patient</th>
<th>1st locus</th>
<th>2nd locus</th>
<th>Deduced haplotype*</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Female</td>
<td>W30</td>
<td>8</td>
<td>W10</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>W15</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>1</td>
<td>W30</td>
<td>8</td>
<td>W10</td>
</tr>
<tr>
<td></td>
<td>Sister</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 8</td>
<td>Patient (a)</td>
<td>Female</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Patient (b)</td>
<td>Female</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous 8</td>
</tr>
<tr>
<td>Family 12</td>
<td>Patient</td>
<td>Male</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>W5</td>
</tr>
<tr>
<td>Mother</td>
<td>2</td>
<td>9</td>
<td>W5</td>
<td>W17</td>
<td>2, W17</td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>9</td>
<td>W5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3—continued

<table>
<thead>
<tr>
<th>Family 15</th>
<th>Patient</th>
<th>Female</th>
<th>1</th>
<th>W32</th>
<th>8</th>
<th>W16</th>
<th>1, 8</th>
<th>Presumed haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Twin Sister</td>
<td>1</td>
<td>W32</td>
<td>8</td>
<td>W16</td>
<td>1, 8</td>
<td>W32, W16</td>
<td>HL-A identical with patient</td>
</tr>
<tr>
<td>Family 16</td>
<td>Patient</td>
<td>Female</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>W10</td>
<td>2, W10/1, 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grandfather (maternal)</td>
<td>11</td>
<td>W28</td>
<td>W5</td>
<td>W27</td>
<td>W28, W5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>1</td>
<td>W28</td>
<td>W5</td>
<td>8</td>
<td>W28, W5/1, 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Haplotypes are arranged as follows. Paternal antigens/maternal antigens, e.g. in family 1 2, 7/1, 8. Where parental attribution is not possible the phenotypes are given as in family 2, i.e. 1, 8.
### Table 4. Families of HL-A8 negative patients

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Father</th>
<th>Mother</th>
<th>Sister</th>
<th>Family</th>
<th>Patient</th>
<th>Mother</th>
<th>Grandfather (maternal)</th>
<th>Grandmother (maternal)</th>
<th>Family</th>
<th>Patient</th>
<th>Husband</th>
<th>Son</th>
<th>Family</th>
<th>Patient</th>
<th>Wife</th>
<th>Son</th>
<th>Daughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>Female</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>W32</td>
<td>7</td>
<td>W15</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W15</td>
<td>7</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W15</td>
<td>7</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W15</td>
<td>7</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Female</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>W29</td>
<td>12</td>
<td>W10</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>W29</td>
<td>3</td>
<td></td>
<td>7</td>
<td></td>
<td>W29</td>
<td>12</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>W29</td>
<td>3</td>
<td></td>
<td>7</td>
<td></td>
<td>W29</td>
<td>12</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>W29</td>
<td>3</td>
<td></td>
<td>7</td>
<td></td>
<td>W29</td>
<td>12</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Female</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>W32</td>
<td>7</td>
<td>W15</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W32</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W32</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>W32</td>
<td>2</td>
<td></td>
<td>9</td>
<td></td>
<td>W32</td>
<td>W27</td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Male</td>
<td>1</td>
<td>1</td>
<td>W28</td>
<td>12</td>
<td>W17</td>
<td>W17</td>
<td>W28, 12</td>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>W28</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>W28</td>
<td>12</td>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>W28</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>W28</td>
<td>12</td>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>W28</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>W28</td>
<td>12</td>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comment:**
- **Thymoma:** Probably homozygous.
- **2:** Probably homozygous.
### Table 4—continued

<table>
<thead>
<tr>
<th>Family 13 Patient</th>
<th>Female</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>( \frac{3, 5}{2, 7} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Husband</td>
<td></td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>W18</td>
<td>( 1, W18 )</td>
</tr>
<tr>
<td>Daughter</td>
<td></td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>W18</td>
<td>( 1, W18/2, 5 )</td>
</tr>
<tr>
<td>Son</td>
<td></td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>( 2, 8/3, 7 )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family 14 Patient</th>
<th>Female</th>
<th>9</th>
<th>10</th>
<th>W5</th>
<th>( \text{Possible parental haplotypes are:} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sister 1</td>
<td></td>
<td>9</td>
<td>10</td>
<td>W5</td>
<td>W19, W17</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>9</td>
<td>10</td>
<td>W5</td>
<td>9, W5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>W19</td>
<td>10</td>
<td>W5</td>
<td>W17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10, W5</td>
</tr>
</tbody>
</table>
Table 5. Sex and age of onset

<table>
<thead>
<tr>
<th></th>
<th>HL-A8 positive</th>
<th>HL-A8 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>With family data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Late</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>5 (7*)</td>
<td>5</td>
</tr>
<tr>
<td>Late</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Without family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early females</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Early males</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two sisters have congenital myasthenia.

the relationship is not a direct one. The existence of HL-A8 negative cases of myasthenia supports this belief, and our family studies demonstrate unequivocally that inheritance of HL-A8 or indeed a particular HL-A phenotype is not an essential precursor to the development of myasthenia gravis. Any gene or genes which affect the likelihood of developing the disease must lie at some distance from the second locus, and indeed might be on another chromosome. The preponderance of HL-A8 positive males with early onset is not even convincing proof of the importance of this antigen as an aetiological factor. In family 3, where we have an HL-A8 positive male patient, his mother is disease free and is actually 8 homozygous. Similarly in family 16, the mother from whom the patient derived his HL-A 1, 8 haplotype does not have myasthenia. It might have been expected that in a family where myasthenia was present, the combination of HL-A8 and female sex would predispose to disease: in family 12, it is the male sibling who has the disease, and his haploidentical sister is healthy. Even homozygosity for HL-A8 does not increase the chance of disease. Family 2 is a clear example of the failure to develop disease, even where the sex preponderance might be expected to operate. Of two HL-A identical sisters, homozygous for HL-A8, only one has myasthenia and two brothers who have both inherited HL-A8 are healthy. The parental attribution of the 8 antigen in this family cannot be deduced; in one sibling at least, the antigen is likely to have come from either parent. The diseases which are frequently found in association with myasthenia also occur in our patients, e.g. thyrotoxicosis, 'rheumatoid' arthritis and presence of various tissue autoantibodies: we did not attempt to establish the prevalence of these conditions in the sibships we studied.

Recent work on the relationship of HL-A antigens to other diseases, particularly multiple sclerosis, has suggested that the association may actually be stronger for at least one histo-
compatibility antigen system other than HL-A. This system is presently detected by mixed leucocyte reaction (MLR) only, and appears to be multiallelic. Jersild et al. (1973) have demonstrated that the frequency of one allele of the MLR locus, 7a, is not only more frequent in multiple sclerosis (MS) patients than in normals, but also tends to show linkage disequilibrium with the serologically detectable antigen HL-A7, which might explain the increased frequency of HL-A7 in multiple sclerosis. The highest correlation may thus be between MLR antigen 7a and disease, and not directly with HL-A7. Because HL-A7 and 7a tend to occur together rather more often than would be expected if inheritance were entirely random and are likely to be the products of linked genes, then HL-A7 will tend to be increased in multiple sclerosis. However, the possession of 7a is not absolutely linked to HL-A7, and thus HL-A7 negative cases of MS will occur (Jersild et al., 1973). The association of 7a with multiple sclerosis is not absolute either, however, suggesting that still other factors, some of which may be related to more distantly placed Ir genes, must be involved.

Studies on MLR genes and myasthenia gravis to identify a similar MLR allele, perhaps associated with HL-A8, are obviously required. The frequent association of HL-A8 with other diseases, including coeliac disease (Falchuk et al., 1972; Stokes et al., 1972), active chronic hepatitis (Galbraith et al., 1974; Mackay & Morris, 1973), juvenile diabetes (Platz et al., 1974), Addison's disease (Platz et al., 1974) and thyrotoxicosis (Grumet et al., 1973) suggests that the presence of an MLR antigen, closely linked to HL-A8, might be of unusual significance in the development of autoimmune disorders. Smith (1974) has pointed out that it is difficult to discriminate between multifactorial models for inheritance of clinical disorders and unifactorial models with low penetrance. The more genetic markers which can be clearly identified to have an association with a specific disease, the greater will be our understanding of the role which inheritance plays in the development of that disease.

ACKNOWLEDGMENTS

Our grateful thanks are due to many of our tissue typing colleagues for generous gifts of antisera. These include Dr G. B. Ferrara, Dr F. Kissmeyer-Nielsen and Mr K. Gelsthorne. We appreciate the skilled technical assistance of Mr R. Wallace, and Miss Nancy Henderson. The work was supported in part by the Muscular Dystrophy Group of Great Britain.

REFERENCES


TOLLERANZA IMMUNOLOGICA E MIASTENIA
IL RUOLO DEL TIMO NELL'ACCRESCIemento
E NELLA DIFFERENZIAZIONE

IMMUNOLOGICAL TOLERANCE AND MYASTENIA
THE ROLE OF THYMUS IN GROWTH AND DIFFERENTIATION

J.A. SIMPSON

Dipartimento di Neurologia dell'Università di Glasgow
Istituto di Scienze Neurologiche e Southern General Hospital
Glasgow - Scozia

SUMMARY. In the period 1955-60 Simpson proposed a hypothesis on myasthenia based on clinical data. He indicated it as a plurisystematic disease with analogies with systemic lupus erythematosus, transmissible to the newborn, responsive to corticosteroids, and with anatomopathological alterations residing in the muscle and thymus, suggesting an immunological disease of the cell-born type (Simpson, 1960). Smithers (1959) held that the myasthenic thymus resembled the thyroid of Hashimoto's struma, assuming autoimmune damage to the thymus, but was unable to account for the neuromuscular disease. Naastuk and coll. (1960) observed that myasthenic serum injured a frog's muscles. The reaction was of the complement fixation type and Strauss and coll. (1960) showed that it was associated with a seizure of the seric globulins at the A threads of the muscular fibres.

Subsequent research showed that this globulin, acting as an antibody, was of high titre in patients with thymus tumour but not closely linked to myasthenia. Myasthenic patients possess a higher number of antibodies against the thyroid, gastric mucosa and other tissues than normal people.

Subsequent developments of the Smithers and Strauss theories led to an «instructive» theory of auto-immunity with thymitis. Goldstein and coll. state they have produced this condition experimentally and explain the neuromuscular disease as due to freeing of a blockage substance by the thymus. These results are not confirmed by other researchers. Simpson's hypothesis is based on the idea of a «breakdown in immunological tolerance». It is the only one that explain both clinical findings and serological data and comes out in favour of a genetic factor (Behan, Simpson, Dick, 1973).

The nature of the neuromuscular damage remains obscure. Simpson's hypothesis regards the immunological function of the thymus and lymphocytes as part of a homeostatic control mechanism over increase and differentiation of the tissues. Theories similar to his advanced by Burnet and by Burwell and Burch are briefly examined.

RIASSUNTO. Nel periodo 1955-60 Simpson propose una ipotesi per la miastenia, basata su dati clinici, indicandola come una malattia plurisistemica con analogie con il lupus eritematoso sistemico, trasmissibile al neonato, rispondente ai corticosteroidi e con alterazioni anatomo-patologiche risiedenti nel muscolo e nel thymus.
Tolleranza immunologica e miastenia


Successivamente le ricerche mostrarono che tale globulina, agente come un anticorpo di elevato titolo in pazienti con tumore del timo ma non era strettamente legata alla miastenia. Pazienti miastenici possedevano un numero di anticorpi contro la tiroide, la mucosa gastrica ed altri tessuti superiori a quello della popolazione normale.

In seguito gli sviluppi delle teorie di Smithers e di Strauss portarono a una "istruttiva" teoria della autoimmunità con timo. Goldstein e coll. affermarono di aver prodotto tale condizione sperimentalmente e spiegano la malattia neuromuscolare come dovuta alla liberazione di una sostanza bloccante dal timo. Altri ricercatori non confermarono tali risultati.

La ipotesi di Simpson è basata sul concetto di un "crollò della tolleranza immunologica". E' l'unica che può spiegare sia le vicende cliniche che i dati sierologici e depone a favore di un fattore genetico (Behan, Simpson e Dick, 1973).

La natura del danno neuromuscolare rimane oscura. L'ipotesi di Simpson riguarda la funzione immunologica del timo e dei linfociti come parte di un meccanismo omeostatico di controllo sull'accrescimento e la differenziazione dei tessuti. Vennero esposte in rassegna brevemente teorie simili a quelle dell'A., esposte da Burnet e da Burwell e Burch.


Altri fattori a favore di tale ipotesi erano:

1) L'età e l'incidenza del sesso dei pazienti, così come l'evoluzione della miastenia erano strettamente simili a quelli del lupus eritematoso sistemico.
2) Il timo dei pazienti miastenici mostrava caratteristicamente dei « centri germinali ». Benché nel 1960 il ruolo immunologico del timo non venisse accettato, sembrò all’A. che facesse parte del sistema reticoloendoteliale correlato con le reazioni immunologiche.

3) Le linforagie riscontrabili nei muscoli dei miastenici, benché considerate dai patologi di allora come « non specifiche », evocavano suggestivamente un tipo di reazione immunologica.

4) Il passaggio di un anticorpo attraverso la placenta poteva spiegare la miastenia neonatale, mentre non si osservava alcun effetto di blocco neuromuscolare da parte del passaggio di siero di miastenici in soggetti adulti.

5) Il cortisone causava notoriamente una remissione della miastenia dopo un temporaneo deterioramento.

6) Mentre si stavano effettuando le indagini su tale ipotesi Nastuk e coll. riportarono la dimostrazione di una citolisi del muscolo di rana determinata dal siero di miastenici.

Nel 1960 sembrò giustificato pubblicare l’ipotesi del meccanismo immunologico della miastenia. E’ da notare che il fattore immunologico fu indicato come un elemento di una catena di eventi controllati geneticamente. Sfortunatamente il resto dell’ipotesi fu sopravalutato dai successivi autori. In tale epoca si ritenne che un anticorpo di origine linfocitaria agisse come sostanza bloccante combinandosi con i recettori acetilcolinici.

Mentre il lavoro era in preparazione, Smithers (1959) segnalò un commento di Castleman (1955) che i centri germinali del timo dei miastenici somigliava alle alterazioni osservabili nella malattia di Hashimoto e Smithers suggerì in tale occasione che « vi era la possibilità che questa malattia potesse essere dovuta anche ad una risposta autoimmune associata in certi casi ad una neoplasia ».

Indipendentemente da questa ricerca e a partire da premesse interamente diverse, un gruppo americano sviluppò una teoria immunologica della miastenia. Nel corso di ricerche su sostanze determinanti il blocco neuromuscolare Nastuk e coll. (1959) trovarono che il sangue dei pazienti miastenici (ed alcuni soggetti-controllo di minor grado) causava la lisi delle cellule muscolari della rana. Successivamente a questa sorprendente osservazione essi riscontrarono che l’attività complementare del siero rientrava nei limiti della norma in alcuni casi. Ciò portava a metterlo in rapporto con le remissioni e con le esacerbazioni rispettivamente (Nastuk e coll., 1960). Nel loro lavoro essi suggerivano la possibilità che un meccanismo autoimmune potesse ave-
re un ruolo eziologico nella miastenia. Il meccanismo che essi ipotizzavano era lo sviluppo di una autoimmunità contro uno dei componenti (M) delle fibre muscolari e che «la terminazione nervosa dei nervi motori poteva essere direttamente o indirettamente interessata». Essi sottolinearono l'evidenza che il timo era in grado di confezionare degli anticorpi. Il gruppo di New York pubblicò poco dopo la dimostrazione di una frazione globulinica legata al muscolo fissatrice di complemento nel siero di pazienti con miastenia (Strauss e coll., 1960).


Van der Geld e coll. (1964) dimostrarono che il siero dei miastenici che reagisce con i muscoli reagisce anche nei confronti delle cellule della midollare timica. Dapprima si pensò che si trattasse di cellule epiteliali ma in seguito furono identificate come cellule mioidi. Da tale osservazione ne deriva la massima diicotomia fra coloro che ritengono che il timo svolga un ruolo pri-
mario nel disordine immunologico della miastenia e chi invece lo ritiene danneggiato immunologicamente. Per il primo gruppo, le cellule mioidi offrono una spiegazione della penetrazione dell'antigene muscolare nel timo nonostante la barriera emato-timica ipotizzata da Marshall e White (1961). Per la scuola che sostiene la «timite», invece gli anticorpi antimuscolo che si sviluppano fuori del timo reagiscono con le cellule mioidi determinando così una lesione autoimmune dello stesso timo.

Allora, nel 1960, vi erano tre conclusioni indipendenti circa il fatto che un'alterazione immunologica fosse alla base della miastenia, che differivano considerevolmente nel loro valore euristico.

Il commento di Smithers circa l'aspetto del timo, suggeriva l'idea di una lesione di natura immunologica, ma non forniva alcuna spiegazione circa i disturbi muscolari. Sarebbe stato impossibile sulla base di quella semplice considerazione predire l'esistenza di alterazioni associate di altri organi o che potesse essere terapeuticamente valida la effettuazione di una timectomia.

I risultati di Strauss furono pertanto estremamente importanti. Veniva ad essere ormai certo che molti miastenici hanno anticorpi antimuscolo per cui si può verificare una lesione muscolare nel corso di una reazione interessante il complemento. Molti clinici rifiutarono di accettare tale evidenza giacché solo una minoranza di miastenici possiede tali anticorpi e perché è difficile accettare come un difetto di trasmissione una lesione che interessa le miofibrille anziché la placca nervosa. La miastenia inoltre può essere assente in pazienti con timoma pur essendo presenti una reattività antimuscolo e antitimo con la gammaglobulina sierica (Strauss e coll., 1966). Nessun ruolo per il timo veniva ipotizzato né potevano essere predette le alterazioni cliniche associate. I modelli di Smithers e di Strauss d'altrparte non esprimevano una spiegazione della miastenia neonatale. L'ipotesi di Simpson era l'unica che spiegasse tutti i nuovi fatti scoperti nella miastenia. Gli elementi più importanti, che non potevano essere previsti con le altre tesi erano i seguenti.

1) Interessamento di molti organi.
2) Miastenia neonatale.
3) L'evoluzione della miastenia.
4) Significato della risposta al cortisone.
5) Segnalazione, per la prima volta, della possibilità di concomitanti disordini del sistema reticolo-endoteliale.
6) L'anatomia patologica del muscolo e del timo nella miastenia.
7) Conclusione di S i m p s o n sul fatto che il timo ha inoltre un ruolo immunologico importante, residuo di una più ampia funzione, sull'accrescimento e sulla differenziazione dei tessuti nel fetto. Una necessaria conseguenza di questo asserito era che la timectomia avrebbe potuto arrestare il danno immunologico e di altri organi se effettuata prima che si realizzassero lesioni permanenti negli organi periferici.

8) Possibile spiegazione della autoimmunità e in generale dell'implicazione di patogenesi genetica e ipotalamo-pituitaria.

Prima di analizzare il ruolo del timo così proposto, è essenziale ricordare che queste ipotesi erano state elaborate nel quinquennio 1955-60 e che furono pubblicate prima che il lavoro di Miller (1961) e che altre ricerche mettessero in chiaro che il timo ha un ruolo importante in immunologia. A quell'epoca infatti era ancora considerato una ghiandola endocrina. Gli immunologi pensavano che le globuline anticroppali derivassero principalmente dalle plasma cellule benché il possibile ruolo dei linfociti fosse già stato ipotizzato. Ciò nondimeno S i m p s o n (1960) congetturava che una secrezione timica potesse « liberare un anticoorpo trasportato dai linfociti » e che la impossibilità (in tale epoca) di identificare un anticoorpo antimuscolo poteva essere dovuta al fatto che « la sostanza ricercata era intracellulare ». Un anticoorpo umorale era una suggestiva spiegazione della miastenia neonatale ed era ipotizzabile che un anticoorpo contro i recettori proteici della placca motrice potesse dar luogo ad un agente bloccante neuromuscolare. Una argomentazione importante contro la versione 1960 della ipotesi di S i m p s o n era il fatto che non fosse possibile identificare un anticoorpo a livello della guanizione neuromuscolare mediante le tecniche di immunofluorescenza (M c F a r l i n e coll., 1966). Nel 1960 nessuna conclusione poté essere tratta sulla natura della autoimmunità. Allora esistevano due dottrine. 1) Che un tessuto normale potesse generare anticorpi da un sistema immunologico normale se questo veniva modificato dalla combinazione con determinate sostanze esterne, quali un virus. 2) Che il riconoscimento immunologico del « proprio » o « non proprio » (« self » e « not self ») potesse venire turbato da malattie del sistema immunologico. L'aggravamento improvviso della miastenia in occasione di malattie delle prime vie respiratorie può diporre a favore della prima interpretazione, ma S i m p s o n (1960, 1964, 1966a) preferì la seconda alternativa perché (a) offriva una migliore spiegazione delle malattie autoimmuni di altri organi così come del muscolo, e (b) vi era una evidenza clinica di un legame genetico fra la miastenia e le altre malattie.
Nella successiva decade altri ricercatori ignorarono questi argomenti e ritengono che il timo può elaborare una sostanza a effetto bloccante neuromuscolare sia «nel corso del suo danneggiamen


Inevitabili conclusioni delle ipotesi di Strauss e di Goldstein sono che la asportazione del timo, può curare la miastenia, impedire la possibility di avere un figlio con miastenia neonatale e prevenire la comparsa di miastenia quando non sia già presente. Nessuna di queste proposizioni è vera e non è necessario documentare che sono false. L’inizio di una miastenia molti anni dopo una timecetmia apparentemente completa rende insostenibile che la malattia neuromuscolare sia dovuta ad una sostanza ad azione bloccante neuromuscolare originata dal timo. D’altra parte una trasmissione alterata, dovuta in qualche modo a un danno immunologico può ancora verificarsi dal momento che risulta ben stabilito che la timecetmia non arresta la successiva reattività immunologica. Goldstein e i suoi collaboratori hanno enunciato di nuovo i loro asserti in una serie di lavori (passati in rassegna da Goldstein e Mangano, 1971) e sono stati sostenuti da Kalde e coll., 1970, ma non confermati da Vettors e coll. (1969) e da altri autori, incluso Jones e coll. (1971) benché di quest’ultimo gruppo facesse parte un antico collaboratore di Goldstein. A meno che questi risultati negativi non siano dovuti a differenze fra gli animali da esperimento, è possibile che il modello di Goldstein rassomigli alla sindrome miasteniforme dei polimiositici piuttosto che alla vera miastenia.

Se il timo soffre di un danno autoimmune perché condiziona un antigene con il muscolo, le alterazioni autoimmuni associate di altri organi non precederebbero la malattia neuromuscolare come in realtà succede comunemente (Simpson 1960, 1964). La concorrenza di un certo numero di alterazioni autoimmuni nel paziente miastenico sembra corrispondere più verosimilmente al fatto che vi sia stato un crollo del normale meccanismo omeostatico che previene la proliferazione dei «forbidden clones» di anticorpi formanti cellule in risposta all’antigene autologo.

Le teorie contemporanee sulla funzione timica non sono adeguate ai dati della esperienza clinica. È indiscutibile che la precoce rimozione del timo arresta il peggioramento della miastenia, ma è difficile conciliare tali risultati con la evidenza sperimentale che la produzione di auto-anticorpi è secondaria alla
deficienza immunologica, o con la ipotesi di Burnet che il timo da solo possa controllare i « clones » delle cellule. Tuttavia l’esperienza clinica sembra indicare che l’organismo sta meglio senza il timo che avendone uno che, presumibilmente, funziona in maniera anormale, almeno per quanto riguarda i soggetti miastenici. D’altra parte altri processi autoimmuni, quali la malattia di Hashimoto (Simpson, 1964), il lupus eritematoso sistemico e la colite ulcerosa (A l a r ç o n - S e g o v i a e coll., 1963) e il pemfigo volgare (H a u s m a n o w a - P e t r u s e w i c z e coll., 1969; V e t t e r s e coll., 1973), possono apparire abbastanza presto dopo una timectomia nonostante la scomparsa del quadro miastenico. La timectomia in età neonatale nel topo nero della Nuova Zelanda, che è particolarmente suscettibile alle malattie autoimmuni non previene né ritarda l’inizio della anemia emolitica autoimmune, ma, anzi, la accelera (H o w i e e H e l y e r, 1966). Queste osservazioni possono essere portate contro l’ipotesi di Burnet e H o l m e s (1964) che i centri germinali del timo sono luoghi di produzione dei « forbidden clones » delle cellule immunocompetenti, capaci di reagire contro i tessuti del proprio corpo. Troppo poco è noto circa la funzione immunologica di altri organi per avere un quadro chiaro della questione. E’ tuttavia giustificabile concludere che la malattia autoimmune può essere associata con un’alterazione del timo ma tale rapporto non è obbligatorio. Ciò è altrettanto vero per il deficit muscolare della miastenia. Inoltre se si arguisce che la giunzione neuromuscolare è danneggiata da una sostanza che non è immunologica in natura, la stessa conclusione si può applicare a che questa sostanza non può essere esclusivamente un prodotto del timo.

Quantunque questo argomento non sia stato presentato in dettaglio altrove, alcuni autori hanno concluso che le alterazioni muscolari e immunologiche sono collegate solo indirettamente. La difficoltà può essere dovuta alla prevalente interpretazione della funzione timica. Dall’essere ritenuto una ghiandola endocrina senza una funzione identificabile esattamente il timo è divenuto improvvisamente il direttore della orchestra immunologica. Può essere che molte sue funzioni siano ignore. Nell’ipotesi originale della miastenia, che precedette questa formulazione, fu ipotizzato che il « riconoscimento » di antigeni proteici e la costruzione di appropriati linfociti e anticorpi, poteva avere una evoluzione specializzata verso un ruolo più o meno ampio del timo nella vita fetale nella regolazione dell’accrescimento e della differenziazione dei tessuti (S i m p s o n,

Rimane aperta la Questione del perché la miastenia sia una malattia relativamente rara. In sede clinica, vi sono elementi, basati sulla ocurrencia familiare della miastenia e con i suoi legami con le malattie della tiroide e con altri processi autoimmuni (Simpson, 1960, 1968; Hermann, 1971; Namba e coll., 1971), che rendono probabile l’esistenza di un fattore genetico responsabile di una diminuita tolleranza immunologica. Studi recenti, effettuati indipendentemente nel mio dipartimento e in
Finlandia hanno dimostrato che si verifica una istoincompatibilità con antigeni HL-A1 e HL-A8 con frequenza sproporzionata in pazienti con miastenia e in minor grado nei membri delle loro famiglie (Firskanen e coll., 1972; Behan e coll., 1973). Questi antigeni sono associati in genere con processi autoimmuni.

Conclusioni

Da diverse osservazioni appare evidente che vi è un crollo geneticamente determinato della tolleranza immunologica nei pazienti affetti da miastenia. Il timo svolge un ruolo chiave ma non esclusivo nell’ambito di una anormalità immunologica mediata attraverso le cellule ed anche nel causare una anormalità morfologica delle placche motrici. Il meccanismo decisivo della lesione neuromuscolare rimane sconosciuto. E’ tuttavia ammesso che tale meccanismo sia immunologico ma nel senso biologico più lato che si riferisce alle reazioni «autoimmuni» come parte di un meccanismo omeostatico normale della morfogenesi.

Bibliografia

Toleranza immunologica e miastenia


WEATHER AND MYASTHENIC FATIGUE

Sir,—Dr. Borenstein and Professor Desmedt are mistaken when they find it "surprising that temperature and weather correlates of myasthenic weakness have not been recognised previously". I have described this phenomenon in 1960 and in every edition of a standard textbook on muscle diseases. It must surely have been known before that date. My own studies along the same lines as your contributors gave inconsistent results on repeated testing of individual myasthenics, and I concluded that the myasthenic patient responded unfavourably to extremes of cold or heat (especially a hot bath or a stuffy atmosphere) in the same way as to emotional stress. I have also recorded that some patients declare that bright sunlight causes generalised weakness in addition to ptosis: the latter being common.

Department of Neurology,
University of Glasgow,
Institute of Neurological Sciences,
Southern General Hospital,
Glasgow G51 4TF.

J. A. SIMPSON.

Myasthenia gravis and myasthenic syndromes

J. A. SIMPSON

University of Glasgow, Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow, United Kingdom

For this specialised audience it is not necessary for me to cover in detail the various signs and symptoms of myasthenia gravis. There is, however, some value in looking again at the myasthenic syndromes with 3 questions in mind. (1) What are the main differences between myasthenia gravis and other myasthenic syndromes? (2) What features do they have in common? (3) Can these clinical data be correlated with laboratory findings to disclose the cause of myasthenia? I hope I may be forgiven for referring to previous personal publications for the benefit of readers requiring more detail than can be provided here.

In some countries the term 'myasthenia' means only muscular weakness. In this paper I am restricting it to abnormal weakness of voluntary muscles which develops following repetitive activation or prolonged tension, with a marked tendency to recovery of motor power after a period of inactivity or lessened muscular tension. The loss of power is progressive if muscular contraction is continued voluntarily, or by indirect electrical stimulation, but is reduced or abolished by administration of anticholinesterase substances or by direct electrical stimulation of muscle. These features are generally considered to indicate a defect of neuromuscular transmission and this definition excludes further consideration of primary myopathies, the periodic paralyses associated with disordered potassium regulation, hypoadrenalism and similar causes of episodic weakness.

For practical purposes we need only consider 4 types of disease with myasthenia as defined above: (1) lower motor neurone diseases, (2) myasthenic syndrome of carcinomatosis, (3) acquired myopathies, (4) myasthenia gravis.

LOWER MOTOR NEURONE DISEASES

I start with this group only to dismiss it rapidly. It is not uncommon in the electromyographic examination of patients with lower motor neurone diseases,
to show a decrementing muscular response to tetanisation of peripheral motor nerves. This has been reported in every lower motor neurone disorder, central or peripheral (for review, see Simpson, 1966). The response to anticholinergic drugs is minimal and it is extremely rare for this type of myasthenia to show progressive 'fatigability' on clinical testing. It is confined to muscles showing atrophic weakness with loss of reflex activity. It is not important diagnostically, but has theoretical importance when we come to consider the nature of the myasthenic phenomenon.

MYASTHENIC SYNDROME OF CARCINOMATOSIS

In 1953 Anderson et al. drew attention to the anomalous responses to neuromuscular blocking agents of patients who had bronchial neoplasm associated with myasthenia. Eaton and Lambert (1957) extended the findings to other malignant tumours and reported an unusual finding on electrical stimulation of the motor nerve to affected muscles. Whereas stimulation at rates below 10/sec caused a decrementing muscular response, at faster stimulation rates there was a marked increase in the responses. When the nerve is stimulated repetitively there is usually a decrease in the response at high and low rates of stimulation. References to the later studies will be found in the papers by Lambert et al. (1961) and Lambert and Elmqvist (1971).

The mean age of patients with this syndrome is 55 years. Weakness is most pronounced in the lower limbs, less markedly in the upper limbs. Weakness of bulbar and extraocular muscles occurs but is much less common than in myasthenia gravis. A most striking sign is that vigorous exercise may temporarily restore power to a weak muscle. Other features which are rare in myasthenia gravis are pain in the lower limbs and loss of tendon reflexes. There may be some improvement with anticholinesterase drugs, but it is inferior to that of myasthenia gravis and the response may disappear at a later stage of the disorder. On the other hand, guanidine is very effective in restoring function in the myasthenic syndrome, but of little value in myasthenia gravis. The special features of the syndrome clearly differentiate it from myasthenia gravis. It has a close similarity to the neuromuscular blockade experimentally produced by magnesium (Elmqvist and Lambert, 1968).

ACQUIRED MYOPATHIES

An apparently identical syndrome (decrementing response to slow stimulation, incrementing response to fast stimulation) was reported in a number of myopathies by Simpson and Lenman (1959) in the absence of known carcinoma and it is now clear that the majority of cases do not have a tumour (Lambert and Elmqvist, 1971). These cases are not common. More frequently patients with acquired myopathy have myasthenic 'fatigability' without the incremental response, which may resemble
Myasthenia gravis and myasthenic syndrome

Myasthenia gravis quite closely. The disorder is usually polymyositis, systemic lupus erythematosus, or other ‘collagen’ disease, but may occasionally be a metabolic myopathy such as thyrotoxic myopathy.

The fatigable response to repeated or sustained voluntary effort is less marked than in myasthenia gravis. It is most prominent in shoulder girdle muscles and in the neck. Dysphagia is common but extraocular muscles have not been involved in my experience. It usually occurs at an early stage of myositis and at that time there may be a good response to edrophonium or neostigmine. On repeated testing, however, the response becomes less and soon disappears. Anticholinesterases play only a minor role in treatment. Where a physician makes an initial diagnosis of myasthenia gravis, at first “confirmed” by a positive edrophonium test but with failure to respond to neostigmine or pyridostigmine in the early stage of the disease, the usual cause is symptomatic myasthenia in polymyositis rather than true myasthenia gravis.

MYASTHENIA GRAVIS

At this point one might well ask whether myasthenia gravis is a separate entity. In the following Reports I believe it will become clear that there is no single item which is exclusive to myasthenia gravis in the pharmacology, electrophysiology and in the neuromuscular pathology. Many people would like to abandon the name and use the term to indicate a disorder with many causes. In my opinion this is a confusion of thought which arises from a regrettable absence in medicine of an agreed systematic method of classification of disease. If we consider the disabilities described as ‘myotonia’ or ‘epilepsy’ we are agreed that there are a number of causes, but we have no mental difficulties in isolating ‘dystrophia myotonica’ and ‘petit mal’ as clinical entities. In the same way, it is already clear that ‘myasthenia’ is a syndrome resulting from loss of the safety factor for transmission at the neuromuscular junction (Simpson, 1969a) which may have a number of causes. Clusters of symptoms and signs (‘syndromes’) with a recognised natural history constitute a disease entity in terms of our present methods of classification and from this point of view I have no hesitation in isolating myasthenia gravis as a disease entity, although I would not disagree with those who feel that disease ‘clusters’ commonly overlap. For instance, there is no doubt that both myositis and denervation occur in this disease and on rare occasions there may be a sensory disorder and an incremental response, like the Eaton-Lambert phenomenon (Simpson, 1960). On the other hand, polymyositis is widely believed to have an immunological cause and may be associated with a tumour of the thymus gland. Nevertheless there is a recognisable ‘cluster’ which is so characteristic as to justify a separate label ‘myasthenia gravis’ – even though the adjective is not well chosen.

Examination of graphs of incidence related to age of onset, sex, and presence
of thymic tumour make it clear that the distribution is neither random nor cumulative with age (Fig. 1). If there is no thymic tumour, the distribution curves show single peaks for each sex, with modal onset age of about 21 years for both sexes. It is twice as common in females owing to a disproportion of 4.5:1 in adolescence, but over the age of 40 years first onset becomes slightly more common in males. There is no significant sex difference in myasthenia associated with thymic tumour. The modal age of onset is 45 years and it rarely starts under 30 years of age. Myasthenia gravis starting at 45 years has an even chance of being associated with thymoma. Starting at 65 years there is almost a 2:1 chance of thymoma being present. The prognosis for life is worse if there is a thymoma, but in other respects there is no essential difference. In both types the myasthenia is associated with germinal centres in the medulla of the thymus gland (Castleman and Norris, 1949). There is no evidence that the thymic changes of the young myasthenic are the precursors of thymoma of the older group, but in view of the common features it is considered advisable to retain both types under the rubric 'myasthenia gravis' for the present.

![Fig. 1. Age at onset of symptoms of myasthenia gravis. Solid line indicates females and broken line indicates males without thymoma. Shaded curve is for both sexes with thymoma.](image)

The natural history of myasthenia gravis also shows characteristic features justifying clinical acceptance as a disease, although the severity of symptoms and the time course vary from one patient to another. Some workers find it convenient to classify cases according to clinical severity. In a large series of cases a definite pattern can be identified (Fig. 2). In Stage 1 ('active stage' of Simpson, 1969b) the severity of clinical symptoms increases, but with a course characterised by relapses and remissions. Fewer than half of the cases have a remission of a month or more and long remissions rarely occur more than once. Stage 1 lasts 5–10 years and during this period occur most of the deaths directly attributable to myasthenia gravis, particularly during
myasthenia gravis and myasthenic syndrome

the first year (Simpson, 1958). It is during this stage that thymectomy may be beneficial (the earlier the better). In Stage 2, the intensity of myasthenic symptoms fluctuates but genuine remission is rare and only a small number of patients benefit from thymectomy although the disorder of neuromuscular transmission may still be compensated for by anticholinesterase drugs. The mortality rate is much lower in this stage but death may occur from asphyxiation.

Stage 3 occurs at 14-20 years from the onset of symptoms. It is a 'burned-out' stage in the sense that the severity of disease does not vary, thymectomy is of no value, deaths are restricted to respiratory accidents and the response to anticholinergic drugs tends to disappear. Many patients find that they can dispense with drugs at this stage but they have a permanent, often 'non-fatigable' weakness with wasting of muscles. The clinical picture closely resembles polymyositis. It has been termed 'myasthenic myopathy' but recent findings suggest that denervation atrophy of muscles has occurred (Oosterhuis and Bethlem, 1973).

This natural history distinguishes myasthenia gravis from the other myasthenic syndromes. Another feature, which is believed to be unique, is that a woman with myasthenia gravis may give birth to a child which is myasthenic for 1-12 weeks and then does not manifest the disease again. This occurs in about 1 in 7 live births to myasthenic mothers, suggesting that a 'myasthenic toxin' may cross the placental barrier and persist in the baby for several weeks after birth. As abnormalities of the thymus gland had been recognised for many years (and 10% of myasthenic patients have a thymic tumour), it was considered that some curare-like substance must be liberated from the thymus which could cross the placental barrier although no substance with this property has ever been isolated from the blood of myasthenics.

Fig. 2. Diagrammatic representation of natural history of myasthenia gravis indicating 3 stages: (1) 'active', (2) 'inactive', and (3) 'burned-out stage'. Shaded area represents severity of symptoms. Solid line is incidence of deaths in each year from the onset. Broken line indicates response to thymectomy.
In 1960 Simpson drew attention to an apparent relationship between myasthenia gravis and a number of disorders of other organs and suggested that myasthenia was a multisystem disease of immunological type. The thymic pathology and the well-known muscular lymphorrhages were reinterpreted as evidence of immunological disorder and the neonatal myasthenia attributed to antibody passing from mother to child. In the next decade the clinical observations were confirmed and many of the associated disorders, including a rheumatoid type of arthritis, pernicious anaemia, Raynaud’s disorder, polymyositis and pemphigus were shown to have an ‘autoimmune’ basis. At the same time Nastuk, Strauss and colleagues (Strauss et al., 1960) observed a cytolytic effect of myasthenic serum on normal muscle and demonstrated a muscle-binding globulin.

I leave to the other speakers the further development of this theme but would summarise the present situation in this way. There is now good clinical and pathological evidence for a disorder of immunological mechanisms in myasthenia gravis and general agreement that the thymus is in some way implicated. The original hypothesis suggested that a circulating globulin could act as a curare-like substance specific to the endplates of a single person or, sometimes, her child. This concept had to be abandoned. There is no evidence of an antibody fixed to the endplate receptors (McFarlin et al., 1966). The presence of anti-muscle and other antibodies in significant titre in serum of myasthenic patients is strong indication of a breakdown of immunological tolerance, but it is clear that this antibody could not cause the transmission defect recognised in myasthenia, although it might be related to the myositis which is sometimes present. Taking special precautions to identify that fluorescent antibody is attached to A-bands rather than I-bands of muscle (Vetters, 1965), we consider that A-band antibody is virtually confined to patients with a thymic tumour. Van der Geld et al. (1964) reported the universal occurrence of anti-muscle and anti-thymus reactivities in myasthenia gravis associated with thymoma. Strauss et al. (1966) confirmed this and also showed similar reactivity in 24% of patients with thymomas (or previous history thereof) who did not have myasthenia gravis.

Correlation between clinical severity and titre of anti-muscle globulin was claimed by Weiner and Osserman (1966) and denied by Oosterhuis et al. (1967). There is no correlation in my own data. Perlo et al. (1971) suggested that the time of onset of remission after thymectomy correlates with the number of germinal centres present in the thymus at the time of operation (delayed remission correlating with many germinal centres). My own material suggests a correlation between a favourable postoperative response and an absence or relative infrequency of germinal centres (Vetters, 1972).

The present position is tantalising. An immunological disorder seems certain, but it is now obvious that the working hypothesis of an antibody against endplates must be abandoned. An alternative concept that the thymus is itself immunologically damaged and releases a ‘myasthenic toxin’ (Goldstein and Whittingham, 1966) finds
Myasthenia gravis and myasthenic syndrome

little support from other workers (Vetters et al., 1969; Vetters, 1972). It ignores the original reasons for postulating an autoimmune disorder and cannot account for the associated disorders of other tissues. More seriously, it cannot be reconciled with the facts that myasthenia may first manifest itself after removal of a thymoma and that thymectomy rarely causes immediate improvement of myasthenic symptoms. Is myasthenia gravis an autoimmune disease? I think it is — but it is more than that.

It is unfortunate that arguments about the nature of disease and its causation so often fail to recognise that the disorder of function is the end-result of a chain of events. To concentrate on one link in the chain is to do less than justice to the others and to ignore the possibility of treating different links in the chain. Many of the arguments about myasthenia gravis and the myasthenic syndromes are of that type. In order to clarify our thinking when we receive the Reports which follow in this symposium I wish to present a simplified account of my present views. The detailed arguments have been presented elsewhere (Simpson, 1969a, b; 1971a, b).

The proximate mechanism of myasthenia

In the past there have been 3 main theories to account for the defect of neuromuscular transmission in myasthenia gravis. (1) A circulating curare-like substance, possibly derived from the thymus. The most recent theory of this type is Goldstein's 'thymin' theory (Goldstein and Manganaro, 1971). All theories of this type fail because of the persistence or first manifestation of myasthenia after thymectomy. Furthermore, no claims to isolate a blocking substance from the blood or thymus have been confirmed. (2) A postsynaptic defect. This theory is based on anomalous responses of myasthenic patients to injection of substances believed to act on postjunctional receptors. (3) A presynaptic defect. This theory is based on a resemblance between postactivation exhaustion of myasthenic muscle and the effect of poisoning by hemicholinium-3, which has its principal activity in blocking the synthesis of acetylcholine.

Note carefully that these theories are analogues based on the action of certain drugs on normal motor endplates. Other aspects of myasthenia gravis are ignored, including the remitting course and the asymmetrical distribution in the skeletal musculature.

In fact, with the possible exception of postactivation exhaustion, the phenomena of myasthenia gravis only indicate reduction of the 'safety factor' for transmission which is normally present. It could occur in various ways and this explains why similar myasthenic phenomena may have different causes.

Lower motor neurone diseases

A decrementing response to repetitive stimulation only occurs shortly before there is complete failure of transmission associated with 'dying back' of the motor neurone. The temporary 'recovery facilitation' so commonly seen in myasthenia gravis is
absent, indicating disease of motor nerve terminals (Simpson, 1966). The neurone is not synthesising enough acetylcholine to sustain a train of impulses. Anticholinesterase drugs will be of little or no benefit. There is no real pharmacological analogue but hemicholinium-3 has some resemblances.

**Myasthenic syndrome of Eaton and Lambert**

In this condition the first muscular response to a maximal nerve impulse is reduced. Subsequent responses decrement progressively with slow stimulation but increment with fast stimulation. This suggests that the number of quanta of acetylcholine liberated are reduced unless release is facilitated by rapid stimulation. A similar state occurs if the Mg\(^{++}\) concentration is increased at the endplate (Elmqvist and Lambert, 1968; Lambert and Elmqvist, 1971). There is limited improvement with anticholinesterases, but restoration to near normal with guanidine, a drug which promotes release of acetylcholine at motor nerve endings.

Similar types of neuromuscular blockade can be produced experimentally by botulinum toxin and by neomycin, one of a group of antibiotics which increase the weakness of some patients with myasthenia gravis. It may be that some toxic substance, possibly a polypeptide, is released by certain types of bronchogenic carcinoma, but it should be noted that there are structural changes at the endplates similar to those found in myasthenia gravis (Bergman et al., 1971).

**Acquired myopathies**

In polymyositis the electrical and pharmacological characteristics of the myasthenic syndromes are the same as in myasthenia gravis or in carcinomatous myasthenia and doubtless the explanations are similar.

**Myasthenia gravis**

I have shown elsewhere that the reduced safety factor for neuromuscular transmission in myasthenia gravis may be accounted for by the altered geometry of the endplates (Simpson, 1969b; 1971a). Certain assumptions are required to account for unusually small miniature endplate potentials reported in biopsy material by Elmqvist (1965) and attributed to lower acetylcholine content of spontaneously liberated quanta. Responses to veratrine, guanidine and anticholinesterase drugs suggest that there is no significant deficiency of stored acetylcholine in this disease. Tetanic facilitation which is prominent in myasthenia gravis, in contrast to the myasthenia of lower motor neurone disorders, suggests that there is no significant failure of acetylcholine release (Simpson, 1966).

Other pharmacological analogies suggest that both prejunctional and postjunctional defects exist. But the analogies cannot be applied if the synapses are abnormal and there is now increasing evidence that this is the case. The detailed ultrastructural studies of Engel and Santa (1971) and Bergman et al. (1971) were not available when my theoretical papers were prepared which suggested that linear
prolongation of terminal knobs over receptor sites more widely spread than normal would account for all observations. The ultrastructural studies stress widening of the primary synaptic cleft, with reduction of secondary clefts. The mean area of each terminal knob and the postsynaptic membrane profile concentration are decreased (Engel and Santa, 1971), but the total area of endplates on one muscle fibre is increased (Woolf, 1966), as they are elongated and commonly made up of serial linear expansions (Coers and Woolf, 1959). In some cases the endplates are shrunken and not elongated, especially in fatal cases responding inadequately to neostigmine (Woolf, 1966). I leave a detailed account of the pathology of the endplate to Dr. Fardeau (This Volume, pp. 427–438), but find it necessary to draw attention to two points of clinical importance.

1. The pharmacological features of myasthenia gravis can be accounted for by the structural changes in the endplates. A search for a better anticholinesterase drug is unlikely to be rewarding and there are cases where, for structural reasons, no therapeutic response is likely. I suspect that similar considerations will apply in the other myasthenic syndromes.

2. The typical light-microscopic appearance of the myasthenic motor nerve terminals, with deformed terminals and ultraterminal sprouting, leading to linearly arranged terminals spread over a wide area of the muscle fibre, suggests that very active regeneration is present, along with a process which is damaging the endplates.

**The aetiology of myasthenia gravis**

If this interpretation is valid, it is obvious that the clinical state represents a balance between two processes— one degenerative, the other regenerative. Obviously no disability is present when the balance is good and so the disease may have been active long before there are clinical manifestations. This would account for the many cases in which no precipitating cause can be found. The most common precipitating factors— infections, emotional disturbances and allergies— could affect either side of the balance. This dynamic bipolar situation immediately accounts for the relapsing-remitting course of the active stage of the disease. But now we must ask the crucial question— what causes the degeneration and regeneration of the endplates?

There is good clinical, pathological and serological evidence that immunological tolerance is decreased in myasthenia gravis. I have reviewed the evidence elsewhere (Simpson, 1960, 1969b) and need not do so again. Instead I wish to emphasise that this is only one aspect of a theory proposed by Simpson (1960) and which I still hold, with some modifications required by the advances in immunology since that date. It is wrong to describe myasthenia gravis as an autoimmune disease as if that were the end of it. Time does not permit a full review but a brief restatement of the theory is worthwhile as an introduction to the papers which follow.

There is some evidence that the mammalian endplates normally undergo cycles of growth and degeneration. Myasthenia gravis is the result of a disturbance of
this mechanism leading to deformed endplates without adequate safety factor for cholinergic transmission. It is postulated that removal of effete tissue is normally carried out by immunological mechanisms. The thymus gland undoubtedly has an immunological role. The differentiation of immunologically competent cells, and of antibody proteins, may be a survival of a wider action on tissue differentiation in the foetus (Simpson, 1960, for references). Szent-Györgyi et al. (1962) claimed to have isolated a growth-promoting hormone (promine) and a growth-inhibiting factor (retine) from calf thymus, which could play a part in the regulation of breakdown and repair of organs showing a regular turnover of cells. Unfortunately these studies were discontinued.

Figure 3 reproduced from Simpson (1971b) summarises my present thoughts on myasthenia gravis as the result of deformed endplates due to an imbalance of the remodelling process controlled by the thymus. The findings of Shapiro et al. (1968) suggest an adrenal role in the remodelling process which may have important implications for treatment. The possible remodelling role of the thymus is, of course, tentative and requires confirmation of Szent-Györgyi's work. Note too that associated autoimmune diseases such as Hashimoto's disease, 'rheumatoid' arthritis, pernicious anaemia and pemphigus, and abnormal humoral antibodies including anti-myosin substances, are considered as para-phenomena. The original paper (Simpson, 1960) suggested a lymphocyte-borne antibody causing a localised myositis with damage to both sides of the neuromuscular junction. Thyroid disorders are not limited
to thyrotoxicosis and evaluation of my own data suggests that all thyroid diseases are linked to myasthenia gravis by a common genetic factor, possibly acting through the hypothalamo-pituitary axis, and by 'autoimmunity' (Simpson, 1968). Histocompatibility antigens HL-A1 and HL-A8, occur with disproportionate frequency in patients with myasthenia gravis and, to a lesser degree, in their families (Behan et al., 1973). I do not believe that the thyroid plays an essential role in the aetiology of myasthenia gravis.

CAUSATION OF MYASTHENIA GRAVIS

How then are we to answer the question - what is the cause of myasthenia gravis? There is no simple answer. It depends on how far back one is prepared to follow the chain of events. My answers to the question are the conclusions of a clinician:

1. Myasthenia of this type is due to morphological abnormality of the motor endplates, which causes a decreased density of charge on receptors and diminished safety factor for transmission.
2. The deformed endplates are due to a disturbance of a normal remodelling process dependent on the thymus and possibly on the adrenal glands.
3. The thymic mechanism is immunological and the disordered function is shown by associated 'autoimmune' disorders in other organs and in serological studies.
4. The reason for the abnormal thymic function is unknown, but correlations with thyroid disease, factors which precipitate myasthenia, and familial studies suggest that it is genetically determined and has a hypothalamo-pituitary link which may be the site of action of stress factors which precipitate the disease.

IMPLICATIONS FOR TREATMENT

Is there any advantage in looking further than the muscle endplates or the thymus? I believe that there is because each of these factors must be considered in the treatment of myasthenic patients.

1. The safety factor for transmission is increased by anticholinesterase drugs and, to a lesser extent, by boosting the muscular response by veratrine-like drugs. There are dose-limiting factors and no response can be expected if the neuromuscular system (on either side) is too severely damaged.
2. The natural history of the disease indicates that the process destroying the motor terminals is self-limiting but can be removed by thymectomy performed during the 'active stage'. It seems likely that immunosuppressive drugs would only be effective during this stage.
3. The process responsible for remodelling the endplates continues into the 'inactive' and 'burned out' stages. There is some evidence that ACTH or corticosteroids may promote this repair if the nerve terminals are not too seriously atrophic. Steroids
have no direct effect on the safety factor of neuromuscular transmission and must not be used as alternatives to anticholinesterases.

4. Thyroid and other associated disorders may require treatment, but this does not affect the management of myasthenia gravis per se.

5. The genetic factor is not dominant and may be ignored in management. The hypothalamic factor is, however, very important. The myasthenic patient should be protected from stress and the most important type is psychological stress. This is a theme which is largely ignored in our symposium. I do not have time to develop it here, but close this contribution by stressing that psychological management is a major factor in treatment.

REFERENCES

Blackwells, Oxford.
Absence of cellular hypersensitivity to muscle and thymic antigens in myasthenia gravis

WILHELMINA M. H. BEHAN, P. O. BEHAN, AND J. A. SIMPSON

From the University Departments of Pathology and Neurology, University of Glasgow

SYNOPSIS Humoral antibodies to skeletal muscle and its components and to thymus have been demonstrated in the sera of patients with myasthenia gravis. A role for cellular hypersensitivity to similar antigens in the pathogenesis of the disease has been suggested by some reports of the presence of cellular immunity. A detailed immunological study using muscle and thymic antigens, including those prepared from the patients' own tissues, failed to confirm these findings. It is suggested that previous reports of cellular hypersensitivity represent the demonstration of an epiphenomenon.

There is good evidence that immunological mechanisms are involved in the pathogenesis of myasthenia gravis (Simpson, 1960, 1975). The strongest argument is the fact that the thymus gland is histologically abnormal in more than 80% of cases; in about 70% medullary germinal centres are found in about 10% a thymoma is present (Castleman and Norris, 1949). The proportion of patients in whom myasthenia is associated with a thymoma has been reported as from 10-30% of cases but the incidence increases with age and the overall percentage is therefore approximately 10% (Castleman, 1955; Simpson, 1958). Medullary germinal centres similar to those found in myasthenia are also seen in other disorders presumed to have an auto-hypersensitivity basis—for example, thyrotoxicosis and Addison's disease (Sloan, 1943), thyroiditis (Gunn et al., 1964), and systemic lupus erythematosus (Goldstein and Mackay, 1967). The centres have also, however, been seen in young normal subjects dying suddenly (Middleton, 1967) or undergoing cardiac surgery for congenital anomalies (Vetters and Barclay, 1973) and therefore their importance seems debatable (Vetters and Simpson, 1974).

There is a definite clinical association between myasthenia gravis and other presumed autoimmune disorders—for example, rheumatoid arthritis (Simpson, 1960), systemic lupus erythematosus (Wolf and Barrows, 1966), Sjögren's disease (Brown et al., 1968), pernicious anaemia (Simpson, 1960, 1964; Bletcher and Williams, 1967), pemphigus (Beutner et al., 1968; Vetters et al., 1973), autoimmune haemolytic anaemia (Cohen and Waxman, 1967; Halperrin et al., 1966), Hashimoto's thyroiditis (Simpson, 1964), and thyrotoxicosis and hypothyroidism (Osserman et al., 1967; Simpson, 1968). Serological abnormalities including rheumatoid factor, antinuclear antibodies, thyroid and haemolytic auto-antibodies have been frequently demonstrated in myasthenia (van der Geld et al., 1963; Adner et al., 1964; Simpson, 1964). Abnormalities in IgA metabolism have also been found in these patients (Behan et al., in preparation). Histologically, skeletal muscles from myasthenic patients show round cell infiltrates (lymphorrhages), non-specific findings which may suggest an immune response.

Humoral antibodies to skeletal muscle or its components have been demonstrated in the serum of myasthenic patients by a variety of techniques, including immunofluorescence (Strauss et al., 1965; Nastuk et al., 1966; Vetters, 1967), tanned red cell agglutination (Djianian et al., 1964), and precipitation in gels (Shulman et al., 1966). Strauss et al. (1965) originally demonstrated that the serum of 30% of myasthenics, and of almost all patients with myas-
Myasthenia gravis and a thymoma, contained antibody to muscle demonstrable on immunofluorescent staining. They used 900 control cases consisting of normal subjects and patients with a great variety of neurological disorders and showed that all but one were negative. Other workers have reported positive immunofluorescence in normal undiluted serum but Strauss used serum at a dilution of 1 in 60 and considered the reaction that he found was specific and characteristic of the disease.

The role of these antibodies in disease pathogenesis, however, is doubtful since antibodies are not present in the majority of patients with myasthenia and, even when present, there tends to be no correlation between the antibody titre and the course and severity of the disease (Oosterhuis et al., 1967). Mothers with myasthenia have been found to show high antibody titres with no symptomatology in the neonate and the reverse has been described (Oosterhuis et al., 1966). Again, patients with a thymoma often have high titres of anti-muscle antibody and yet show no evidence of myasthenia even on detailed electrophysiological testing (McFarlin et al., 1966). Theoretically, it is also difficult to account for a transmission defect produced by anti-muscle antibodies involving myofibrils rather than by a process which affects the motor end-plates.

The original suggestion of Simpson (1960) of a cell-mediated immunity in myasthenia gravis has, therefore, been reconsidered. Several reports have suggested that such mechanisms are involved but the results have been conflicting. It was decided to investigate cellular hypersensitivity to a variety of muscle and thymic antigens in myasthenia using a standardized and reproducible in vitro technique (Soborg and Bendixen, 1967; Rosenberg and David, 1970). These antigens were prepared similarly (1) to those used in other reports which had claimed positive cell-mediated immune responses (Alpert et al., 1972; Armstrong et al., 1973; Kott et al., 1973; Goust et al., 1974), or which had been claimed to induce an experimental thymitis and a partial neuromuscular block in guinea-pigs (Kalden et al., 1973), and (2) to those antigens which react specifically with the sera of patients with myasthenia (Aarli, 1972). In addition, control non-muscle antigens were used to which the patients demonstrated delayed hypersensitivity by skin testing. Finally, a group of patients were tested immediately before and within a week after thymectomy with antigens prepared from their own thymus and skeletal muscle so as to obviate the possible effect of histocompatibility antigens.

**METHODS**

**SUBJECTS**

**Patients A** Fifteen patients with myasthenia gravis of varying stages of severity, none of whom had undergone thymectomy.

**Patients B** Nine patients with myasthenia gravis who were tested before and after thymectomy.

** Patients C** Thirty control subjects consisting of 10 normal subjects and 20 patients with a variety of neurological disorders affecting the central and peripheral nervous system: sciatica (five), peripheral neuropathy (two), meningioma (two), glioma (three), cerebrovascular haemorrhage (two), cerebrovascular thrombosis (four), subarachnoid haemorrhage (two).

**ANTIGENS**

1. **Streptokinase/streptodornase** (Vari-dase, Lederle Laboratories) (SKSD) This was used at a concentration of 300 units/ml tissue culture fluid.

2. **Muscle antigens** Three categories of muscle antigens were prepared from skeletal muscle obtained at thymectomy, other operations, or necropsy and dissected as free as possible of connective tissue, fat, and blood. One of the five antigens listed below was then prepared.

**a. Preparation of muscle homogenate** Fresh muscle was placed in a sterile dish, diced with a scalpel blade, and then homogenized in phosphate buffered saline pH 7.2, as a 20% w/v homogenate, in a Sorval Omnimix Blender. It was then centrifuged for 15 minutes at maximum speed and aliquots of the supernatant were stored at −20°C and used within three weeks of preparation. Before use, the antigens were frozen and thawed once, then diluted with RPMI 1640 tissue culture fluid to obtain the desired dilution. Dose response curves were used initially to determine cytotoxicity of the antigen. The concentrations finally used were 1/100 and 1/50 dilutions of the original 20% w/v homogenate.

**b. Preparation of soluble and microsomal fractions of muscle** The method of Kalden et al. (1973) was followed. Fresh skeletal muscle tissue was homogenized (20% w/v) in phosphate buffered saline...
The homogenate was centrifuged for 10 minutes at 2,500 g and the supernatant then used to prepare the fractions needed. First the supernatant was centrifuged for 30 minutes at 10,000 g at 4°C. The sediment was discarded and the supernatant further centrifuged in a Beckman vacuum ultracentrifuge at 105,000 g for one hour. The final sediment obtained (so-called microsomal fraction) and the supernatant (so-called soluble fraction) were lyophilized. The lyophilized fractions were prepared for use by dissolving in RPMI 1640 tissue culture fluid and making up to concentrations of 500 µg/ml (microsomal fraction) and 500 µg/ml and 250 µg/ml (soluble fraction). These concentrations were determined to be non-cytotoxic by previous dose-response measurements.

c. Preparation of citric acid extract of muscle Skeletal muscle was thawed, minced, and washed in cold phosphate buffered saline repeatedly until the supernatant was free of all discoloration. A citric acid extract was then prepared as described by Espinosa and Kaplan (1968) and used by Aarli (1972). The procedure included repeated extractions with 0.85% NaCl before treatment with 0.05 M citric acid. The final extract obtained was lyophilized. It was easily soluble in RPMI 1640 and concentration response curves were used in order to obtain a concentration of the extract which did not produce non-specific inhibition in the controls. In most cases the concentration used was 50 µg/ml.

3. Thymus antigens Human thymus obtained at operation for thymectomy was dissected free of connective tissue, fat, and blood vessels. It was washed repeatedly in cold phosphate buffered saline and then frozen to −20°C until use. It was used at once to prepare the antigens.

a. Preparation of thymus homogenate Fresh thymus tissue was minced, then homogenized in 20% w/v in RPMI 1640 using a Sorval Omnimix blender. It was filtered once through cotton gauze and then frozen and thawed once before being stored at −20°C until use. It was used at a final concentration of 1/20 of the original 20% w/v homogenate.

b. Preparation of thymus soluble antigen The method of Goust et al. (1974) was used. Fresh thymus was minced and suspended in 10% w/v in 0.5 M saline and then homogenized in a Sorval Omni-Mix blender at maximum speed in bursts of 20 seconds. The homogenate was then spun at 1,000 g for 15 minutes and the lipid-free supernatant lyophilized. The above operations were all carried out at 4°C. Finally, protein concentration was adjusted to give a final concentration of 100 µg/ml.

SKIN TESTING Patients and control subjects were skin tested by an intradermal injection into the volar surface of the arm of 0.1 ml of a normal saline solution containing 5 units and, if unreactive to this, a saline solution containing 10 units of streptokinase/streptodornase. The results were examined at 24 and 48 hours and were considered to be positive when the raised indurated area was greater than 1.0 cm in diameter.

In vitro technique of macrophage inhibition The leucocyte migration test was performed according to the technique of Soborg and Bendixen (1967) with modifications as indicated. Essentially 50 ml venous blood was taken under sterile conditions into a syringe containing 1,000 units of preservative-free heparin and 10 ml dextran. The blood was then allowed to sediment at 37°C for 20 minutes. All but the bottom 0.5 cm of leucocyte-rich plasma was removed and the suspension then centrifuged at 250 g for 10 minutes. Nine volumes of 0.83% ammonium chloride were added and the cells resuspended and left in this solution for four minutes exactly. (The ammonium chloride lysed the red cells present.) The cells were then centrifuged at 200 g and washed twice in RPMI 1640 tissue culture fluid and then once in RPMI 1640 to which 10% fetal calf serum had been added and 1.0% penicillin and streptomycin. After the final wash the cell pellet was suspended in the residual fluid and a trial capillary filled and sealed with Critoseal. This capillary was centrifuged at 200 g for four minutes and the concentration of the cell suspension remaining was adjusted so that after dilution with antigen (1/5 dilution) a length of 2–3 mm of the capillary would be filled with packed cells. Usually between 2.2 and 2.8 ml of cell suspension of the required concentration was obtained, meaning that a total of between 22 and 28 chambers (each containing two capillaries) could be set up. The cell suspension was then divided into at least nine aliquots of 0.2 ml each aliquot was incubated with 0.05 ml of one of the various antigens (and one aliquot had 0.05 ml of RPMI 1640 alone added) for two hours at 37°C. Thus a minimum of two chambers was set up for each antigen and the controls. If the number of cells permitted, the aliquots of each cell suspension and the antigen were increased accordingly. At the end of the two hour incubation period haemocytometer tubes were filled with the cell suspensions sealed at one end with Critoseal and centrifuged at 200 g for four minutes. The tubes were cut at the cell-fluid interphase and the cell bearing ends of two capillaries placed in each small culture chamber.
Wilhelmina M. H. Behan, P. O. Behan, and J. A. Simpson

(planchette: Univers, Meckaniska, Sweden) secured at one end with silicone grease (Dow Corning, vacuum silicone grease). The chambers were filled with tissue culture medium RPMI 1640, sealed with a greased cover slip, and incubated for 18 hours at 37°C. The migration surfaces were then examined under a Leitz Diavert inverted microscope with drawing attachment and the area of migration drawn on white paper. This area was measured with a planimeter. The migration index was then calculated thus:

\[
\text{migration index} = \frac{\text{surface area in presence of antigen}}{\text{surface area in absence of antigen}} \times 100
\]

Each result is the average of at least four capillary measurements: unless the measurements agreed within 15% the result was discarded. An inhibition of migration of more than 30% was considered to be a significant result.

**AUTOLOGOUS ANTIGENS** Group B patients were tested for possible cell-mediated immunity to their own muscle and thymus. Their peripheral blood was withdrawn before thymectomy and set up as described in the presence of crude homogenates of muscle and thymus obtained at operation. The experiment was repeated one week later using the same antigens.

**RESULTS**

Delayed hypersensitivity, as demonstrated by inhibition of macrophage migration greater than 30%, was observed in all patients tested with the SKSD antigen. Excellent correlation was observed between this finding in vitro and the demonstration of cutaneous reactivity to SKSD with thickened, indurated lesions greater than 1.0 cm in vitro.

No inhibition of any significant degree (greater than 30%) could be found in normal subjects or in the three groups of patients, to any of the antigens prepared from thymus and muscle (Figs 1, 2, and 3).

It can be seen that thymectomy had no influence on the results of testing with SKSD antigens, as measured in vitro. Similarly, thymectomy did not influence the negative results to muscle and thymic antigens in the patients tested with autologous antigens before and after operation.

**DISCUSSION**

We were able to demonstrate that the in vitro technique of macrophage migration inhibition

---

**FIG. 1** Migration Index of peripheral blood leucocytes on contact with muscle and thymic antigens, in 10 normal controls and 20 patients with neurological disorders other than myasthenia gravis. △ = normal controls. ● = patients with neurological disorders.
Absence of cellular hypersensitivity to muscle and thymic antigens in myasthenia gravis

**FIG. 2** Migration Index of peripheral blood leucocytes on contact with muscle and thymic antigens, in 15 non-thymectomized patients with myasthenia gravis.

**FIG. 3** Migration Index of peripheral blood leucocytes on contact with muscle and thymic antigens, in nine patients with myasthenia gravis, before and after thymectomy. ◆ = before thymectomy. △ = after thymectomy.
detected cell-mediated immunity to streptokinase/streptodornase (SKSD) but we were unable to show similar sensitivity to any of the skeletal muscle or thymic antigens employed in our experimental subjects. These results, therefore, conflict with those of other workers who, using the same technique, claim to have found hypersensitivity to muscle and thymic antigens in patients with myasthenia.

Alpert et al. (1972) obtained positive inhibitory responses to crude skeletal muscle and myosin-containing fractions in 14 of 21 myasthenics. They considered inhibition of migration of more than 30% to be significant: 12 of their patients showed this degree of inhibition but inhibition to the same antigens of up to 10% was also present in their control subjects. No positive control antigens were used in the system—for example, PPD, candida, or SKSD but with non-specific proteins, such as meconium extract, they obtained greater than 20% migration inhibition in some patients. A strong positive correlation was noted between the degree of cell-mediated immunity and the titre of humoral muscle antibodies.

Armstrong et al. (1973), using thymic lymphocytes, found cellular hypersensitivity to crude muscle and crude thymus antigens in myasthenic patients but also reported similar sensitivity to muscle in nearly 50% of their control subjects. In addition, they obtained a positive response in a patient with a thymoma who did not have myasthenia gravis. Kott et al. (1973) showed positive in vitro responses to crude muscle and myosin-containing fractions as antigen in 63% of their patients with myasthenia gravis. They noted, however, poor correlation between cell-mediated immunity and the presence of humoral antibodies but they were able to demonstrate a correlation between the stage of the disease and cell-mediated or humoral immunity. The authors stated that tuberculin was used as a positive control antigen but these particular results were not presented.

Gouste et al. (1974) found cell-mediated immunity to muscle and thymic antigens in 45 of their 46 patients. They used monkey muscle and thymus and human muscle as antigens. They demonstrated that some of their patients responded only to human muscle and others only to monkey muscle and one to monkey thymus only. They noted that 'no correlation appears to exist between the clinical and pathological data' and presence of a thymoma, thymectomy, or a changing clinical state did not influence the results of their test. No control antigens of any sort were used and some of their control subjects had inhibition of 20–35% to the muscle and thymus used as antigen. Positive responses to the muscle extracts were also found in 12 of 14 patients with polymyositis but without myasthenia gravis.

A criticism that can be made of all these reports is that positive control antigens were not used. In addition, dose response curves were not employed to measure cellular hypersensitivity when present. In our experiments, increasing the dose of antigen had no effect, whereas if sensitivity were present one would expect some increase in inhibition with an increase in antigen concentration. Furthermore, Goust et al. (1974) noted that enhancement of macrophage migration was present in 10 cases at one or both of the antigen concentrations used and they stated that 'in myasthenia gravis, with this technique, an augmentation of the migration index seems as significant as a depression'. Augmentation is found in the macrophage migration inhibition test when no sensitivity exists, so that this statement is open to criticism.

A non-specific inhibition of macrophage migration may occur due to differing histocompatibility antigens. Falk et al. (1970) showed that non-sensitized lymphocytes were stimulated to produce macrophage inhibiting factor on contact with different histocompatibility antigens. We used autologous antigens in some of our experiments in order to eliminate this effect but the phenomenon may help to explain, in part, the inhibition noted by other workers.

Our findings do agree with those of experimenters who used different in vitro methods to try to detect cell-mediated immunity to muscle and thymic antigens in patients with myasthenia gravis. Housley and Oppenheim (1967) and Lisak and Zweim (1975), employing the technique of measuring lymphocyte proliferative responses, were unable to show any sensitivity in patients with myasthenia. Similarly, Abramsky et al. (1975), using a lymphocyte transformation method, found no response to crude muscle antigen in the myasthenic patients they investigated.
The inhibition which has been observed is most likely an epiphenomenon. Indeed, Alpert et al. (1972) point out in the interpretation of their results that the findings need not reflect a primary immunological effect but rather 'may simply reflect a cellular response to tissue alteration resulting from another cause'. This was also the opinion of Lisak and Zweiman (1975) who stated 'it would be somewhat surprising if such reactivity (cell-mediated immunity) was pathogenic in the face of the disparate clinical features of the two disorders' (the other condition they referred to was polymyositis in which cell-mediated immunity has been shown by Currie et al. 1971).

The specificity and significance of cell-mediated immune responses to muscle in myasthenia gravis is questioned because of the demonstration of similar sensitivity in patients with muscular dystrophy (Caspari et al., 1971), polymyosalgia rheumatica (Esiri et al., 1973), and Guillain-Barré syndrome (Caspari et al., 1971). Furthermore, Kott et al. (1971) have reported sensitivity to central nervous system antigens in patients with myasthenia gravis and Caspari et al. (1971) have found similar reactivity to these same antigens in patients with muscular dystrophy.

The possibility that muscle and thymic antigens might be involved in the pathogenesis of myasthenia gravis, however, is also suggested by the work of Goldstein and Whittingham (1966) and Kalden and Irvine (1969) who claim that myasthenia gravis can be produced in guinea-pigs by immunizing them with these antigens. Several other investigators, however, have been unable to confirm their findings (Kaufmann et al., 1969; Namba and Grob, 1969; Vetters et al., 1969; Behan, 1974). None of the animals immunized by the former workers developed clinical disease and subtle electrophysiological changes were used as the criteria for diagnosis of myasthenia. These changes are non-specific and open to differing interpretations (Vetters et al., 1969; Brooks, 1971). The antigens used by Kalden et al. (1973) failed to elicit any positive responses in our system.

The occurrence of myasthenia gravis in association with diseases in which there is anergy—for example, chronic lymphatic leukaemia (Cohen and Waxman, 1967), lymphosarcoma (Simpson, 1960), Hodgkin's disease, systemic lupus erythematosus (Wolf and Barrows, 1966), and sarcoidosis (Simpson, 1960; 1964)—militates against cell-mediated hypersensitivity being involved in the pathogenesis of the disease. Fudenberg (1971) has postulated that there is a depression of T-cell function rather than a hyperallergic state in the autoimmune disorders. In fact, myasthenic patients show impaired immune responses to dinitrochlorobenzene (DNCB) sensitization (Adner et al., 1964).

An association between certain histocompatibility antigens and myasthenia has also been demonstrated recently (Pirskanen et al., 1972; Behan et al., 1973). HLA-8 has been found to occur with a very high frequency in myasthenia gravis, gluten enteropathy, dermatitis herpetiformis, and autoimmune thyroiditis (Daoust et al., 1974). It is claimed that HLA-8 is associated with disorders of impaired immunity (Da Costa et al., 1974).

Our work, therefore, provides no evidence to support the theory that cell-mediated immunity to muscle or thymic antigens is involved in the pathogenesis of myasthenia gravis. The most likely explanation for the previous reports of such cellular hypersensitivity is that these findings represent an epiphenomenon and not true cell-mediated immunity.

The encouragement and advice of Professor J. R. Anderson are gratefully acknowledged.

REFERENCES


Absence of cellular hypersensitivity to muscle and thymic antigens in myasthenia gravis


ROSETTE TESTS FOLLOWING THYMECTOMY

Sir,—Dr Charriere and Dr Bach (Aug. 3, p. 259) observe that approximately 1% of normal mouse spleen cells form rosettes with autologous thymocytes (auto-E) and that the percentage of autologous-forming cells (A.R.F.C.) is increased in thymic-deficient (thymectomised, Nude, NZB, and old CBA) mice. They report also autorosette formation by 0.12% of human peripheral blood lymphocytes (P.B.L.) from young adults and by 0.45% of P.B.L. from older people. They suggest that autorosette formation is related to the thymus and that T lymphocytes may play a suppressor role in autoimmune responses: this has also been postulated by Allison et al.1

We reported a autorosette formation by 0.5-10% of human P.B.L. from normal individuals, and an increased percentage of A.R.F.C. in some patients with cancer. This might seem to support Dr Charriere and Dr Bach's suggestion, for there may be suppression of T-cell function in cancer patients.4

To obtain more direct information on the role of the thymus in rosette formation, we have performed rosette tests with auto-E, sheep E, and with chicken erythrocytes (chicken EA) on the P.B.L. from 14 patients with myasthenia gravis, 8 of whom had undergone thymectomy 1-8 years ago. The techniques have been described.6,7 The results, together with tests on P.B.L. of 23 normal subjects of similar age and sex distribution to the myasthenic patients, are shown in table 1.

Although a minority of tests were performed with 0.5% suspensions of auto-E, which we have since shown to be below the optimum concentration, the percentages of A.R.F.C. in all three groups are many times greater than those reported in man by Dr Charriere and Dr Bach, almost certainly because of differences in technique. We have regarded as A.R.F.C. those lymphocytes binding 3 or more auto-E; if lymphocytes binding 1 or 2 auto-E are included, the total approximates to 10% of the P.B.L. The mean percentages of A.R.F.C. were not significantly different for the three groups, nor does consideration of the mean lymphocyte counts (not shown) suggest a significant rise in the absolute numbers of A.R.F.C. following thymectomy.

We have also compared the percentages of A.R.F.C. in the P.B.L. of normal subjects of different age-groups, using optimum (1.5%) suspensions of auto-E, and have observed no significant differences between the ages of 18 and 55 years (table II).

These results provide no evidence that thymectomy or ageing are associated with an increase of A.R.F.C. in the P.B.L. We suspect that autorosette formation by P.B.L. may be of little immunological significance, partly because we have observed rosette formation by small percentages of human P.B.L., using erythrocytes of various species, (guinea-pig, mouse, rat, and chicken EA), and partly because human P.B.L. in culture are apparently not stimulated to transformation by human E.8

Lastly, we are unaware of reports on the proportions of T and B lymphocytes following thymectomy. Accepting the sheep-E and chicken-EA rosette tests as indications of T and B cells, respectively, our results suggest that there has been no significant fall in the T-cell population up to 8 years after thymectomy (table I). Case was taken, at operation, to remove the anterior mediastinal fat tissue, and it seems likely that thymectomy was complete or nearly so, in which case maintenance of the T-cell population in these patients appears to be largely independent of the thymus. It is not known, however, whether they are capable of normal immune responses to previously unencountered antigens.

We thank Mr K. Fitter, who performed the thymectomies, for permission to report these findings.

G. P. SANDILANDS
KATHLEEN GRAY
ANNE COONEY
J. R. ANDERSON.


TABLE I—ROSETTE TESTS IN MYASTHENIA GRAVIS AND NORMAL SUBJECTS

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Mean age (yr.)</th>
<th>Percentage of lymphocytes forming rosettes with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sheep E</td>
</tr>
<tr>
<td>Myasthenia gravis,</td>
<td>8</td>
<td>37</td>
<td>66</td>
</tr>
<tr>
<td>Thymectomised...</td>
<td></td>
<td>(20-50)</td>
<td>(60-72)</td>
</tr>
<tr>
<td>Non-thymectomised...</td>
<td>6</td>
<td>19</td>
<td>69</td>
</tr>
<tr>
<td>Normal individuals...</td>
<td>25</td>
<td>25</td>
<td>69</td>
</tr>
</tbody>
</table>

TABLE II—PERCENTAGES OF AUTOROSETTE FORMING Lymphocytes AT DIFFERENT AGES

<table>
<thead>
<tr>
<th>Age group (yr.)</th>
<th>16-29</th>
<th>30-39</th>
<th>40-55</th>
<th>60-72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoreactive-forming cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>39</td>
<td>28</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Range</td>
<td>0-5-9</td>
<td>0-5-9</td>
<td>0-5-6</td>
<td></td>
</tr>
<tr>
<td>No. of individuals</td>
<td>34</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

with IgG heteroantibody (chicken EA) on the P.B.L. from 14 patients with myasthenia gravis, 8 of whom had undergone thymectomy 1-8 years ago. The techniques have been described.6,7 The results, together with tests on P.B.L. of 23 normal subjects of similar age and sex distribution to the myasthenic patients, are shown in table 1.

Although a minority of tests were performed with 0.5% suspensions of auto-E, which we have since shown to be below the optimum concentration, the percentages of A.R.F.C. in all three groups are many times greater than those reported in man by Dr Charriere and Dr Bach, almost certainly because of differences in technique. We have regarded as A.R.F.C. those lymphocytes binding 3 or more auto-E; if lymphocytes binding 1 or 2 auto-E are included, the total approximates to 10% of the P.B.L. The mean percentages of A.R.F.C. were not significantly different for the three groups, nor does consideration of the mean lymphocyte counts (not shown) suggest a significant rise in the absolute numbers of A.R.F.C. following thymectomy.

We have also compared the percentages of A.R.F.C. in the P.B.L. of normal subjects of different age-groups, using optimum (1.5%) suspensions of auto-E, and have observed no significant differences between the ages of 18 and 55 years (table II).

These results provide no evidence that thymectomy or ageing are associated with an increase of A.R.F.C. in the P.B.L. We suspect that autorosette formation by P.B.L. may be of little immunological significance, partly because we have observed rosette formation by small percentages of human P.B.L., using erythrocytes of various species, (guinea-pig, mouse, rat, and chicken EA), and partly because human P.B.L. in culture are apparently not stimulated to transformation by human E.8

Lastly, we are unaware of reports on the proportions of T and B lymphocytes following thymectomy. Accepting the sheep-E and chicken-EA rosette tests as indications of T and B cells, respectively, our results suggest that there has been no significant fall in the T-cell population up to 8 years after thymectomy (table I). Case was taken, at operation, to remove the anterior mediastinal fat tissue, and it seems likely that thymectomy was complete or nearly so, in which case maintenance of the T-cell population in these patients appears to be largely independent of the thymus. It is not known, however, whether they are capable of normal immune responses to previously unencountered antigens.

We thank Mr K. Fitter, who performed the thymectomies, for permission to report these findings.

G. P. SANDILANDS
KATHLEEN GRAY
ANNE COONEY
J. R. ANDERSON.

DECREASED SERUM-IgA IN MYASTHENIA GRAVIS

Sir,—Immunoglobulin A (IgA) is the major immunoglobulin found in external secretions after the first three years of life, and its function appears to be to protect the external microbial surfaces against infections.1 Another suggested function is exclusion of antigens, and IgA deficiency has therefore been said to result in atopic illnesses.3 Relative IgA deficiency is the commonest isolated immune deficiency, occurring in 1/500 normal individuals and in association with a miscellany of disorders. In apparently normal individuals with IgA deficiency, however, there is a conspicuous predisposition to develop respiratory infections, allergies, intestinal malabsorption, autoimmune disorders,1 and malignancy.3

The most frequent association of decreased serum-IgA and disease is seen in patients with autoimmune disorders—chronic arthritis, systemic lupus erythematosus,1 rheumatic fever,1 chronic active hepatitis,2 Addison's disease,3 Sjögren's syndrome,4 and dermatomyositis.5 Other disorders in which decreased concentrations of IgA have been found include chromosomal abnormalities,6 pulmonary hemosiderosis,7 and persistent viral and fungal infections.8 9

In a detailed immunological investigation of a large group of patients with myasthenia gravis, we found that some patients had decreased serum-concentrations of IgA. A study was carried out using the radial immunodiffusion technique with monospecific antiserum to IgA (Hyland Laboratories) in 51 patients, with appropriate age and sex matched controls.

13 of the 51 patients had decreased concentrations of IgA, with a very low concentration in 1 serum and no IgA detectable in 2 other sera (see figure). Of the 2 sera with no IgA, 1 was from a patient with congenital myasthenia gravis and the other from a patient with myasthenia gravis and systemic lupus erythematosus. The latter had a mother having celiac disease and no detectable serum-IgA. The congenital-myasthenic patient had been found (6 years previously, before thymectomy) to have a very low concentration of serum-IgA. Serum samples before and after thymectomy were available in 12 patients; the immunoglobulin levels were not affected by this procedure. Decreased concentrations of IgA were found in 6 patients, 2 having very high levels. The significance of these findings is unknown.

There is evidence that IgA production is related to intestinal function.10 Neonatal thymectomy in the mouse and rat interferes with production of IgA.11-12 resulting in subnormal concentrations of IgA but not complete absence. Congenitally athymic mice have IgA deficiency, but many IgA-bearing B lymphocytes12 are present. Similarly, IgA deficiency has been found in ataxia telangiectasia,6 but there is again evidence that IgA-bearing B lymphocytes are present in this condition.6 These lymphocytes will release IgA on antigenic stimulation in vitro.13

Increased IgA is usually seen in association with inflammatory disorders, but paradoxically, very high levels are often found in patients with immunodeficiency diseases. These increased concentrations have been recorded in both ataxia telangiectasia and the Wiskott-Aldrich syndrome.

IgA deficiency has been reported in association with congenital myasthenia gravis,14 and a low concentration of all immunoglobulin classes has been found in myasthenic patients with thymoma.15 There seems to be a definite association between deficiency of IgA and depressed cell-mediated immunity. It has been suggested that a pre-existing deficiency in the cellular immune (T-cell) system, associated with impaired expression of cell-mediated immunity, predisposes the patient to the development of autoimmune diseases. For instance, there is an increased incidence of autoimmune diseases in children with immunological deficiency, and neonatal thymectomy in animals predisposes to various autoimmune states.16 The presence of the thymic-dependent immunological deficiency could facilitate the entry to the host of an environmental agent, presumably a virus.

We have previously reported subtle impairment of thymus-derived lymphocyte function in patients with myasthenia gravis17 and suggested that the disease had an immunodeficience-like basis.18 Further work is clearly required to elucidate the role of IgA in this disease.

References

ciency basis. The present findings of abnormal immunoglobulin-A metabolism are further evidence to support this hypothesis.

This work was supported by the Muscular Dystrophy Society of Great Britain.

University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow, and University Department of Pathology, Western Infirmary, Glasgow

PETER O. BEHAN
JOHN A. SIMPSON
WILHELMINA M. H. BEHAN
STUDIES ON THE NATURE OF AUTOIMMUNITY IN MYASTHENIA GRAVIS
EVIDENCE FOR AN IMMUNODEFICIENCY TYPE

J. A. Simpson, P. O. Behan, and Heather M. Dick
STUDIES ON THE NATURE OF AUTOIMMUNITY IN MYASTHENIA GRAVIS.
EVIDENCE FOR AN IMMUNODEFICIENCY TYPE *

J. A. Simpson, P. O. Behan, and Heather M. Dick

Department of Neurology
University of Glasgow
Glasgow, Scotland

Institute of Neurological Sciences
Southern General Hospital
Glasgow, Scotland

Department of Bacteriology
Royal Infirmary
Glasgow, Scotland

In 1955-60, one of us proposed a hypothesis for myasthenia gravis based on clinical data indicating a multisystem disease with resemblances to systemic lupus erythematosus, transmissibility to the newborn, response to corticosteroids, and pathological changes in muscle and thymus suggestive of immunological disease. The hypothesis was that a genetically determined breakdown of immunological tolerance associated with abnormal thymic function permitted development of antibody against end-plate receptors of skeletal muscle, the antibody possibly being carried by lymphocytes, as again suggested by Alpert et al. Smithers considered that the myasthenic thymus resembled the thyroid of Hashimoto's disease, suggesting autoimmune damage of the thymus, but did not attempt to explain the neuromuscular disease. Nastuk et al. noted that myasthenic sera lysed frog skeletal muscle. The reaction was complement-fixing and was shown by Strauss et al. to be associated with binding of a serum globulin to A bands of muscle fibers. Later work showed that this globulin, acting as antibody, was at high titer in patients with thymic tumors but was not closely related to myasthenia. Myasthenic patients also have more serum antibodies against thyroid, gastric, and other tissues than a control population.

Later developments of the Smithers and Strauss theories lead to an "instructive" theory of autoimmunity with production of thymitis. Goldstein and collaborators claim to have produced thymitis experimentally by injection in a range of laboratory animals with skeletal muscle or thymic tissue, and these workers account for the neuromuscular disease as the release of a blocking substance from the thymus. Their experimental animals do not show clinical evidence of myasthenia and do not produce autoantibodies other than those to muscle or thymus. Other workers dispute the interpretation of their electrophysiological findings. The Goldstein hypothesis cannot account for persistence or first appearance of myasthenia after thymectomy, though these facts are well

* We are grateful to the Muscular Dystrophy Group of Great Britain for supporting this research.
Simpson et al.: Evidence for an Immunodeficiency Type

documented. On the contrary, autoimmune disease may flare up soon after thymectomy. This has been reported for systemic lupus erythematosus and chronic ulcerative colitis,6,11 Hashimoto's disease8 and pemphigus vulgaris.14,10 It would also be predicted from the Goldstein hypothesis that the response of myasthenia gravis to thymectomy should be, in some measure, proportional to the histological reactivity of the thymus. Opinions on this point are conflicting. Vettes and Simpson16 review previous reports and in our material find better response from removal of relatively unreactive glands, but the tendency was not statistically significant.

The explanation for immunological tolerance to "self" as advanced independently by Weigle13 and Allison18 is that tolerance is dependent on healthy thymus-dependent lymphocytes. In their theory, the formation of autoantibodies against self-antigens, such as thyroglobulin and muscle, requires cooperation of T and B lymphocytes. When self-antigens are present in the blood in small amounts, T cells fail to react although sensitized B cells are present in normal numbers. Bypassing the T lymphocytes can occur in several ways, particularly in genetically predisposed individuals with specific but subtle T-cell defects and who are exposed to certain viruses. The confirmed findings of Abdou and collaborators25 of increased B cells in the thymus, and the demonstration of new antigenic determinants on the surface of thymocytes strongly suggest the presence of an occult virus.

The Simpson hypothesis is a "breakdown of tolerance" type of theory. It is the only one that accounts for the clinical and serological overlap and that postulates a genetic factor. Persistence or relapse of myasthenia gravis after thymectomy is not surprising as we have shown apparent persistence of T cells up to eight years after thymectomy.20 The heuristic values of the three major hypotheses were reviewed by Simpson21 and the concept of genetically determined defective immunological tolerance was advanced. After 15 years of research all over the world, it is only recently that evidence of antiaacetylcholine substance has been published.25 Recent attention has turned from humoral to cell-borne immunological mechanisms as suggested in the original hypothesis.1

Thymus-Dependent Cellular Function

Synthesis of new protein, as part of increased metabolic activity of resting thymus-derived lymphocytes, is produced by stimulation of these cells with phytohemagglutinin, which leads to blast transformation, DNA synthesis, and cell division. Measurement in vitro of increased protein synthesis when phytohemagglutinin (PHA) is applied to resting lymphocytes offers advantages over conventional methods that measure blast transformation, DNA synthesis or both, to determine lymphocyte function. We have applied this technique to study the peripheral blood lymphocytes of 37 myasthenia gravis patients and healthy age- and sex-matched control subjects. This method is more accurate and sensitive than measurement of PHA stimulation at three days.29 There is a subtle but highly significant impairment of lymphocytic function in patients with myasthenia gravis (Figure 1). This impairment is similar to that found in patients with malignant disease and patients in the early stages of Hodgkin's disease. Furthermore, this abnormal response is discernable in myasthenia gravis patients when skin tests with ubiquitous antigens such as streptokinase/streptodornase (SKSD), candida, and purified protein derivative (PPD) are strongly
positive and the PHA stimulation at three days, as measured by thymidine uptake, is normal.

The cause of this T lymphocyte abnormality is unknown and could be explained by defective PHA receptors or decreased receptor density on the lymphocytes, or by an abnormal serum factor. We have demonstrated that the population of T cells in the peripheral blood of myasthenic patients is normal and uninfluenced by thymectomy. The number of T cells in the peripheral blood, as measured by auto-E-rosettes was found to be normal eight years after thymectomy despite a good clinical response.

Studies on cell-mediated immunity (CMI) to muscle and thymic antigens in myasthenia gravis have been conflicting. Inhibition of macrophage migration by skeletal muscle fractions was reported by Alpert, Armstrong, Kott, and Goust and their collaborators, but these workers disagree in regard to correlation between the degree of cell-mediated immunity, the titer of humoral muscle antibodies, and the stage of the disease, and the experimental controls may be criticized. A nonspecific inhibition of macrophage migration may occur
because of differing histocompatibility antigens.\textsuperscript{28} Using similar techniques with muscle and thymic antigens but with more rigorous controls, including the use of antigens prepared from the patient's own muscle and thymus, we have found that there was no inhibition of macrophage migration of any significant degree (greater than 30\%) in normal subjects and in myasthenic patients either before or after thymectomy though delayed hypersensitivity was observed in all patients tested with a streptokinase/streptodornase antigen. The SKSD response was not influenced by thymectomy.\textsuperscript{57} Our findings agree with the lack of sensitivity of CMI shown in myasthenics by lymphocyte proliferative responses.\textsuperscript{29-31}

As cell-mediated immune responses to muscle in myasthenia gravis resemble those reported in other neuromuscular diseases\textsuperscript{32,33} they may, like the antibodies previously studied, be epiphenomena and markers of cellular damage, or be active only at the initiation of the disease or its relapses. Nonetheless, the clinical and immunopathological data still point strongly to a disorder of immunological tolerance in myasthenia gravis. A possible solution to the dilemma is suggested by the hypothesis of Fudenberg\textsuperscript{34} that autoimmune diseases are, in fact, manifestations of either generalized or selective immunological deficiencies that, in the genetically predisposed, permit microorganisms, normally handled readily by the immune system, to circulate freely and to attack tissues for which they have a tropism. He has suggested that antibodies, far from causing autoimmune disease, may be part of a normal protective mechanism designed to help the body eliminate dead or damaged tissue and may, in fact, help protect an organ from increased damage. Adner \textit{et al.}\textsuperscript{35} have previously shown that myasthenic patients show impaired responses to sensitization with dinitrochloro benzene. It may also be considered as mitigating against hypersensitivity in the pathogenesis of myasthenia that it occurs in association with diseases in which there is anergy, e.g., chronic lymphatic leukemia,\textsuperscript{36} lymphosarcoma,\textsuperscript{37} Fudenberg's disease, systemic lupus erythematosus,\textsuperscript{38} and sarcoidosis.\textsuperscript{39}

\textbf{Immune Response Genes in Myasthenia Gravis}

Simpson \textit{et al.}\textsuperscript{4,8,21} proposed that a breakdown of immunological tolerance, probably genetically determined, may be manifested either as myasthenia gravis or other autoimmune disorders, with occasional overlap, a view supported by Fudenberg.\textsuperscript{24} Genetic determination of defective tolerance is supported by an immunogenetic analysis of our patients. In preliminary reports\textsuperscript{39,40} we noted that histocompatibility antigens HL-A1 and HL-A8 occurred with disproportionate frequency in patients with myasthenia, and to a lesser degree in their families. Other preliminary reports\textsuperscript{40,41} showed similar findings but in both an increased frequency of HL-A8 was present only in female myasthenic patients. Feltkamp and coworkers\textsuperscript{42} showed a positive correlation between HL A8 and myasthenia gravis that was significantly higher in women than in men. In their experience the prevalence of HL-A8 was less and that of HL-A2 more in patients who had serum antibodies to skeletal muscle or a thymoma or both. A similar incidence of HL A8 antigen has been found in other autoimmune disorders and in malignancies (Hodgkin's disease) that share a hypoallergenic state. It may be, however, that specific immune response genes with close linkage to HL A antigens may be more important with respect to disease susceptibility.\textsuperscript{43}

It is probable that there is linkage disequilibrium between HL-A second
locus antigens and mixed lymphocyte culture (MLC) antigens. For example, the HL-A haplotype HL-A 1-8 is very frequently associated with one particular MLC antigen. Thus two unrelated individuals with these HL-A antigens may also have identical MLC antigens. In preliminary studies we have found that there was restricted MLC inheritance in the families of myasthenics. We have only looked at three such families to date and an alternative explanation may be a high frequency of homozygotes for MLC in myasthenic families.

**Immunoglobulins**

Most patients with myasthenia gravis show grossly normal plasma protein fractions, an increase in gamma globulin being associated with concomitant autoimmune disease. We have previously stressed the low incidence of rheumatoid factor in the "rheumatoid arthritis" of myasthenics. We have now demonstrated a selective decrease in IgA in 10 of 50 myasthenic patients, which was not influenced by thymectomy. Three of these patients who showed the lowest levels were all cases of congenital myasthenia who had undergone thymectomy. In addition, IgE levels were determined and these proved normal in all cases.

Selective IgA deficiency, found in 1 in 700 normal subjects, occurs more commonly in patients with autoimmune diseases and in immunodeficiency states, particularly those with defective CMI. (Hobbs prefers to use the term "aberrant immunity" in connection with IgA deficiency).

**Serum Complement**

A role for complement in the pathogenesis of myasthenia has been suggested by several workers. They measured serum complement levels serially in patients and stated that it was lower during active disease, rising to normal or supernormal levels in remissions. It was considered that an autohypersensitivity reaction during the exacerbation of the disease utilized complement. Plescia and colleagues found reduced levels of C2 and C4 and inhibitors to these components but were unable to state how these phenomena were related to myasthenia.

A detailed analysis of serum complement was carried out in 30 of our patients. This included measurement of C1q, C3, C4, C7, and C3 proactivator, and C3 conversion products with C3 activator levels, and the estimation of CH50 units. Anticomplementary assays were also performed. All were normal though we examined active phase and remittant cases. A possible explanation for the discrepancy of these findings may be that cases of myasthenia found to have low levels of complement had other autoimmune disorders such as SLE or rheumatoid arthritis in which complement metabolism is disordered.

**Summary**

Clinical and laboratory data continue to support the concept of a genetically determined breakdown of immunological tolerance in myasthenia gravis with immunological damage to the motor end plates. The demonstration of impaired
function of thymus-derived lymphocytes and of IgA deficiency correlate well with the clinical data in which there is an increased incidence of autoimmune diseases associated with anergy. Whilst the exact pathogenesis of myasthenia gravis is unknown, the available data support the concept of an immune deficiency disorder.

References

39. DICK, H. M., P. O. BEHAN, J. A. SIMPSON & W. F. DURWARD. 1974. The in-
Evidence for an Immunodeficiency Type


At the 1973 Barcelona Congress I ended a review by indicating some factors which should influence the treatment of myasthenia gravis at different stages. This paper provides an opportunity to elaborate the points indicated briefly (Simpson 1974).

A question frequently put to me is - "What is the best treatment for myasthenia gravis?". The question implies that:

1. There are several alternatives,
2. They can be put in order of rank.

This is an over simplification due to faulty analysis of the disease and of the role of drugs. In a review of "The Defect in Myasthenia Gravis" (Simpson, 1969) I have pointed out how misleading was the belief of the previous 50 years that all disease can be "explained" given an adequate knowledge of normal physiology or biochemistry. In this approach, each disease is seen to represent increased or decreased activity of a normal function. The "pathophysiological" approach has the inevitable result that unthinking clinicians began to take note of only those things which fit that conceptual framework. All thinking about myasthenia gravis was concentrated on neuromuscular transmission in 1934 when Mary Walker's demonstration of the dramatic relief afforded by physostigmine coincided with Dale and Feldberg's confirmation of the role of acetylcholine in neuromuscular transmission.

But there are different levels of "causation" in medicine, and explanations of disease processes may concentrate on different levels without necessarily being incompatible, and therapeutics aimed at one level is not necessarily better than treatment aimed
at a different level. To treat disease rationally it is therefore necessary to be clear what level of "causation" is being treated. As already pointed out (Simpson, 1969) a corollary of the different levels of "causation" is that an "explanation" at the most superficial level will be adequate only in so far as it takes account of the deeper levels. Thus five levels of "causation" of a disorder may be recognised.

(i) Increased or decreased function of an organ ("pathophysiology")
(ii) The disorder of function may be secondary to a deformity of structure ("pathoanatomical")
(iii) Intracellular disorder of function ("molecular biological")
(iv) Cellular or body fluid disorders may be secondary to disturbance of a fundamental biological process ("dyshomoeostatic")
(v) First cause of each of the above (e.g. genetic, infective, psychosomatic.)

In the review, I concentrated on the evidence that

(i) The clinical, electrophysiological and pharmacological evidence available in 1969 all indicated that the functional disorder in myasthenia gravis was due to a loss of the safety factor for transmission which is normally present at the mammalian neuromuscular junction. I will return to the various possibilities later.

(ii) The same data pointed strongly to the presence of a defect which is both pre-synaptic and post-synaptic. There was already histological and electron microscopic evidence for a morphological abnormality of both sides of the neuromuscular junction and also of the muscle fibres. At a meeting at
which I further elaborated this analysis (Simpson, 1971) the ultra-structure was more fully described by Engel and Santa (1971). A clear understanding of the abnormalities (more fully described by ) is necessary to understand treatment at the lowest level.

Concepts of the myasthenic defect based on analogies with the action of drugs on morphologically normal synapses must be wrong. They are essentially "models" built according to the ideas fashionable at the appropriate time. The conceptual model available to Oppenheim (1887) and Jolly (1895) was curare poisoning. Jolly (1895) suggested physostigmine as an antidote. Apparently it was used but abandoned as ineffective until reintroduced by Remen (1932) and Walker (1934). The synthetic analogue, neostigmine was used by Walker (1935) just after Dale and Feldberg (1934) had confirmed the role of acetylcholine in neuromuscular transmission. It was natural that the concept of a circulating myasthenic toxin like curare, or possibly inadequate production of acetylcholine at the motor nerve terminal, should gain wide acceptance. Later concepts based on the action of hemicholinium-3 and on ad hoc interpretation of the abnormally small miniature endplate potentials (the "small quantum" theory) were in turn 'models' based on the reaction to drugs of endplates of normal animals. Their authors were unaware of the fact that the geometry of the myasthenic endplate is abnormal, or they ignored that fact. At this date the morphological changes are fully accepted and as I have stressed (Simpson, 1960, 1969, 1971) these changes are sufficient to
account for the loss of safety factor and abnormal drug responses which characterise the myasthenic endplate. Any drug which raises the safety factor will be beneficial to the myasthenic but it can never cure him. This is symptomatic treatment only, and I will return to it later.

The second level of explanation for myasthenic transmission must be an account of the pathology which distorts the neuromuscular junctions. I have interpreted the morphological changes as indicative of an interaction between a process which destroys the motor terminals and a remodelling process. This is a dynamic interaction which could account for the natural history of the disease, with relapses and remissions during an 'active stage', followed by 'inactive' and 'burned-out' stages (Simpson, 1971b, 1974). Short term variability and the neonatal myasthenia occurring in some babies with myasthenic mothers are more likely to be due to a blood borne factor. In 1960 I suggested that this might well be an antibody against the receptor substance of the motor endplates, or a cellular reaction of immunological type (Simpson, 1960). After 15 years of failure to find definitive evidence for this concept it is now clear from experimental studies with antibody raised against homologous tissues, electroplaque organs of various fish, that a myasthenic syndrome can in fact be produced in this way in mammals, including primates ( ). Furthermore, new techniques for detecting acetylcholine labelled with isotopes have shown that serum from myasthenic patients contains globulin which can block endplate receptors in the manner postulated. In addition, we have shown subtle impairment of function of thymus-derived lymphocytes in myasthenic patients. It is not unreasonable to postulate that the
morphological abnormality of the neuromuscular junctions is also immunologically determined and that, as originally suggested by Simpson (1960) both abnormalities are due to a breakdown of immunological tolerance due to a disorder of function of the thymus gland during the 'active' stage of the disease. Whether the continuing disease of the 'inactive' stage is due to secondary changes elsewhere in the immune system is a question which remains to be solved.

In the original hypothesis it was tentatively suggested that the thymic abnormality may be genetically determined, possibly through a hypothalmo-pituitary mechanism. In common with others, we have reported evidence based on clinical (Simpson, 1960) and histocompatibility studies (Behan et al, 1973; Dick et al, 1974) to support a genetic predisposition to myasthenia gravis though this may not be an obligatory cause. It is, for instance, possible that genetic predisposition might affect the response to a virus or other infective agent. This is certainly not excluded. Obviously the question which is so often put, "is myasthenia gravis an autoimmune disease?" is meaningful only at one level of causation. To return to the opening section of this paper, according to the level of causation considered, myasthenia gravis may be regarded as

(i) a genetically controlled disease
(ii) possibly due to viral infection
(iii) a disease of the thymus
(iv) part of a breakdown of immunological tolerance
(v) a disorder of neuromuscular transmission due to morphological abnormality of the junctions and probably receptor blockade by antibody.
(vi) associated disorders of immunological type affect the thyroid, blood cells, joints and other tissues.

It is now possible to study the rationale of treatment.

**Genetics, viral infection or other precipitating factors**

In the present state of knowledge it is not practical to attack the predisposing or precipitating factors directly. However, this may be an appropriate place to comment on the significance of emotional disturbance on the precipitation of the first clinical episode and subsequent relapses (Simpson, 1960, 1970). Other factors which appear to be significant in this respect are infections, allergy, pregnancy, the pre-menstrual state, and extremes of heat or cold. It was a consideration of these aspects which made me consider the possibility of a hypothalamic-pituitary factor, in addition to some experimental evidence of pituitary control of the thymus (Simpson, 1960). They are mentioned here to stress the importance of appropriate management of the patient's psychological and physical state.

**Thymus gland and abnormal immunological state**

It is clear from the chain of causation that the highest level of causation which can be tackled therapeutically concerns the thymus and the immune system.
(i) **Thymectomy**

Controversy about the value of thymectomy was resolved by the analysis of Simpson (1958) which showed a clear benefit in favour of thymectomy, regardless of age or sex of the patient, provided that the disease was still in what I later termed 'the active stage' and that there was no thymic tumour. It is still controversial whether the pathology of the thymus, at least so far as germinal centres are concerned, is important. In my cases the presence of large numbers of germinal centres has not been associated with a better response to thymectomy, contrary to the opinion of some authors (Vetters and Simpson, 1974).

Even in the active stage, thymectomy is not a cure. Rather it appears to cause a "shift to the left" (Fig. ) and the further course becomes more benign. Recovery processes appear to be potentiated, whatever they may be. Presumably the state of abnormal immunological tolerance is at first thymic dependent but later becomes autonomous. One could speculate about the possible reasons for this but I leave that aspect to those more expert in immunology. Nevertheless the clinical lesson is clear. Thymectomy is beneficial if the operation is carried out before these secondary changes take place, and so it should be advised as an early treatment and not postponed in favour of medical treatment which is aimed at the next level of causation. These conclusions have been substantiated by many later authors (Perlo et al, 1971).

Alternative methods of attacking this stage of causation are radiotherapy, carotid sinus denervation, immunosuppressive drugs, or thoracic duct drainage.
(ii) **Radiotherapy**

In my opinion radiation of the thymus is merely a less certain method of destroying thymic function. It has been stated by Keynes (1955) that pre-operative radiotherapy should be carried out before removal of a thymoma. Perlo, Schwab and Castleman (1966) agree that it increases the duration of post-operative survival. I do not consider that this has been proved and have had no cause to regret abandoning radiotherapy 15 years ago. Radiation of the thymus may be considered in a patient who is "unfit" for surgery but it should be stressed that the myasthenic state may be temporarily exacerbated. I never use it.

(iii) **Carotid sinus denervation**

This operation has had some advocates in France (Thevenard, 1954) because of evidence from animal experiments that adrenocortical hypertrophy and thymic atrophy occurred after bilateral denervation of a carotid sinus. As the beneficial effect claimed in myasthenia gravis is delayed for a year one may be forgiven for being sceptical. Mertens (1955) was not impressed with his results. It is certainly an inferior method of destroying the thymus if such be its rationale.

(iv) **Immunosuppressive drugs**

On first consideration this would appear to be one of the most logical weapons against the active process causing the disease. Delwaide, Salmon and van Cauwenberge (1967) used azathioprine, Nouga and Sonat (1968) used cyclophosphamide and Mertens, Balzereit and Leipert (1969) used 6-mercaptopurine and amethopterin. These authors claimed good results. The reports published up to 1970 were reviewed by Rowland (1971). Evidence in favour of this form of treatment may be dangerous. Matell et al. (1976) claim a 'positive effect' in 78% of patients. When improvement does occur, it may not be obtained for
several months after starting treatment. This is
not necessarily a serious criticism as the same may be true of
thymectomy. A more serious consideration is that the breakdown of
immunological tolerance present in myasthenia gravis is not necessarily
of the hyperergic type. We have recently published evidence that,
like some other autoimmune disorders, the myasthenic may be in a state
of immunological deficiency (Simpson et al, 1976). Eddleston and William s
(1974) have suggested that histocompatibility antigen HL-A8 may be a
marker of defective suppressor T cell function. It remains uncertain
whether purine analogues and folic acid antagonists are beneficial in
this type of autoimmunity. At first sight it would seem that they are
not likely to be of value, but there are possible immunological factors
such as suppressor and K-cells which might be favourably affected.
Transfer factor would also be worth investigating.

It is fair to say that after a decade of use,
the value of immunosuppressive drugs has not been established.
Nevertheless I must draw attention to the natural history of the
disease. If my comments about the timing of thymectomy are valid,
it should also be expected that immunosuppressive treatment would be
beneficial only during the 'active stage', (Simpson, 1974a).
In the series of Mertens et al. (1969) the disease responded better to
immunosuppression if recently detected. I do not have personal
experience because I prefer the certainty (and safety) of thymectomy.
Furthermore, even in cases of myasthenia successfully treated by
thymectomy, we have found that the number of T cells in the peripheral
blood, as measured by auto-E-rosettes, is normal eight years after the
operation (Sandilands et al, 1975) and Matell et al. (1976) found
only a slight decrease in T cells after several months of treatment with
ACTH or azathioprine. It may be that the functional capacity of the
T cells is altered but at least we can conclude that the numbers do not
correlate with the clinical state.
(v) **Thoracic duct lymph drainage**

By cannulation of the main thoracic duct it is possible to remove lymph cells and immunoglobulins (Bergström et al., 1975). According to Matell et al. (1976) there is a rapid decrease in T-cells in the blood and all their patients improved within 48 hours, returning to the pre-treatment state in about the same time when drainage was stopped. Nevertheless 13 of 14 patients so treated were in nearly complete remission 1 year later. Again the stage of the disease is not mentioned - the Swedish authors use the Ossermann classification which is not relevant in this context. Unfortunately they cannot be certain of the long term value of lymph drainage as several of their patients received ACTH and azathioprine after drainage. Furthermore, in seven of their patients retransfusion of homologous cell-free lymph worsened the myasthenic symptoms and so did an immunoglobulin-containing fraction of the lymph in three patients.

(vi) **Steroids**

It is widely assumed that steroids modify myasthenia gravis by their immunosuppressive action, but I have chosen to discuss this form of treatment separately because of some important theoretical implications. It is not the new form of treatment that many young people believe. In the very early days of steroid therapy Torda and Wolff (1951) reported remission of myasthenia following the use of ACTH but it did not become popular at that time because it was recognised that initial deterioration was common and could be fatal (Grob and Harvey, 1952) and that a favourable response may not occur until the drug is withdrawn (Westerberg and Magee, 1955). In 1952 and 1953 I was fortunate in being allocated some of the first ACTH and cortisone imported into Britain. The results were not impressive in the four patients treated. No severe deterioration was seen but it was confirmed that the patients felt better after withdrawal of the drug, though less than with the subsequently administered neostigmine. The initial deterioration and subsequent
improvement were cited in the paper presenting the autoimmune hypothesis (Simpson, 1960). Steroid therapy was considered too dangerous and was generally abandoned until we learned how to cope with crisis situations in the myasthenic, and intensive therapy units became commonplace. In these changed circumstances von Reis et al. (1966) in Sweden were able to report that ACTH in larger dosage than we had used could induce rapid and often fairly complete remission for prolonged periods of time, provided that the pronounced initial deterioration could be overcome. They gave about 1000 I.U. in 5-8 days. Since their paper there have been many others on ACTH, cortisone, or prednisolone using a variety of dosage regimes. Namba et al. (1970) give an excellent review. Genkins et al. (1971) reported on 100 patients and some 300 courses of treatment in a period of 5 years and there is now widespread adoption of steroid treatment. It does not seem to matter which of the above drugs is used (Brunner et al., 1976) or whether the dosage is daily or on alternate days (which is claimed to avoid the adrenal-suppressive effect). In general, there is increased weakness starting on the second or third day and greatest by the 6th or 7th day, with some recovery after 10 days. Muscle strength continues to improve for about a week after a 10 day course of treatment and may be maintained for 2-3 months. Benefit may be prolonged by further single injections of ACTH (100 units) once or twice weekly for many months. Despite the acknowledged risks (5% mortality) the treatment is justified in severe cases who are not adequately controlled with anticholinesterase drugs. What is more, the benefit may be striking in patients in the 'inactive stage' or even in the 'burned out' stage. Muscles (eg. extraocular) which have not responded to anticholinesterase for months or years, commonly show return of power and renewed response to these drugs. The response cannot be thymocytolytic as it occurs in patients who have had thymectomy. Indeed the response may be greater in those who have had thymectomy (Genkins et al, 1971). Nor is there good evidence for action on humoral or cellular immunity reactions. It is for these reasons that I have
described steroid treatment under a separate heading and have previously speculated (Simpson, 1971b) that adrenocortical hormones may play a role in the remodelling of the endplates which are deformed. The findings of Shapiro et al. (1968) suggest an adrenal role in the remodelling process and this is being further studied in my laboratory. (For fuller discussion cf Simpson 1969, 1971a, 1974).

My own experience of steroid treatment is limited, being a late convert because of my unfavourable experience in 1952. I have not yet seen the striking and prolonged remissions described by others but the effect on end-stage ocular palsy has been quite remarkable and justifies the undoubted risk of high dosage steroid therapy. It seems most unlikely that activity of this type is due to facilitation of neuromuscular transmission, Pinelli et al. ( ) considered that transmission was directly affected but Namba (1972) and Howard et al. (1976) found no such effect.

**Neuromuscular transmission**

It will be apparent that drugs which alter the safety factor of neuromuscular transmission play an important role in the management of the myasthenic patient but cannot be considered to be treatment of 'the cause' except at the lowest level of causation. The safety factor is raised by drugs which increase the production or release of acetylcholine (guanidine, 4-aminopyridine, ephedrine), inhibit its hydrolysis by cholinesterase (physostigmine and its analogues; organophosphorus compounds; alkaloids), or potentiate the response of the muscle to endplate depolarisation (ephe drine; veratrine alkaloids; potassium). I have listed these under their principal actions; some act at more than one site. Drugs which reduce the safety factor for transmission must be used with caution in patients with myasthenia gravis (vide infra)
Potentiation of ACh release + sensitisation of ACh receptors

(i) Guanidine hydrochloride Guanidine has both pre-synaptic and post-synaptic facilitatory actions on neuromuscular transmission (Feng, 1940, 1941). It is rarely used for the treatment of myasthenia gravis but is very effective in the management of carcinomatous myasthenia (Lambert, 1966) in a dose of 20-50 mg/kg body weight/day, divided into three doses.

(ii) 4-Aminopyridine sulphate or chloride.

(ii#) Adrenaline and ephedrine sulphate

Despite exceedingly weak anticholinesterase activity, adrenaline and many of its primary and tertiary amine analogues potentiate the transmitter process. Bowman and Raper (1966) considered that the facilitating actions of adrenaline and noradrenaline are due to their effects on the motor nerve terminals where they augment the output of transmitters. However, it should be emphasised that the structure of catecholamines, like some other drugs, is appropriate for both facilitation and depression and the action is dependent on dosage and on other drugs administered. There is controversy regarding the action of adrenaline on the muscle fibre membrane. It is of no practical value in the management of myasthenia gravis. On the other hand, ephedrine, an inhibitor of the amine oxidase which oxidizes adrenaline, has been claimed to have an antimyasthenic action since the report of Edgeworth (1930) from her personal experience. (A myasthenic patient, she used ephedrine for her asthma and found that both disorders were improved.) An oral dose 10-25 mg thrice daily is used. Large doses should be avoided as they may aggravate the weakness I have not been convinced that ephedrine has any useful clinical action on skeletal muscle in myasthenia gravis and it may be that Ringqvist and Ringqvist (1971) are correct when they suggest that clinical improvement w
Ephedrine may to a significant extent be explained by its bronchodilator action, which could be very important in a patient taking anticholinesterase drugs. Ephedrine may be used to supplement anticholinesterase drugs but is of little or no value independently.

Veratrum alkaloids lower the stability of membranes of excitable cells, causing repetitive firing in response to threshold stimulation and hence 'amplifying' the stimulus-response relationship. This occurs both pre- and post-synaptically at the neuromuscular junction. Thus veratrine and germine diacetate (Flacke et al., 1971) may temporarily increase the efficiency of neuromuscular transmission in myasthenia gravis, but only at the price of decreased efficiency of oxidative metabolism of the muscle and nerve fibres and loss of intracellular potassium in addition to action on other organs causing bronchiolar constriction and occasional cardiac arrhythmia. Veratrine causes hypotension but germine is said to be without this action. I have not used this group of drugs. Their value is certainly marginal, probably not persistent and entirely inferior to the anticholinesterase drugs.

Anticholinesterase drugs
Acetylcholinesterase (AChE) localised in the subneural apparatus of the motor endplate of a skeletal muscle fibre hydrolyses acetylcholine (ACh), thus freeing receptors for occupation by further molecules of ACh released by subsequent nerve impulses. Inhibitors of this enzyme prolong the occupancy of receptor sites by ACh, thus potentiating its action and raising the safety factor for transmission, where this is diminished for any of many possible reasons. If carried to excess, so that ACh persists at receptor sites, the endplate remains depolarized or else becomes desensitized, a condition which has been termed 'cholinergic blockade'.
The first effective anticholinesterase substance, extract of Calabar bean, is an aboriginal antidote to curare poisoning. Because of the resemblance of myasthenia gravis to that condition, Jolly (1895) suggested that the active substance physostigmine (eserine) might be tried in myasthenia. (He also suggested veratum alkaloids but there is no evidence that he actually used them.) Physostigmine, a carbamate ester and a tertiary amine first used in the treatment of myasthenia gravis by Walker (1934), is a potent cholinesterase inhibitor, but as it crosses the blood-brain barrier and acts on synapses within the central nervous system, it has been replaced by one or other of the synthetic analogues.

Another tertiary amine, galanthamine (Nivalin) isolated from the Bulgarian snowdrop is said to be useful (Ugunov, 1966). Walker (1935) was also the first to use neostigmine, one of a number of quaternary ammonium compounds with anticholinesterase activity. The most suitable for clinical use have been neostigmine, pyridostigmine and ambenonium.

Hydroxyanilinium salts or esters may have this property. The one established for clinical use is edrophonium (Tensilon).

All of these drugs probably also act on the motor nerve terminals and perhaps directly on the post-synaptic membrane but it is generally accepted that the anticholinesterase activity is the more important clinically.

Edrophonium (Tensilon). This drug, an anticholinesterase administered intravenously (2.0 - 10 mg) is used to confirm the diagnosis of myasthenia gravis or to differentiate between underdosage and overdosage with anticholinesterases. As an abbreviated account of this test could be misleading and possibly dangerous the reader is referred to Osserman and Kaplan (1953). The potentiation of ACh is substantially over in 2-5 min but the test should not be repeated without awareness that
residual activity can be demonstrated electro-physiologically at least 30 min after intravenous injection. The clinically detectable anti-myasthenic action is too brief to use in treatment.

**Neostigmine** (Prostigmin) may be given orally, or by subcutaneous or intramuscular injection. Absorption is rapid and it should rarely be necessary to use the intravenous route, with greater danger of bradycardia. Although it is incompletely and irregularly absorbed from the gut, neostigmine should normally be given by mouth. The 15 mg tablet is approximately equivalent to 1 mg by injection. If dysphagia prevents normal administration the crushed tablets can be given by gastric tube. Following a dose there is a surge of muscular power for 30 - 60 minutes followed by continued activity at a lower level for 2-6 hours, after which strength is lost rapidly. This makes it difficult to adjust timing of dosage for a smooth control but the 'boost' effect is valuable if the tablets are given 30 minutes before a meal or in anticipation of a special effort. Most myasthenics do not require this and prefer to avoid the 'let down' between doses by selecting pyridostigmine as the drug of choice.

**Pyridostigmine** (Mestinon) Although introduced as a long acting anticholinesterase substance, the duration of action of pyridostigmine is not substantially longer than that of neostigmine. There is less swing from the possible overdose of the peak effect to the 'let down' stage characteristic of neostigmine. Slower warning of activity allows a sustained level to be achieved by judiciously timed dosage. The 60 mg tablet is approximately equivalent to 15 mg neostigmine.

The useful duration of activity of neostigmine and pyridostigmine varies in individual patients from 1-8 hours or more. For effective treatment, the spacing of dosage should first be established by having the patient keep a written record of the effect of one tablet.
This is repeated four or more times, with a gap of at least one hour after the patient considers that power has relapsed to the pre-dosage level. Once the time interval is established, dosages are timed throughout the day so that a dose is taken half-an-hour before each major meal. Each dose is then increased by half-tablet increments until maximum improvement occurs. The amount of each dose may be the same, or adjusted according to the activity of the succeeding epoch. It may, for instance, be preferable to have uninterrupted sleep rather than have a dose in the early hours of the morning. Other patients must have the dose at regular intervals, day and night.

The most difficult decision is regarding the dose required by a patient. Those unfamiliar with myasthenia gravis, but aware that overdosage with anticholinesterase drugs leads to increasing weakness ("cholinergic block"), assume that between myasthenia and cholinergic weakness there must be a stage of full restoration of transmission with normal strength. In many cases, possibly in most, this does not happen and there is then considerable danger of overdosing the patient. Furthermore, as the safety factor for transmission is not equally reduced in all muscles, it follows that some muscles will require a dose of anticholinesterase which is an overdose for other muscles. Thus, in chronic (stage 2 or 3) myasthenia it is common to find fasciculation of lower limb muscles when facial/bulbar muscles are still underdosed. The edrophonium test with suitable precautions for control of respiratory failure is valuable in assessing dose levels, particular attention being paid to important functions such as chewing, swallowing and breathing. When in doubt, it is always safer to have the patient slightly underdosed. If muscarinic effects are prominent it is customary to use atropine or propantheline (Pro-Banthine). I prefer not to do so if possible because the most reliable indicator of an early cholinergic state is the
pupil size and this guide is lost if anticholinergic drugs are used.

As indicated above, pyridostigmine was introduced as a long-acting anticholinesterase (and in some countries there is a "time-span" formulation with longer action) but the 'plateau of activity' is little difficult from that of neostigmine. However the activity passess off more slowly and so this drug is preferred by most patients. In fact, a prolonged low level activity can be demonstrated 48 hours after a dose of pyridostigmine (Simpson, unpublished observations). For this reason, very gradual cumulative effects may occur and a patient who seemed to be correctly dosed on discharge from hospital may return in 2-3 weeks with signs of overdosage. It is my contention that the general demand for an anticholinesterase drug with 24 hour action is misguided. All drugs act for longer than pyridostigmine have proved unsuitable because of cumulative effects.

**Ambenonium** (Mytelase)

This drug is widely used in the USA though rarely in Britain. Its duration action is slightly longer than pyridostigmine. Central actions are more common but as muscarinic side effects are less frequent it may be difficult to detect the onset of cholinergic crisis. A 25 mg tablet is about equal in potency to 15 mg neostigmine or 60 mg pyridostigmine.

**Bis-neostigmine compounds** Still longer activity is obtained from anticholinesterases combining two neostigmine radicles in one molecule, separated by a polymethylene chain of different lengths. Distigmin (Ubretid), 5 mg, is promoted for use in myasthenia gravis though the danger cumulative effects was recognised in Vienna twenty years ago (Pateisky et al, 1955). I do not recommend it.
Alkyl-phosphates  Organophosphorus compounds, which are potent anti-
cholinesterases, are no longer used because of the cumulative effects
and because they have more action on central synapses than the
quaternary ammonium compounds, giving rise to headaches, nightmares,
and personality disturbance. Cumulative effects go pari passu with
long duration activity. A short acting drug is much safer when used
at near-toxic levels of dosage.

Potassium and aldosterone inhibitors

Potassium is used as an adjuvant in the treatment of myasthenia
gravis. I am not convinced that it is beneficial and it may cause
nausea and diarrhoea resembling cholinergic crisis.

Spironolactone, given to conserve potassium (Gottlieb and Laurent,
1961) is of no proven value though it gives a sensation of wellbeing.
Provision of potassium to counteract the loss of intracellular potassium
during steroid therapy is quite another matter and its use for that
purpose is rational.
Seventh Symposium on Current Research in Muscular Dystrophy

held at
The University of Birmingham
5-7 January 1977

ABSTRACTS OF COMMUNICATIONS
Myasthenia Gravis: Passive Transfer from Man to Mouse.

REES, D., BEHAN, P.O., BEHAN, W.H., SIMPSON, J.A.

Toyka and co-workers (Science vol. 190; 397-399, 1975) claimed that isolated immunoglobulin from pooled sera of patients with myasthenia gravis reduced the amplitude of miniature end-plate potentials (MEPPS) in mice. BDF1 strain of mice were used in their experiments and inoculated daily with 0.4-0.65 ml of the fraction containing 1.2/2.6 gm per 100 ml of IgG and they also received cyclophosphamide 24 hours after the first injection to reduce immune responses to the human immunoglobulin. "The mice showed reduced amplitudes of miniature end-plate potentials and reduced number of acetylcholine receptors at the neuromuscular junctions. Some mice showed typical decremental responses on repetitive nerve stimulation with reversal by neostigmine." Some of these effects were found in mice as early as 3 days after receiving human IgG. The authors claimed that this was evidence of a circulating factor in the serum of patients with myasthenia gravis which reproduces some of the features of the disease in mice.

The present study was carried out to try and confirm the above findings. BDF1 mice and LACA mice were used and inoculated with 7 mg of isolated and purified IgG from myasthenic sera given intraperitoneally daily for 14 days and the mice examined electrophysiologically between the 5th and 26th day. Examination in vitro of sciatic nerve triceps surae preparation and nerve diaphragm muscle preparation, and internal intercostal and peroneus muscle preparations showed no abnormalities. In fact the electrophysiological properties were as found in specimens removed from healthy mice and were preserved for periods of up to 30 hours. In a parallel experiment several nerve muscle preparations were perfused with 0.01-0.05 mg/ml of isolated IgG in order to test direct curare-like action of the IgG fraction on the acetylcholine receptor complex. These preparations survived also up to 30 hours and exhibited no abnormal pattern in their electrophysiological characteristics.

Our data are in direct conflict with the findings of Toyka and his co-workers and it is difficult to give an explanation. The sera were obtained from patients with myasthenia gravis in all stages of the disease and some having had a thymectomy and others a thymoma. Possible reasons for our failure to confirm Toyka's results will be discussed.
MYASTHENIA GRAVIS—VALIDATION OF A HYPOTHESIS

J. A. Simpson
Glasgow University Department of Neurology, Institute of Neurological Sciences,
Southern General Hospital, Glasgow

The use of hypotheses is the method of science. To suppose we can make discoveries by the Baconian method is a delusion. A hypothesis or supposition is not a conclusion; it is only a starting point for methodical observation and experiment, the endeavour being not only to prove it, but to disprove it.

Hughlings Jackson

When I first became interested in myasthenia gravis in the early 1950's the important problem seemed to be the defect in neuromuscular transmission and the general opinion was that it was due to a biochemical lesion at the neuromuscular junction. I built myself an electroneyograph but very soon found that the electrophysiological changes showed only that there was a loss of safety factor for neuromuscular transmission which could be due to any of a number of disorders (Simpson, 1960; 1966; 1969). It could not settle the then current arguments as to whether there was defective formation or release of acetylcholine, abnormal endplate responses (as suggested by studies with the new drug decamethonium), or a 'curare-like' substance from an extrasynaptic source such as the thymus or released from active muscle, as suggested by the Mary Walker phenomenon. In short, there were pre-synaptic, post-synaptic, and myasthenic-toxin theories.

I went to Queen Square in 1953 to build a DC amplifier and develop the new glass microelectrode for human muscle studies. Bernard Katz told me it couldn't be done in mammals, and certainly I could not get it to work with the technique available at that time, before transistor amplifiers and commercial micromanipulators. So I spent a lot of time talking to myasthenic patients—and this turned out to be a more sensitive probe as I will describe!

There is no time to give a detailed analysis of the various theories on the transmission defect and I have presented this elsewhere (Simpson, 1971; 1975). But I think it is important to stress that they are all models, usually based on pharmacological analogues which are currently fashionable. Thus Willis (1672), in the first description of the disease in the era of humors and animal spirits, concluded: 'Without doubt in these, although the Animal Spirits do after a manner actuate and irradiate the whole nervous stock, yet their numbers are so small, and in so few heaps, that . . . before noon the stock of the Spirits being spent, which had flowed into the Muscles, they are scarcely able to move Hand or Foot'. This is a model based on the topical view of nervous activity and it is clearly a presynaptic theory.

Two centuries later when Erb and Goldflam established the well known clinical picture of muscular 'fatigability' on repeated or continuous effort, the transient paralysis caused by curare was known and this became the model for Oppenheim (1887) and Jolly (1895). They suggested using physostigmine but there is no evidence that it was tried until the famous report of Mary Walker (1934) who soon also used the analogue neostigmine (Walker, 1935). In the same year Dale and Feldberg confirmed the role of acetylcholine in neuromuscular transmission and from that time all thinking turned to the model of a biochemical lesion. In the early 1950's I was working with the early methonium compounds when Churchill-Davidson and Richardson (1952) reported anomalous effects of decamethonium in myasthenics. I have found that this is not confined to myasthenia gravis but that is another story. The important point was the clear evidence for abnormal receptor response—i.e. a post-synaptic disturbance, if the model is valid. The next pharmacological model was hemicholinium-3 which Desmedt (1957; 1958) considered to be a better model than curare, particularly with regard to 'post-activation exhaustion' which is certainly an important feature of myasthenia gravis. At that time hemicholin-
Simpson

readily-releasable

or packaging of ACh or its transfer to a readily-releasable store. Unfortunately, like most pharmacological probes, the action of hemicholinium is not exclusively pre-synaptic and it is not true that curare does not cause post-activation exhaustion (Bergmans, 1975).

When technical advances and superior skills permitted microelectrode studies of biopsied human muscle, Elmqvist (1965) reported that spontaneous miniature endplate potentials (MEPP) were reduced in size. It is a general principle of interpretation of MEPP studies that the frequency of MEPPs is controlled entirely by the conditions of the pre-synaptic membrane, while their amplitude is controlled by the post-synaptic element (Katz, 1962). These results should therefore point to a post-synaptic disorder in myasthenia, but the Swedish workers preferred to interpret them as indicating lowered quantum content of ACh, also on the analogy of hemicholinium studies.

There are 2 major flaws in the pharmacological models. Firstly, the drugs do not have single sites of action. Secondly—and this is the major unrecognised flaw—the known effects are those of drugs acting on normal synapses. If the neuromuscular junction is not morphologically normal the model has little or no validity. In 2 theoretical papers I have shown that all the known electrical and pharmacological features of myasthenic muscle, including the small MEPP, can be accounted for by geometrical changes of the neuromuscular junction (Simpson, 1969; 1971).

Structure of the neuromuscular junction

When I started my work the textbooks were emphatic that there were no pathological changes in myasthenia gravis and so the lesion must be 'biochemical'—the appropriate model for the 1940-60 era. Mott and Barada used intravitral staining of muscle with methylene blue as early as 1923. They did not detect any abnormality but contemporary critics suggested that the diagnosis was erroneous. Coers and Woolf (1954; 1959) used this method along with staining of the subneural apparatus for cholinesterase (which incidentally is not present in excess). They demonstrated florid morphological changes of the intramuscular nerve endings. There is no time to discuss their findings, which were described at our Second Symposium by the late Dr A. L. Woolf (1963), or the electronmicroscopic studies of the 1960's, but the precise morphological studies of Engel and Santa (1971) make it clear that in addition to the elongated simplified terminals with ultra-terminal sprouting shown by intravitral stains, there is significant disorder at the ultrastructural level, fitting very well with my simultaneous theoretical analysis. The mean area of each terminal knob is reduced but multiple small nerve terminals appear over an extended region of the muscle fibre. In some regions the primary synaptic cleft is widened and the postsynaptic region is abnormally simple. Secondary synaptic clefts are sparse, shallow, abnormally wide or absent. No abnormality of number or size of synaptic vesicles is reported—certainly no morphological support for the 'small quan- tum' theory which was so widely and uncritically accepted.

Still more recently it has been possible, by α-bungarotoxin which is a specific molecular probe for the nicotinic AChR molecule (or by an immunoperoxidase method), to show that endplate binding in myasthenic muscle averages 80 per cent below normal (Fambrough et al., 1973). Contrary to expectation, the receptors appear to be at the crest of the folds and not in the secondary clefts.

Quite obviously the previous 'models', based on pharmacology of normal neuromuscular junctions, must be rejected. But 4 questions immediately present themselves: 1. What causes the morphological changes I have described? 2. Why is the disease characterised by relapses and remissions? 3. How can we account for the fact that 1 in 7 of all live children born to myasthenic mothers have a short lasting myasthenia? 4. What is the role of the thymus?

I have described how my electrophysiological studies in the early 1950's led me to believe that this type of study could not take me further than a demonstration of loss of safety factor. They were completely incompetent with respect to these 4 crucial questions. Fortunately I was trained as a general physician educated to look at disease as
biology out of control. While I was building my apparatus at Queen Square, Dr Arnold Carmichael asked me to review the myasthenic patients of the National Hospital to sort out the transatlantic argument about the value of thymectomy. Sir Geoffrey Keynes had been writing serial reports on his encouraging results with thymectomy for myasthenia gravis whereas the American workers who had started it were abandoning the procedure as useless. Keynes had always maintained that the results were good if there was no thymoma (Keynes, 1954). The American teams in Boston, Baltimore and the Mayo Clinic were selecting severe cases and particularly those with a thymoma. My survey vindicated Keynes and showed that the published data for America were in accordance when these cases were regrouped. The rest is history and I need not go into the other findings regarding the influence of age, sex and duration of illness on the results of operation. Nothing that has been reported in the many studies since Simpson (1958) has contradicted the findings. I want only to make 3 points: (1) the best results are obtained if operation is within the first 5 to 7 years from the onset of illness; (2) the disease ceases to progress but actual improvement may be delayed for 2 or 3 years; (3) late relapse remains possible. Indeed there are many reports of myasthenia first presenting years after a thymus has been removed on other indications. These facts make it impossible that the thymus is the source of a myasthenic-toxin, be it 'curare-like' or a hormone released by thymitis, in a more recent version. But the thymus obviously plays a key role.

When I began to examine some 250 cases of myasthenia and the records of 150 more from the National Hospital and Sir Geoffrey Keynes's series I decided to forget all that the books had to say about chemical lesions and to listen to the patients. I prepared a proforma and noted every fact I could elicit about the previous history of these patients, including every illness whether it seemed relevant or not. I soon realised that certain features recurred in the histories. On returning to Glasgow in 1955 I conceived the idea that instead of rejecting as 'irrelevant' any data which were 'not statistically significant', it would be worth considering whether there was some correlation between them—i.e. that the muscular disorder might be part of a multi-system disease. I have already described at the Third Muscular Dystrophy Symposium (Simpson, 1968) my finding that myasthenia was correlated not only with thyrotoxicosis, but with all other non-tumour types of thyroid disease (Simpson, 1960; 1968). This was also the first report of the linkage between myasthenia gravis and 'rheumatoid' arthritis, pernicious anaemia, reticuloendothelial disorders and diabetes mellitus, and the first suggestion of a genetic factor with alternative forms of expression.

It immediately seemed appropriate to regard the pathological features from the same point of view. Histological changes in muscle and other organs had been described for many years but were always dismissed as 'non-specific' on the grounds that similar changes occurred in other diseases. Weigert (1901) was probably the first to describe focal collections of mononuclear cells and lymphocytes within the muscle. His suggestion that they were metastatic tumours was disproved by Buzzard (1905), who believed that the cells originated from small blood vessels ('lymphorrhages'). Russell (1953) considered that lymphorrhages were 'non-specific' as they occurred in thyrotoxicosis, Addison's disease, rheumatoid arthritis and other diseases. It seemed to me that instead of dismissing the lymphorrhage as 'non-specific' it would be more profitable to consider what factor might be common to each of these diseases. As lymphocytic infiltrations are common in the target organs of allergic disorders, an immunological reaction suggested itself.

In the same way as the pathology was being dismissed as 'non-specific', the recent description of myasthenic syndromes in some muscular diseases had given rise to the opinion in the 1950's that myasthenia was a type of muscular reaction not confined to one disease and so myasthenia gravis might not be a clinical entity. As soon as the concept of a disease which could occasionally be generalised and which had pathological features in common with connective tissue diseases, was
considered, the hypothesis of an immunological disorder resembling systemic lupus erythematosus (SLE) suggested itself. In 1954 when Harvey et al. published their classical paper on SLE I immediately recognised the similarity in the age and sex incidence and the fluctuating natural history. They even described myasthenia in SLE patients. I also remembered that before going to Queen Square I had the opportunity to give some of the first cortisone in the UK to myasthenic patients, and was aware of the early deterioration and 'rebound' improvement. All the pieces were falling into place, and the jigsaw looked like an autoimmune disease. Immediately I could see how a 'personal' neuromuscular blocking substance could pass through the placenta to affect the fetus (and persist for much longer than curare could) and yet person to person transfusion did not transfer myasthenia and no animal transfer had been achieved (Nastuk et al., 1959).

But what of the thymus? Castleman and Norris (1949) had recently emphasised the importance of the germinal centre, even in glands with a thymoma. During the period I am describing, the thymus was believed to be an endocrine gland with an obscure function which might be related to fetal growth and development. It seemed to me that it was more like a lymphoid organ.

The logic of my argument led me to conclude that the thymus was responsible for cellular and humoral immunity and that when defective, because of a genetically determined breakdown of immunological tolerance, it would release lymphocytes which would damage the neuromuscular junctions and form antibody to the ACh receptors—the personal blocking substance that I needed—but which could also damage other organs.

It is very difficult in biology, and especially so in medicine, to publish a theoretical study. Unlike the physicists we value our manual workers more than our thinkers. And so it was not until 1960 that the opportunity to present it was given by a Honyman-Gillespie Lecture in Edinburgh. Note that this was the year before Miller (1961) published his seminal work on the immunological function of the thymus and the whole story of the thymus in autoimmune diseases. The lecturer was encouraged to speculate about his topic. Unfortunately he was forbidden to discuss experiments on animals, and so I could not relate that on my return to Glasgow in 1955 I obtained the co-operation of John Anderson in an attempt to produce an 'autoimmune' myasthenia in mice by inoculating them with an emulsion of homologous muscle with Freund's adjuvant. Well, we were 20 years too soon. I now know that the mouse is a poor subject for this type of experiment, the antigen must have contained very little AChR protein, and the mouse has such a high safety factor for n-m transmission that we could not have detected any change with the techniques of that time.

However, the point about a hypothesis is its heuristic value. This is something I wish to emphasise as its importance has been overlooked. By the coincidence which is so common in science, other people described autoimmune theories of myasthenia at about the same time. Their premises were different and it is impossible to predict the later findings by extrapolation. I have given a more thorough analysis of the rival theories in a recent Robert Schwab Memorial Lecture in Boston (1975) and do not have time to repeat the arguments.

In 1956 I moved to Edinburgh and with collaborators there (W. R. M. Alexander) and in Glasgow started a search for antibodies against the endplates. They were very tolerant of my unorthodox ideas. The experiments became more urgent in 1959 when Smithers suggested that the myasthenic thymus might be immunologically damaged (drawing attention to a comment by Castleman (1955) that the germinal centres resembled the thyroid changes in Hashimoto's disease). In the same year Nastuk et al. (1959) searching for a neuromuscular blocking substance in blood from myasthenic patients (and a few control subjects in lesser degree) found that the serum caused lysis of frog muscle cells. I still had no direct evidence of antibody when I gave the Honyman-Gillespie Lecture in the spring of 1960, but one month after it was published the New York group announced the discovery of a complement-fixing antibody against the myosin of skeletal
muscle in the blood of many myasthenic patients. It was a long time before we in Edinburgh could confirm the findings of Strauss et al. (1960). The reasons are important. It turned out that the New York work used pooled sera and in every pool there was at least one sample from a patient with a thymoma—now known to be high titre serum. We had always used single samples and for a long time had no patient with a tumour. Furthermore, our experience on control sera indicated that only A-band fluorescence (as distinct from I-band) was significant. When I returned to Glasgow, John Vettes (1965) showed that the A-band antibody was very highly indicative of thymoma.

The American workers went on to speculate that an antibody developed against skeletal muscle found a similar antigen in the thymus which became damaged and released a neuromuscular blocking substance (Strauss et al., 1966), an unrecognised return to the concept of a 'curare-like substance' although it was their final refutation of this concept that had started their immunological studies (Nastuk et al., 1959).

Later in Australia and America, Goldstein and a series of collaborators purported to show that the thymus was immunologically damaged ('thymitis') and released 'thymin'. Their theory, based on animal experiments which I cannot confirm (Vettes et al., 1969), would be incompatible with the occurrence of human myasthenia years after removal of the thymus. There is no reliable evidence for a neuromuscular blocking substance produced by the thymus. The skeletal muscle antibody discovered by Strauss is inconsistent and is directed against myofibrils but it was clear evidence of immunological disturbance. Indeed for many years the failure to find an antibody against ACh receptors appeared to be fatal to the Simpson (1960) hypothesis. Critics failed to see the essential difference from the Strauss and Goldstein models. I had clearly postulated a breakdown of immunological tolerance of genetic origin with alternative expression. The thymus was regarded as a controller of immunological competence and the neuromuscular damage was due to cellular and humoral antibody.

My clinical evidence on the stages of the disease (Simpson, 1974) indicated that after 5 to 7 years the immunologically competent cells no longer required the thymus and that there were 2 types of lesion at the junctions, one an antibody block, the other morphological. The hypothesis also emphasised an important regenerative factor, later postulated as adrenal in origin.

It could be predicted that myasthenics would produce antibodies against other organs, have associated autoimmune diseases (not only personally but in close relatives), that thymectomy must be carried out within a certain period, that the disease would be phasic and that antibody against AChR would be found.

I showed the first correlation with pernicious anaemia, rheumatoid arthritis, reticuloendothelial disease, sarcoidosis and diabetes mellitus in 1965, with Hashimoto's disease in 1964 and polycythaemia vera in 1976, and confirmed other reports of pemphigus, epilepsy and Sjögren's disease. Antibodies were found against nuclear factor, gastric parietal cells, thyroid, liver and rheumatoid factor (Feltkamp et al., 1963; Simpson, 1964) and later against skin. Subtle disorders of lymphocyte function were detected (Behan et al., 1976). The genetic linkage was shown to be associated with the HLA-A8 antigen in many but not all cases (Behan et al., 1973; Dick et al., 1974) and finally we showed that the autoimmune disorder was of the immune-deficiency type which is being increasingly recognised (Simpson et al., 1976).

Only the Simpson hypothesis can account for these later findings. As we have seen, the electrophysiological and ultrastructural findings of the decade 1965-75 were falling into line. Only the AChR antibody remained. At this point 2 important discoveries opened up the field.

I have described our unsuccessful attempts to produce an experimental myasthenia in mouse, using homologous muscle as antigen and the consistent failure of all workers in the 1960's to find an antibody against any junctional tissue in human myasthenia gravis (McFarlin et al., 1966). In 1966 Goldstein and Whittingham claimed to produce an
experimental myasthenia in guinea pigs inoculated with fresh calf thymus, muscle or lymph node. I have already criticised the 'thymitis' concept developed by Goldstein and must now say that with few exceptions other workers are unable to reproduce his findings. In our own experiments the phenomena reported by Goldstein were shown to be experimental artefacts (Vetters et al., 1969).

The position was transformed when Patrick and Lindstrom (1973) observed that repeated immunisation of rabbits with nicotinic AChR protein purified from the electric organ of *Electrophorus electricus* caused the development of muscular weakness and a decrementing electromyogram response to repeated nerve stimulation, reversible by neostigmine. They were preparing an antibody for other purposes but fortunately they rapidly realised the significance of their observation for myasthenia gravis. The antigen is certainly from the endplate receptor tissue though not necessarily the receptor substance itself. It does, however, block the depolarising response of the electroplaque to carbamylcholine. Their findings were rapidly confirmed and extended to other species including primates (Tarrab-Hazdai et al., 1975) in which the resemblance to human myasthenia is very striking. Engel et al. (1976) have shown that the morphological and electrophysiological changes at the neuromuscular junctions in experimental autoimmune myasthenia gravis (as it is now termed) in the rat closely resemble those of human myasthenia gravis in the chronic phase though there are differences in the acute phase of the experimental disease.

So there is now strong evidence that the neuromuscular junction can be selectively damaged by antibody raised against homologous tissue. It still remains to demonstrate that antibody can compete for receptors. In the last 2 years it has been shown that serum from human myasthenic patients can block the binding of α-bungarotoxin to endplate receptors either extracted (Almon et al., 1974) or in situ (Bender et al., 1975; 1976) and antibody against human AChR has been measured in myasthenics by a number of techniques involving radioimmunoassay of antibody—AChR—bungarotoxin complex (Lindstrom et al., 1976) or by complement—fixation (Aharanov et al., 1975).

The final validation of the peripheral part of the hypothesis should be a passive transfer of the disease. Although neonatal myasthenia suggested that this should be possible to a histocompatible subject, it has not been achieved post-natally. In 1975, Toyka et al. from the Johns Hopkins University in Baltimore reported that they had succeeded in passive transfer from man to mouse. To achieve this they had to immunosuppress the mice with cyclophosphamide so that they would tolerate 14 daily injections of IgG separated from myasthenic serum. These mice showed some of the electrophysiological changes of myasthenia gravis (including small MEPPs) and the numbers of ACh receptors were reduced by 42 per cent on some but not every muscle. Only one animal was weak, and only one was tested with neostigmine. The safety factor was restored. I have already warned about the limitations of the electrical and pharmacological studies. Nevertheless this report is very exciting. I had hoped that we would be reporting confirmatory results from our own experiments at this meeting. Sadly, we have not yet been able to do so (Rees et al., 1976). Possibly we have again been unlucky with low titre sera. But this has been a story of an idea waiting for techniques. In 1960 the thymus was an endocrine gland and microelectrodes could not be used in mammalian muscle. In the next decade techniques for detecting antibody were too primitive. Immunology knew nothing of suppressor cells as the role of the thymus gradually became clearer. Genetic linkage had to wait for the transplant workers to get excited about histocompatibility antigens. Then a biologist needed an antibody against a tissue from an exotic animal. A hypothesis has slowly become a theory and parts of it are accepted dogma. I feel that Hughlings Jackson would have approved. The quotation at the head of this paper concluded my 1960 paper in this Journal. The reader must judge how far the hypothesis has been validated.

References are in 2 lists. The first gives those other workers cited. The second is a chronological list of 'local' papers which may
be helpful to others studying myasthenia gravis.

REFERENCES TO TEXT


Bergmans, J. (1976). Personal communication


Desmedt, J. E. (1957). Nature of the defect of neuromuscular transmission in myasthenic patients. 'Post-tetanic exhaustion'. *Nature*, 179, 156


---

**Publications Related to Myasthenia Gravis**

**By Professor J. A. Simpson and Colleagues**


209


4 Myasthenia gravis: a clinical approach to pathogenesis

JOHN A. SIMPSON

Institute of Neurological Sciences, Southern General Hospital, Glasgow

In teaching the logic of clinical diagnosis to medical students it is valuable to group the data obtained from questioning and examining patients as answers to three questions ‘Where, What, and Why?’ – more formally, the localization, the pathology, and the aetiology of the disease process. The scientifically trained student will also ask ‘How does the lesion produce the functional defect?’ Concentration on the last question has, paradoxically, retarded understanding of myasthenia gravis.

Where is the lesion?

Myasthenia is a term to describe muscular weakness which increases with maintained or repeated contraction of skeletal muscle and is reduced by rest. The contraction may be voluntary, reflex or electrically provoked, but only if the electrical stimulus is applied to the motor nerve. The response to direct stimulation of the muscle fibre is normal, and the propagated action potential of the motor nerve fibre is also normal. It is therefore a reasonable conclusion that the disorder is at the neuromuscular junction, a conclusion supported by the therapeutic finding that anticholinesterase drugs such as neostigmine decrease the weakness whereas sensitivity to D-tubocurarine is increased.

A myasthenic disorder may be found in a number of diseases involving lower motor neurones or muscle (Simpson, 1966a). It is a sign of loss of the safety factor for transmission which is normally present and which can be reduced in several ways. The disease entity termed Myasthenia Gravis is characterized by a severe defect of neuromuscular transmission without clinical evidence of a motor neurone disease or of a myopathy in the usual sense. (There is a little evidence of dysfunction of the contractile mechanism but it is not the primary disorder). There is recent evidence from single fibre electromyography and the new methods for endplate receptor microscopy that the disease process may involve all motor endplates in skeletal muscle but the disorder is more severe, or involves more endplates, in certain muscles. This gives rise to a characteristic distribution though the
exact pattern differs from patient to patient. The (statistical) order of appearance and the severity of clinical weakness are shown in Fig. 1 (Simpson, 1960). Thus the typical patient has drooping eyelids, double vision, weakness of face and jaw muscles, then of the neck and proximal muscles of the upper limbs, spreading ultimately to the muscles of respiration and swallowing — a combination dangerous to life (Fig. 2). The distribution is rarely symmetrical and the weakness varies remarkably. In one week the right eyelid may droop and in the next it may be the left. Weakness is increased by emotional stress or by exercising the appropriate muscle, and relieved by rest. Complete remission for weeks, months or years may occur. Atrophy of muscle is found in 10% of cases but is not an early feature and virtually confined to certain muscles, not within the distribution of any particular peripheral nerve. Furthermore the muscle stretch reflexes are retained and often unusually brisk. With rare exceptions sensation is not affected. Power is temporarily restored by anticholinesterase drugs.

Fig. 1. The left of the key shows the percentage of various muscle groups involved at the onset, and the right of the key the percentage involved at some time during the course of myasthenia gravis. Note the early onset and frequent involvement of extra-ocular muscles and orbiculares oculi, then bulbar, neck and shoulder girdle muscles.
Myasthenia gravis: a clinical approach

All these features lead to the conclusion that the disease causes a defect of transmission at the neuromuscular junctions which is disproportionate in certain muscles. On the other hand the function of other cholinergic synapses in the central nervous system and the peripheral autonomic nervous system is apparently normal.

How does the functional deficit occur?

From the time the disease myasthenia gravis was identified by Erb and Goldflam a century ago, the main interest has been in the mechanism of the muscular weakness. It is unnecessary to review the theories, all of which are essentially pharmacological analogues or models based on topical interests, an approach which led to bitter arguments between supporters of presynaptic, postsynaptic and 'myasthenic toxin' theories. Critical analysis leads to the conclusion that there is a disorder of all three types (Simpson, 1969) though recent developments have concentrated attention on the lesion of the receptors and on the antibody 'myasthenic toxin' so long denied since I first suggested it in 1960. The fatal flaw in all the models has been the failure to recognize that the pharmacology of the normal neuromuscular junction does not apply to one which is morphologically abnormal (Simpson, 1971). Having reiterated this warning, I leave further discussion on the mechanism of transmission failure to other speakers in this Symposium, with the observation that concentration on the proximate mechanism was responsible for failure to appreciate the true nature of the disease. The clues were already in the literature of the early 20th century. They disappeared after 1934 when it was confirmed that acetylcholine is a neuromuscular transmitter and this concentrated all attention on biochemical models of myasthenia gravis. The fallacies immediately become obvious if we proceed to the other elementary questions taught to the clinical student.

What is the pathology of the lesion?

The physician at the bedside makes a conclusion about the hidden pathology of disease by reading the 'pathological clock'. In this stage of the diagnostic logic the details of localization take second place to the natural history of the illness (how it has evolved) which is then related to the time course of the major categories of pathological processes. In addition the physician looks for the tissue specificity of the disease. Does it involve a number of organs or is it confined to one (or a few) cell lines, which would suggest a biochemical disorder?

Since the early descriptions of myasthenia gravis it has been recognized that severe illness may be followed by complete remission and then by later relapses. I have pointed out that it is unusual to have more than one or
two complete remissions and that these are more likely to occur during the first 5-10 years of the illness. Later remissions are usually less complete and the disease then enters a stage of relative stability, still responsive to anticholinesterase medication but with less danger to life but, conversely, with less potential for improvement by the operation of thymectomy. Finally there may be a stage of permanent weakness with little response to anticholinesterases (Fig. 3, Simpson, 1974). In this stage atrophy may occur, especially in the extraocular muscles, the tongue (Fig. 2) and the triceps humeri muscles. Previously termed 'myasthenic myopathy', modern histochemical studies indicate that the atrophy of stage 3 is of denervation type (Fenichel and Shy, 1963). There is increasing evidence that the pathological process may be arrested or reversed, even at stage 3, by long-term administration of corticosteroids.

Fig. 2. (a) A myasthenic patient showing her teeth. Note the vertical snarl, left ptosis and paresis of left external rectus oculi. (b) Same patient. Early stage of the triple grooved tongue.

In 1960 I pointed out that this natural history (early relapses and remissions with progressively irreversible change) has resemblances to allergic disease and connective tissue diseases (notably systemic lupus erythematosus) which were then beginning to be recognized as immunological in nature. At the same time (Simpson, 1960) I drew attention to previously unrecognized correlations with a number of disorders of other organs — 'rheumatoid' arthritis, pernicious anaemia, sarcoidosis and other
lymphoproliferative disease, and diabetes mellitus. At the same time it was pointed out that the known linkage between myasthenia gravis and thyrotoxicosis was only part of the story. Myasthenia gravis is linked (in the same individual and also in siblings) with all non-malignant diseases of the thyroid and there is no necessary temporal sequence, making it unlikely that myasthenia is caused by thyrotoxicosis as had been suggested by many earlier authors. In a Honyman-Gillespie Lecture in Edinburgh on 28 April 1960 in which I made the first complete proposal that myasthenia gravis was an autoimmune disease, I showed a section of thyroid gland as possible Hashimoto's disease. This was omitted from the published version as the pathologists were undecided, but I later published it (Simpson, 1966b) and also a confirmed case of Hashimoto's disease (and incidentally vitiligo) in myasthenia gravis after thymectomy (Simpson, 1964). Reviewing the relationship between myasthenia gravis and disorders of the thyroid gland (Simpson, 1960, 1968) it is apparent that they are genetically-linked diseases with a related immunopathology. More recently other 'autoimmune' diseases have been recognized in myasthenics such as Sjögren's disease (Downes et al., 1966; Simpson, 1966b), pemphigus vulgaris (Wolf et al., 1966; Vetters et al., 1973) and ulcerative colitis (Alarcon-Segovia et al., 1963).

It was the natural history and the related non-muscular diseases that suggested to me that myasthenia gravis was a disease of the same type as systemic lupus erythematosus which is sometimes associated with myasthenia (Harvey et al., 1954). Lymphorrhages in muscles had been described for many years in myasthenic patients but were considered non-specific by Russell (1953) because of their prevalence in other diseases. Most of those listed by Russell would now be regarded as having immunological abnormalities. It was also believed that there were no histological abnormalities at the neuromuscular junction until Coers and Desmedt (1959) demonstrated consistent morphological changes, a finding recently extended by electronmicroscopy. This review does not require a full account of these changes. It is enough to point out that (i) they are sufficient to account for loss of the safety factor of transmission and certain abnormal responses to drugs in myasthenics (Simpson, 1969, 1971) and (ii) they withdraw validity from pharmacological 'models' of myasthenia (Simpson, 1971), (iii) the changes indicate cycles of degeneration and active but abnormal regeneration of motor nerve terminals as well as of endplates of muscle. This is important as the variability of the clinical state could indicate a balance between a degenerative lesion (possibly immunological) and a restorative one.

So the tentative answer to the question 'what is the lesion?' is that the symptoms and signs can be accounted for by an active lesion on both sides of the neuromuscular junction, but also in other organs, with a natural history which suggests an immunological disease.
Why has it occurred?

The question of aetiology is the crucial one for designing therapy and providing a prognosis. At this point in the diagnostic logic the clinician should ask four questions: (i) is the disease of the presenting organ(s) secondary to abnormal function of another organ? (ii) does it occur randomly or is it age or sex linked? (iii) is the primary organ damage determined genetically or by an environmental factor – or both? (iv) is the disease transmissible to other human or animal subjects?

The primary defect. It has been recognized since the beginning of the century that 10-15% of myasthenic patients have a tumour of the thymus gland of the mixed epithelial lymphocytic type. In others the organ was considered to be ‘hypertrophic’. In fact the glands removed from myasthenic patients are rarely larger than normal and the change of size with age is within normal limits. Castleman and Norris (1949) pointed out that the significant abnormality is the unusual number of ‘germinal centres’ in the cortex and medulla of the thymus, even in those cases with a thymoma. Furthermore, the early conflict of opinion regarding the therapeutic value of thymectomy was resolved when it was shown (Simpson, 1958) that the best results obtained from removal of the non-tumour gland, and especially if this was removed during Stage 1 (Fig. 3). However, myasthenia may relapse years after thymectomy or may even occur for the first time after removal of the gland (for other indications), and there is no correlation between the therapeutic benefit from thymectomy and the number of

![Fig. 3. The three stages of myasthenia gravis. The time scale is an average one. In the active stage (1) there are major relapses and remissions, most of the mortality, but also best response to thymectomy. In the inactive stage (2) there are fewer deaths or severe relapses but also fewer remissions and less response to thymectomy. In the burned out stage (3) there is no response to thymectomy and resistance to anticholinesterase drugs but improvement may occur spontaneously or with steroids.](image-url)
germinal centres in the gland (Vetters and Simpson, 1974). These facts make it impossible to accept that the gland produces a neuromuscular blocking substance as in earlier theories and more recently advocated by Goldstein (1968, for review). Although it was then regarded as an endocrine gland, Simpson (1960) was impressed with its lymphoid structure and suggested a cellular and humoral mediated immunological function leading to structural damage of neuromuscular junctions and production of antibodies against AChR protein and other tissues. As this hypothesis preceded the paper by Miller (1961) which established the immunological role of the thymus, it is necessary to examine the reason for postulating a receptor-blocking antibody (vide infra). The immunological work of the next 10 years established that immunological reactivity could persist after removal of the thymus and this aspect will not be pursued in this paper.

Age and sex distributions: Myasthenia gravis occurs in every race. Estimates of prevalence range from 1 in 50,000 to 1 in 10,000 of the population. There is a distinct difference between the tumour and non-tumour types. If the age at onset of symptoms is charted for those without a thymoma (Fig. 4), both sexes show a modal age of 20 years with 4.5 females to 1 male in the under 30's, changing to an equal incidence or slight male preponderance in later life – a distribution found in systemic lupus erythematosus and other connective tissue disease. Patients with a thymoma, on the other hand, have a modal age of 45 years and account for 30% of cases starting over the age of 40 years. There is no significant sex difference for the latter group. Whatever ‘causes’ myasthenia gravis, young women are more susceptible, but the relative insusceptibility of later life is lost if a thymoma occurs.

Fig. 4. Age at first symptoms of myasthenia gravis (percent of total cases). The solid line indicates age of onset for females without thymoma, the dotted line the onset age for males without thymoma. The hatched area indicates the ages of onset of cases of both sexes with a thymoma.
Genetic or environmental factors? Cases of myasthenia gravis in more than one generation of a family are rare. Less rare are same generation cases starting in early childhood (Congenital Myasthenia). If these are excluded, because some workers do not accept that they have 'true' myasthenia gravis, the existence of a genetic mechanism is considered to be not definitely established (Herrmann, 1966; Jacob et al., 1969). However, I have drawn attention to the possibility of alternative gene expression (as thyroid disease or non-myasthenic autoimmune disease), a concept given limited support by the study of Bundey (1972). Recently it has become clear that there is an association between myasthenia gravis and at least one of the human histocompatibility antigens HLA-B8, though this is not an essential precursor of myasthenia gravis (for review, see Dick et al., 1975). Eddleston and Williams (1974) suggest that the HLA-B8 antigen may be a marker of defective suppressor T-cell function. Genetic factors probably constitute risk factors for autoimmune diseases but the relative scarcity of familial cases indicates that the causation is multifactorial. We have no evidence for the nature of manifesting factors, but this is now the most important aspect of research.

Is the disease transmissible? The possibility of a 'curare-like substance' in the blood stream has been considered for a century and, in the last half of that era, that it could be formed in the thymus. There has never been acceptable evidence of transfer of the disease from one adult human to another by plasma or serum, and Nastuk et al. (1959) discredited all claims to cause block of neuromuscular transmission in vitro. Nevertheless there is one important fact which must be accounted for in any theory of myasthenia gravis. A myasthenic mother commonly (1 in 7 live births) has a baby which is myasthenic for the first few weeks of life and then recovers. The duration of this effect and the apparent restriction of passive transfer to her own child suggested to me that the neuromuscular block could be due to an antibody raised by the mother against her own ACh receptors and active only against those of her genetically similar offspring (Simpson, 1960). It could not be the 'thymin' postulated by Goldstein as this neonatal type of myasthenia still occurs after thymectomy. For many years it was impossible to test the hypothesis because the postulated antibody could not be detected and isolated, or else the test preparations were too insensitive to detect the presence of blocking substances in the serum of myasthenic patients. Using the more sensitive indicators now available (reduction in amplitude of miniature endplate potentials, or reduction in number of acetylcholine receptors at neuromuscular junctions), it is now claimed that serum IgG from myasthenic patients induces characteristic changes in the muscle end-plates of mice when passively transferred (Toyka et al., 1975, 1977). Though we have not been able to reproduce this work (Rees et al., 1977) it looks very convincing. It is exactly the mechanism I postulated in 1960. Is it immunological?
The immunological status of myasthenia gravis

It would need another chapter to do justice to this aspect. But for the present purposes some short conclusions will be sufficient to concentrate attention on the essentials which remain for the research of the '80s. (i) It is now fully accepted that there is clinical overlap between myasthenia gravis and many diseases now recognized as ‘autoimmune’ (Simpson 1960, 1964, 1977). (ii) For 16 years we have known that myasthenic patients produce antibodies against skeletal muscle, thyroid, rheumatoid factor, anti-nuclear factor and other tissues (Strauss et al., 1960; van der Geld et al., 1963). Only recently, with new techniques described elsewhere in this book (Chapter 9), is it confirmed that the blood of myasthenics has a high titre of anti-AChR globulin (Almon et al., 1974; Lindstrom et al., 1976). (iii) There is some evidence of cell-mediated immunity against muscle and thymus (Behan et al., 1975). (iv) An experimental model of myasthenia gravis can be produced in a wide range of mammals immunized with AChR protein from the eel electroplax (Patrick and Lindstrom, 1973; Engel et al., 1976). These are, of course, exciting events. Placed in a long term perspective they show (a) that the proposed immunological lesion of neuromuscular junctions is reasonable and likely, and (b) that it is part of a breakdown of immunological tolerance involving a number of tissues. Some alterations in plasma proteins (Simpson, 1966c) and particularly a low level of serum IgA (which is T-cell dependent) in some myasthenics (Simpson et al., 1976; Behan et al., 1976) point to an immunodeficiency type of autoimmune disease with defective T-cell function. This could be genetic or secondary to neoplasia or other disorder of the thymus.

Conclusions

It has not been possible in the available space to give a full discussion of many important aspects. For that reason the bibliography of this chapter refers to personal and other papers with fuller discussion of controversial points, or to the first description of significant findings. At the time of this review I feel reasonably certain that we have the answers to Where, What, and How? The ‘Why’ question remains the important one. What is the link between genes, thymus and autoimmunity? Is there a trigger that fires a pre-loaded gun? Is it a virus, hormonal, even psychosomatic? These are now the important questions in myasthenic research. For decades it was side tracked into arguments about neuromuscular transmission and then about antibodies which are epiphenomena. Now that we can mimic the end stage of the immunological lesion it would be tragic if we are again side tracked from the only questions that will lead to prevention or cure of myasthenia gravis.
References
Myasthenia gravis: a clinical approach


MYASTHENIA GRAVIS: A PERSONAL VIEW OF PATHOGENESIS AND MECHANISM, PART 1

JOHN A. SIMPSON, MD

Myasthenia gravis (MG) has been defined by Viets as a specific muscular disease characterized by the development of an abnormal amount of muscular weakness in voluntary muscles following repetitive activation or prolonged tension, with a marked tendency to recovery of motor power after a period of inactivity or lessened muscular tension. Viets did not require a response to anticholinesterase drugs as part of a primary definition of MG, despite his long experience, since 1913, with Robert Schwab using the neostigmine test in the treatment of this disease.

MG is readily recognized when well established, but it is often missed in the most frequent patient—a woman in her early 20s—because the symptoms seem too severe for the patient’s apparent well-being at the time of medical consultation and when the history of emotional provocation is obtained. Indeed, some patients are diagnosed as suffering from hysteria or from multiple sclerosis for many years before the true diagnosis is reached. Factors that appear to bring on the first attack or relapses of MG, in addition to emotional upset, are infection, allergy, and pregnancy. Once the disease is established, weakness is greater just before menstruation, in exciting or embarrassing situations, or with extremes of temperature. Once the possibility of myasthenia gravis is considered, the diagnosis is simple and can be easily confirmed by demonstrating abnormal fatigability of muscle using simple bedside tests or an ergograph such as Schwab’s bulb ergograph, and by restoring muscle strength to normal with the use of an anticholinesterase drug such as edrophonium or neostigmine. Electromyography is used to demonstrate failure of neuromuscular transmission.

The name given to the disease (for I consider it to be a clinical entity and not a symptom complex of multiple causation) is derived from the characteristic weakness of voluntary muscles which occurs after exercise and which may disappear after a short rest. The most common, and usually the earliest, muscles involved are those of the eyelids (including orbicularis oculi), the extraocular muscles, the bulbar muscles, the neck muscles, and the proximal muscles of the limbs. The hand, lower limb, and trunk muscles are usually involved later, though in some cases they are the first to be affected.

Muscular atrophy is more common than earlier descriptions would suggest. In my experience, permanent weakness with moderate wasting occurs even-
NATURAL HISTORY

MG occurs in every race. Estimates of prevalence range from 1 in 10,000 to 1 in 50,000. Women are affected twice as frequently as men, but the female: male ratio changes from 4:3:1 in MG beginning before the age of 30 years, to an equal incidence or slight preponderance of males when the disease begins in later life. 

The most common age of onset for both sexes is about 20 years. Approximately 10% of patients have a thymoma; the modal age for such patients is 13 years. This tumor is rare in cases with onset before 30 years of age but is present in 30% of cases beginning after the age of 10.

The course of the illness can be divided into three stages. In stage 1 (“active stage”), the severity of clinical symptoms increases but with a course characterized by relapses and remissions. Fever than half the cases experience a remission of a month or more, and long remissions rarely occur more than once. This stage lasts 5 to 10 years; it is the period (particularly the first year) during which most deaths, directly attributable to MG occur. This is the stage at which thymectomy is usually beneficial, though the improvement may be delayed.

In stage 2 (“inactive stage”), the intensity of the myasthenic symptoms fluctuates but genuine remission is rare. Only a small number of patients benefit from thymectomy, although the transmission disorder may still be partially corrected by anticholinesterase drugs. In this stage, death may occur from apneumonization, but the mortality rate is much lower than in stage 1.

Stage 3 (“burned-out stage”) occurs 14 to 20 years from the onset of symptoms. The severity of the disease either does not vary or tends to decrease. Thymectomy is of no value, deaths are restricted to respiratory accidents, and the response to anticholinergic drugs tends to disappear. There is a permanent weakness, often without the myasthenic characteristics defined by Nits and with wasting of some muscles. Although previously named “myasthenic myopathy” because of clinical and EMG resemblance to polymyositis, this atrophy has recently been found to be caused by denervation.

It is my belief that in the active stage (stage 1) immunological damage of the neuromuscular junction results from a breakdown of immunological tolerance. The thymus plays a significant role in this process. Antibody blockade and structural changes at neuromuscular junctions cause loss of the normal safety factor for transmission. This can be compensated for by anticholinesterase drugs during a prolonged “inactive stage,” but it is not reversible by thymectomy, though it may be benefited by corticosteroids. In stage 3, the patient has become adapted to his disorder, the remaining endplate damage may not be capable of compensation, and denervation atrophy of muscle may occur.

To me, this classification by stages is more valuable than a classification by severity or distribution of symptoms such as that of Oslerman, except that it is generally agreed that if subjective symptoms are limited to the extracerebral muscles and remain so for two years, the prognosis is good. Additionally, if a thymic tumor is present, the myasthenia is often difficult to control either by drugs or by thymectomy.

DISORDERS OF OTHER ORGANS

It has been recognized for many years that there is a relationship between MG and disorders of the thyroid gland. In my experience, about 9% of males and 18% of females with MG show signs of a thyroid disorder at some time during their life; however, there is no regular temporal relationship (“see saw” or other) between these disorders. The disorder need not be thyrotoxicosis; in fact, nontoxic goiter and, more rarely, primary myxedema may be found. Indeed, most of the early reports refer to “lymphadenoid goiter.” It is now recognized that many myasthenia patients have Hashimoto’s thyroiditis.

It was clearly shown by Engel that myasthenia could not be caused by hyperthyroidism, as has been suggested. Only a minority of myasthenics have abnormal thyroid function tests, and these patients are as commonly hypothyroid as hyperthyroid. I have shown that the link between MG and thyroid disorders is probably genetic.

The incidence of diabetes mellitus may also be slightly higher than normal in myasthenics and their relatives: an arthropathy resembling rheumatoid arthritis or anklyosing spondylitis is not uncommon. The arthropathy is often transient and seronegative, and it may precede or follow the myasthenic illness. A familial linkage has also been shown between rheumatoid arthritis, MG, and thyroid disease.

I have noted cases of rheumatoid arthritis and also of pernicious anemia in relatives of myasthenics. Per-
nicious anemia is a much more common blood dyscrasia in myasthenics than the better-known aplastic anemia associated with thymic tumors. Epilepsy is not uncommon, neither is acrocyanosis, sarcoidosis, Sjogren disease, pemphigus vulgaris, or ulcerative colitis. There is increasing evidence that the factor common to these disorders is a disturbance of immunology. In drawing attention to these conditions and their possible immunological link, I have proposed an analogy with systemic lupus erythematosus, a disease that also occurs in association with myasthenia.

GENETIC ASPECTS
The possibility of a genetic link for disorders has been mentioned. Familial MG is rare but well recognized: yet only one of a pair of identical twins may be affected. Neonatal myasthenia differs in that the affected parent is always the mother and the disorder persists for only one to eight weeks, with a mean duration of 18 days. It occurs in one in seven live children born to myasthenic mothers, even if birth takes place after the mother has had a thymectomy. This linkage is apparently caused by passage through the placenta of a substance that may be an antibody.

A genetic study by Jacob et al (1968) showed no secondary cases of MG in 448 relatives of 70 myasthenic patients, and no association was revealed between MG and the ABO or Rhesus blood groups, secretor status, or the ability to taste PTC. Herrmann (1971) was unable to establish a definite genetic mechanism in his study of familial myasthenia. Bundey (1972) recognized an early-onset form of childhood myasthenia, with autosomal recessive inheritance of the trait; her study gives limited support to the concept of alternative gene expression that I proposed in 1960.

In recent years there have been many reports of striking alterations in the frequency of at least one of the human histocompatibility antigens (HL-A) in patients with MG. We find a frequent association of the HL-A8 antigen and MG, but it is clear that the relationship is not a direct one (there are HL-A8-negative cases). This association is interesting because the HL-A8 antigen may be the marker of a defective suppressor T-cell function. However, our familial studies do demonstrate unequivocally that inheritance of HL-A8, or indeed of a particular HL-A phenotype, is not an essential precursor to the development of MG.

Another histocompatibility antigen system, such as the LD antigens, detectable only by mixed leukocyte reaction (MLR), may exhibit a stronger association with MG and with autoimmune disorders. Genetic factors probably constitute risk factors for autoimmune diseases, but the relative scarcity of familial cases indicates that causation is multifactorial. We have no evidence to explain the nature of the factors that lead to manifestation of the disease in a susceptible individual.

AUTOIMMUNE HYPOTHESIS
In 1960 I proposed a genetically determined autoimmune basis for MG founded on clinical and pathological evidence. I have already noted that the incidence of the disease by age and sex, its natural history, and the evidence for multisystem or linked disease are strikingly similar to these same parameters in systemic lupus erythematosus, as it was described by Harvey et al. There is an apparent disparity between the occurrence of neonatal MG and the failure to transmit the defect to another adult by cross-transfusion. This disparity could be resolved if the toxic substance were an antibody against some tissue—muscle or nerve—with common antigens in mother and child. The duration of neonatal myasthenia would fit this autoimmune concept admirably.

Other factors leading to the hypothesis were the pathological changes in the thymus and the skeletal muscle in this disease. The thymus gland is usually abnormal, but descriptions such as "thymic hypertrophy" or "thymic atrophy" are unsatisfactory. The glands removed from myasthenic patients are rarely larger than normal, and the change of size with age is within normal limits. The characteristic abnormality is the presence of "germinal centers" in the cortex and medulla. The epithelial cells do not proliferate unless there is a tumor. Even if there is a thymoma, the surrounding lymphoid tissue commonly shows the germinal centers typical of the disease.

By 1966 the thymus had generally been accepted as an endocrine gland, but it seemed to me to have the appearance of an active lymph organ associated with immunological reactions. For the first time, this provided a rationale for thymectomy as a form of treatment. Follow-up studies had indicated that the best results occurred when thymectomy was performed as soon as possible after the onset of myasthenic symptoms. Thus it was strange that Castelman and Norris and others found no relationship between operative response and the histological appearances of the thymus and, in particular, that Alpert et al and Perlo et al found that a favorable response was more likely with the removal of those glands with fewer germinal centers. The report of Mackay et al that favorable response correlated with the presence of numerous germinal centers seemed more likely to be correct. I was therefore surprised to find that the
Glasgow cases supported Alpert and his colleagues. Whatever this means, it is evidence against the importance of “thynitis,” which has been claimed by other workers.

The muscles commonly but not invariably show the lymphocytic infiltration described by Weigert and named lymphorrhages by Buzzard. Although labeled by many pathologists as “nonspecific,” these lymphorrhages suggest an immunological reaction in the muscles. Many other diseases previously associated with lymphorrhages, which caused Russell to regard them as nonspecific, are now recognized to be associated with immunological reactivity.

Nastuk et al. reported in 1959 that, in common with most earlier experimenters, they had not succeeded in demonstrating a curarelike substance in myasthenic serum. In the course of their experiments, however, they had noticed that the serum caused lysis of frog muscle cells, a reaction later shown to be associated with fixation of complement.  

In 1960 Strauss et al. published additional studies in which they used a fluorescence technique to demonstrate binding of a myasthenic serum globulin to skeletal muscle. At this same time (1956–1960), my colleagues and I had been searching—unsuccessfully—for such an antibody. It is now clear that we were unfortunate in not using serum from patients with a thymoma, who, it is now known, develop higher titers of antibody. Our incidence of positive binding results still remains lower than in most centers because we differentiate A-band from I-band fluorescence. We believe that A-band fluorescence is virtually confined to cases with a thymoma, whereas I-band fluorescence is not restricted to MG.

Aarli confirmed that the γ-G globulin fixing to muscle from some myasthenic serum was indeed reacting immunologically, whereas the binding sometimes found with normal sera was nonspecific. We were able to show, in common with other workers, that many myasthenic patients have abnormal serum antibodies against thyroid and gastric parietal cells, and an antinuclear factor is commonly present in their serum. I have agreed with Strauss et al. and others that the antimuscle antibodies demonstrated by so many workers were epiphenomena in the sense that they cannot account for the disorder of transmission. It is clear, however, that these multiple antibodies are evidence of disordered immunological function. The techniques available in 1968 were inadequate to demonstrate antibody bound to acetylcholine receptors (AChRs), though such binding was predicted on the basis of the hypothesis. Only in the last three years have new and more sensitive methods made demonstration of antibody binding to AChR possible.

**IgA Levels in MG.** My study showed that plasma proteins (estimated electrophoretically) were normal except when myasthenia was associated with Hashimoto’s disease, thymoma, or one of the other complications linked with humoral antibodies. When methods for fractionating immunoglobulins became available, we found some cases with low levels of IgA, a globulin that is T-cell dependent. These were mostly cases of congenital myasthenia, as also reported by Bundey et al. Lisak and Zweiman (1975) found no abnormalities of IgA, but their series did not include congenital myasthenia patients. Bramis et al. (1976) have confirmed that serum IgA concentration may be subnormal in myasthenic patients, and in their series of 107 patients the lowest concentrations were associated with thymoma. Low concentrations were also found in myasthenic patients without thymomas who had associated extrathymic neoplasms. Decreased IgA concentration tended to be associated with many prominent germinal centers in the thymus, and serum IgA levels tended to increase slowly after thymectomy.

**Cellular Immunity and MG.** During the period 1965 to 1970, interest passed to cell-mediated mechanisms of immunity. Alpert et al. and Kott et al. obtained positive inhibitory responses to crude skeletal muscle and myosin-containing fractions of leukocytes from two-thirds of their myasthenic patients. However, we have criticized the controls in these papers and in that of Goust et al. A nonspecific inhibition of macrophage migration may occur because of differing histocompatibility antigens. Armstrong et al. using thymic lymphocytes, found cellular hypersensitivity to crude muscle and crude thymus antigens in myasthenic patients, but they also reported similar sensitivity to muscle in nearly 50% of their control subjects. Using different in-vitro methods, other workers have shown abnormal lymphocyte proliferation or transformation responses to crude muscle antigen in MG patients.

We have reviewed the published work in this field and have concluded that the cellular reactions demonstrated are, like the humoral antibody reactions, not pathogenic. We have, however, found a subtle but significant impairment of the ability of T-cells from myasthenics to synthesize new protein in response to stimulation by phytohemagglutinin. The hypothesis of Fudenberg may provide a solution to the apparent dilemma of a disorder of immunological tolerance without evidence of cytopathic antibodies or cells. In 1968 he proposed that autoimmune diseases are manifestations of either generalized or selective immunological deficiencies. This concept is supported by a clinical correlation between MG and some anergic diseases.
Although there are still many details requiring clarification, I submit that there is no doubt that MG is a disease of disordered immunity, and the evidence is growing that the neuromuscular junction is immunologically damaged. Thus, it is now commonplace to read about "the autoimmune theory of myasthenia gravis." Yet it should be remembered that there are a number of theories of this type for MG, and arguments against one should not be viewed as contradicting all.

**COMPARISON OF AUTOIMMUNE THEORIES**

In 1960, the conclusion was drawn independently by three groups that an immunological disorder was associated with MG. A fourth "theory" was added in 1966, but they all differ considerably in their heuristic value.91

1. **Smithers.** In 1959, Smithers94 emphasized a comment by Castelmon (1955)28 that the germinal centers of the myasthenic thymus resembled the thyroid changes in Hashimoto's disease. Smithers suggested that "there is a possibility that this disease may also be due to an autoimmune response associated at times with neoplasia." The suggestion was that the thymus was immunologically damaged, and Smithers did not offer any explanation for the neuromuscular disorder. From this presentation, it was impossible to account for the occurrence of associated disorders of other organs; for neonatal myasthenia; or for the beneficial effects of thymectomy.

2. **Strauss et al.** Independently, an American group developed an immunological theory of MG. Searching for a neuromuscular blocking substance, Nastuk et al. (1959) found that blood from myasthenic patients, and to a lesser degree from a few control subjects, caused lysis of frog muscle cells.25 Following up this surprising observation, they found that the serum complement activity was within the normal range in most myasthenic patients but was outside the normal range in a few cases. The increased or decreased complement levels tended to correlate with remissions or exacerbations, respectively.62

Nastuk et al. suggested that an immune mechanism might play an etiological role in MG.62 The mechanism they visualized was the development of autoimmunity against one of the components (M) of the skeletal muscle fibers, and they stated that "the terminal arbor of the motor nerve might be indirectly or directly involved." They drew attention to evidence that the thymus gland was capable of manufacturing antibodies. This group soon published evidence demonstrating the presence of a muscle-binding, complement-fixing globulin fraction in the serum of patients with MG.63 and the subsequent studies of a similar nature have already been described.

These were extremely important findings, compatible with an immunological abnormality, but Strauss and his colleagues realized the difficulty of accounting for a transmission defect with a lesion involving myofibers rather than endplates. Because of this apparent inconsistency, clinical workers refused to accept the findings as evidence of an immunological cause of MG, especially since not all myasthenics demonstrated such antibodies. In addition, there may be no myasthenia present despite documented antiskeletal muscle and antithymus reactivity in the serum of some thymoma patients.86

The New York group could not at first demonstrate a role for the thymus, but when it was shown to have, in its myoid cells, an antigen in common with skeletal muscle,89 Strauss and his colleagues considered that it might elaborate a neuromuscular blocking substance "in the course of its derangement."90 This was an unrecognized return to the old concept of a circulating "curarelike substance" released from the thymus, although ironically it was the firm refutation of this concept by Nastuk et al. (1959)86 that had led initially to their immunological studies. They abandoned the concept of cytolysic antibodies and concluded that antithymocytes were merely additional evidence of disordered thymic function. They speculated further that such antibodies might inactivate a supposed "neurohumoral inhibitor" which could be, at the same time, antigenic.

3. **Goldstein.** The theories of Goldstein and his collaborators derive from those of Smithers and Strauss. Goldstein's hypothesis maintains that an autoimmune reaction in the thymic medulla results in "thymitis," which then causes the release of a humoral substance that produces the characteristic neuromuscular block.67 This humoral substance was later named thymus and characterized as a polypeptide.88

This thymitis theory originates from experimental observations, and its validity depends on the reliability of the experiments, which will be discussed in the next section. It should be emphasized that it is the same type of theory as that of Strauss. Inescapable conclusions from the Strauss and Goldstein hypotheses are that thymectomy: (1) should cure MG, (2) could negate the possibility of having a child with neonatal myasthenia, and (3) could prevent the occurrence of myasthenia when it has not already been present. None of these logical extensions is true; nor is it necessary to document that they are false. The documented onset of MG many years after apparently complete thymectomy87 makes it untenable that this
4. Simpson. This hypothesis was elaborated to account for the following clinical and pathological facts:

a. As recognized initially in 1960, MG is associated with a number of diseases, which were then or have subsequently been shown to be immunological in nature.

b. The incidence of MG by age and sex, and its natural history, were strikingly similar to those of systemic lupus erythematosus.

c. The thymus of myasthenic patients characteristically showed “germinal centers.” Although in 1960 the immunological role of the thymus was not yet accepted, it seemed to me that it was part of the reticuloendothelial system and associated with immunological reactions.

d. Lymphorrhages in muscles, though considered by contemporary pathologists to be “nonspecific,” were very suggestive of an immunological reaction.

e. Passage of an antibody across the placenta could account for neonatal myasthenia. Later writers have reported this observation without noting that the significant points are the duration of the neonatal myasthenia and the inability to achieve passive transfer of myasthenia between adult humans.

f. Cortisone was known to cause remission of myasthenia after a temporary deterioration.

g. Nastulc et al reported cytolysis of frog muscle cells by human myasthenic serum. It must be remembered that it was not until 1961 that Miller published the work that convinced most people of the immunological role of the thymus. In 1960 it was still regarded as an endocrine gland. Therefore, although it seemed justifiable to publish the hypothesis, because it was so novel it had to be shown to be compatible with known facts. It was for this reason that I postulated that thymus-derived cells produced antibody against endplate receptors, and ultimately structural changes of the neuromuscular junction (described by Coërs & Woolf). It should also be noted that the immunological factor was postulated as one of a chain of genetically controlled events.

EXPERIMENTAL MYASTHENIA

Once an immunological hypothesis for MG had been formulated, it was natural to attempt to produce experimental myasthenia by immunological means. In 1953, John R. Anderson and I inoculated mice with myasthenic muscle tissue and Freund’s adjuvant but without success. Our hypothesis was that the thymus would produce antibodies or immunologically active cells in response to this antigen that would attack the animal’s own muscle. In retrospect, the failure of the experiment was inevitable since little of the antigen would be derived from endplates.

Marshall and White (1961) showed that injection of an antigen into a guinea pig thymus caused histological reactions resembling the thymus pathology of MG which were not present after immunization by other routes. They suggested that there is a blood-thymus barrier that may be deficient in MG. Alternatively, they suggested that a segregated (free) antigen may have arisen within the gland, or that the cellular reactions of the germinal centers may have arisen spontaneously as a “forbidden clone.”

Goldstein and Whittingham (1966) inoculated young guinea pigs with antigens prepared from fresh calf thymus, muscle, or lymph node along with Freund’s complete adjuvant, and reportedly produced a myasthenic EMG response to nerve stimulation in all the experimental animals except those subjected to prior thymectomy. There was no “clinical” myasthenia, and rapid nerve stimulation was necessary. Goldstein and a series of collaborators have repeatedly reported similar findings in a number of animal species. Goldstein’s hypothesis proposes that an autoimmune “thymitis” in the thymic medulla causes the release of a humoral substance which produces the myasthenic neuromuscular block. He later claimed to have isolated a substance from normal bovine thymus that produced myositis and myasthenic neuromuscular block in guinea pigs.

The basic findings of Goldstein’s papers were supported by Kalden et al and by Kawanami, who later reported passive lymph-node-cell transfer of the waning phenomenon on evoked EMG. Other workers, including my own group, have not been able to reproduce Goldstein’s findings, and some attribute his observations to artifact. The uncertainty of these results—combined with the absence of evidence, using fluorescence techniques, of an antibody acting against endplates rather than against other parts of the muscle fiber—produced a tantalizing gap in the theory.
The immunological hypothesis was further advanced by the work of Patrick and Lindstrom (1973). They were the first to observe that repeated immunization of rabbits with nicotinic AChR, purified from the electric organ of Electrophorus electricus, produced muscular weakness and a decrementing electromyogram response to repeated nerve stimulation, which was reversible by anticholinesterase. Cross-immunoelectrophoresis, the lack of catalytic activity, and the antigen's amino acid composition and binding properties have shown that it is not acetylcholinesterase. There seems no doubt that the substance isolated is from the endplate receptor tissue though not necessarily the receptor substance itself. Nevertheless, it is antigenic and can raise antibodies which also act against the host's endplate receptors.

The electric organs of E. electricus, Torpedo marmorata, and T. californica also possess antigens in common with the endplate receptors of many avian and mammalian species, including man, and also with calf thymus. Serum from rabbits immunized with these antigens has been shown to contain antibody to purified AChR, and this antibody is capable of blocking the depolarizing response of the electric plaque to carbamylcholine. These findings have been easily confirmed and extended to other species.

Some investigators have successfully produced experimental MG in animals that are close clinical models of the disease. The experimental myasthenia produced in monkeys by Tarrab-Hazdai et al is a strikingly good model of human MG that includes ptosis reversible with edrophonium. Engel et al have shown that the morphologic and electrophysiologic changes at the neuromuscular junctions in experimental autoimmune MG in the rat closely resemble those of human MG in the chronic phase, though there are differences in the acute phase of the experimental disease.

The demonstration that experimental autoimmune myasthenia can be transferred passively by lymphoid cells suggests that the disease involves T-cell reaction.

**Measurement of Immune Factors.** The principle used to isolate and separate AChR is its reaction with one of the specific binding toxins from Naja naja siamensis or from Bungarus multicinctus (a-bungarotoxin). It is also possible to measure the antibody titer to AChR by incubating serum with isotope-labeled AChR linked to toxin. The antibody-AChR-toxin complexes are then precipitated along with carrier immunoglobulin by the addition of anti-immunoglobulin, and the radioactivity of the resulting pellet is measured.

Using this technique, Lindstrom et al have been able to measure antibody against human muscle AChR. They have also found that rat thymus contains a small amount of AChR (about 0.5% of the concentration in rat muscle). When the same radioimmunoassay (RIA) was used with human sera, they found significant titers of anti-AChR antibody in 82 of 84 sera from 50 patients with active MG, whereas none of the 82 sera from persons without MG had anti-AChR antibody. Later the same authors found significant titers in 82% of 71 patients with MG. No anti-AChR antibodies were detected in any other neuromuscular diseases tested, including patients with the Eaton-Lambert syndrome. The antibody was found in a myasthenic mother and in both of her twin children, who were suffering from neonatal myasthenia.

Titters of antibody correlated well with clinical severity, including response to treatment with corticosteroids, and with spontaneous remission, and titers were increased in MG patients with thymoma. The RIA method is claimed to detect a 300-fold difference in antibody titer from sera of most MG patients compared with normals, and thus is of diagnostic value. The diagnostic yield obtained was much higher with this method than with the test for inhibition of toxin binding described below.

The authors caution against using AChR from other species to assay for anti-AChR antibody from humans, as they were unable to detect cross-reaction between antihuman muscle AChR and eel AChR. Lindstrom's radioimmunoassay measures only those antibodies directed at determinants other than the ACh binding site. There is some evidence that the antibody will block receptor response to acetylcholine, but the slow time course of blockage in experimental autoimmune myasthenia suggests that immune responses to AChR result in morphologic alterations of the nerve-muscle junction; these alterations have been demonstrated. Thus, Lindstrom's work supports the concept of immunological damage of muscle endplate with morphologic change of the latter (as the Simpson hypothesis suggested) in addition to providing evidence for blocking antibodies to receptors.

A sensitive assay for those serum factors inhibiting the binding of isotope-labeled a-bungarotoxin to the AChR has been described by Almon, Appel, and colleagues. The basis of the test is the measurement of the serum's ability to block the uptake of labeled a-bungarotoxin by AChR extracted from denervated rat muscle. The serum of at least 5 and possibly 11 out of 15 patients with MG showed inhibitory activity that was localized to the globulin fraction. Serum globulins from normal individuals and from a patient with polymyositis had no inhibitory effect. The serum factor
Role of Immune Factors in MG. Using α-bungarotoxin as a specific molecular probe for the nicotinic AChR molecule, Fambrough and colleagues have found reduced endplate binding—averaging 80% below normal—in muscle from MG patients. This is unlike denervated muscle in which there is a spread of AChR to the entire sarcolemmal membrane. These researchers have now confirmed that, by blocking the AChRs of rat muscle by the intravenous administration of the α-toxin of the Formosan cobra (Naja atra), it is possible to produce electrophysiologic and pharmacologic changes typical of MG.

The studies of Fambrough and colleagues support the proposal that either antibody blockade or a reduction of available AChRs would account for the characteristics of MG, including the observation that miniature end-plate potentials may be reduced in amplitude (see part 2). As Satyanarayanan et al point out, however, it is not possible from these studies to say whether the receptor protein itself is defective, the receptor packing in the postsynaptic membrane is abnormal, or the receptor is damaged or blocked, perhaps by autoimmune mechanisms.

Bender and his colleagues have shown that serum from MG contains a factor that blocks α-bungarotoxin binding to AChR of human muscle receptors as detected by immunoperoxidase staining of α-bungarotoxin. This technique, which complements that of Alm et al., demonstrates that toxin binding is inhibited at neuromuscular junctions. Extrajunctional sites were found only in denervated muscle. The staining technique shows that AChRs are located mainly at the postsynaptic membrane folds (and only at the upper portions of these folds); also, a slight amount of staining of the presynaptic membrane is always seen. In one patient the blocking factor was localized to the IgG fraction.

Engel et al have devised a technique that demonstrates localization of IgG and C3 at motor endplates in MG by reacting biopsied muscle with staphylococcal protein A (which binds to the Fc region of human IgG subclasses 1, 2, and 1) and rabbit antihuman C3 conjugated with peroxidase. The immune complexes were detected both at the sites of AChR and on degenerate material in the synaptic space. Staining of presynaptic sites was considered to be secondary to the diffusion of reaction product from the postsynaptic membrane. More immune complexes were detected at the endplate in the less severe than in the more severe cases of MG, the criterion for severity being morphometric analysis of electron micrographs of the endplates.

Engel and his colleagues interpret these findings as indicating that the myasthenic weakness is caused by AChR destruction by the autoimmune reaction. This proposal assumes that IgG binding to AChR has resulted in completion to C3 of the activation phase of the complement reaction sequence, and that subsequent sequential activation of C5 to C9 would complete the attack phase and set the stage for lytic destruction of the postsynaptic membrane. They consider that immunopharmacological blockade and IgG-induced modulation of AChR may also contribute to the deficiency of AChR at the myasthenic endplate. Passive transfer of myasthenia immune complexes would help to differentiate among these possibilities.

Tovka et al have reported the successful passive transfer of myasthenia from man to mouse using alan immunoglobulin fraction of serum from myasthenic patients. The mice showed reduced amplitude of miniature endplate potentials and reduced numbers of AChRs at the neuromuscular junctions. Some showed a decreasing response on repetitive nerve stimulation at 3/sec, with reversal by neostigmine. Only one of the animals was clinically weak in the original report, but in a later study 12 mice injected with crude immunoglobulin from five different MG patients showed marked clinical weakness beginning two to seven days after the first dose. Although we have not succeeded in repeating their results, the Baltimore work is very convincing; it is surprising, however, that no mention was made of the responsiveness of the weak mice to edrophonium or neostigmine. The active fraction was identified as an IgG which was enhanced by C3 but not by C5, and hence unlikely to be a cytolytic effect.

The latent period of the clinical effect may be considered unfavorable to the concept of immunopharmacological block, but these animals were not tested by refined electrophysiologic techniques.

Whatever the blocking mechanism and the resultant cellular reaction associated with these immune complexes at AChR sites is, the evidence supports the Simpson hypothesis rather than the Goldstein concept of a polypeptide thymus released by thymitis. However, Aharanov et al maintain that there is cross-
reactivity at cellular and humoral levels between the AChR (from *E. electricus*) and calf thymus fractions. They agree with Goldstein in viewing the thymus as a target organ for autoimmune disease. They recognize that this poses a problem in accounting for the therapeutic effect of thymectomy unless this effect results from the general immunosuppression caused by that treatment.

The thymectomy question was further explored by Lennon et al., who found that in rats with experimental autoimmune myasthenia gravis (EAMG), thymectomy, carried out at the stage associated with a 75 globulin antibody to both eel and muscle AChR, had no significant effect on the myasthenia. At that time, however, they were able to transfer AChR-immune cells to normal recipients using lymph nodes of donor rats. It may already have been too late for thymectomy to arrest the disease, and any resultant immunosuppression was of no clinical importance. On the contrary, normal rats depleted of T-cells by thymectomy and X-irradiation before challenge with AChR/adjuvant failed to develop EAMG. Susceptibility to EAMG was not restored by transfusing thymus cells, but these animals did develop anti-AChR antibody at a titer that was not significantly different from the usual response. Transfusion of B-cells alone was much less effective in restoring immunological responsiveness. These experiments strongly suggest that the immunological damage to the rat's endplate receptors depends on T-cells which rapidly populate lymph nodes, that these cells can transfer EAMG to normal rats, and that the thymus does not otherwise produce a receptor-active substance.

Namba et al have shown that the circulating lymphocytes and lymphoid cells of the lymph nodes and spleen of myasthenic patients produce stronger graft-versus-host reactions in mice than do similar cells from normal subjects. However, the lymphoid cells of the thymus of myasthenic patients with lymphoid hyperplasia do not exhibit such activity. When lymphocytes from both myasthenic patients and normal subjects were injected intramuscularly in rats, they produced local infiltration of polymorphonuclear and lymphoid cells, including lymphorrhages, and later fibroblasts and eosinophils. The motor endplates of the host animal showed a biphasic increase in the mean diameter accompanied by changes in fine structure and innervation of motor terminals. Terminal branching was the same regardless of whether the human lymphocyte donors had MG, and the changes probably represent a normal lymphocyte transfer reaction. Nevertheless, the increased diameter of endplates, though transient, was confined to rats injected with lymphocytes from myasthenic donors.

**SUMMARY**

These experimental studies may be summarized as follows. Serum antibodies and circulating T-cells raised against an acetylcholine receptor substance produce a good experimental model of myasthenia gravis. New methods of assay which test the affinity of endplate receptors for toxins believed to react specifically, indicate the presence of complement-fixing humoral neuromuscular blocking globulin in myasthenic subjects and not in normal controls. There is some evidence that lymphocytes may attack motor endplates. If the lymphocytes are from myasthenic donors or from electropole-immunized animals, the damage to endplates reflects this difference in source; in the latter case, they passively transfer a myasthenia-like disease.

It is premature to describe these experiments as definitive, but I submit that they provide considerable support for the autoimmune theory of myasthenia and, in particular, for the first part of my hypothesis. There is now no doubt that the neuromuscular junction can be modified structurally and physiologically by immunological mechanisms that are probably cell-mediated, and the evidence for antibody blockade of AChR in the human disease is very strong. The hypothesis that this is caused by a genetically determined breakdown of immunological tolerance remains, after 17 years, compatible with all the evidence.

End of part 1; part 2 will appear in the March/April 1978 issue of *MUSCLE & NERVE.*

**REFERENCES**

6. Abranov-Segovia D, Galbraith RF, Maldomando JE, Howard EM: Systemic lupus erythematosus following thymectomy for...
Myasthenia Gravis

MUSCLE & NERVE

Jan-Feb 1978 55


A review of our current knowledge of the etiology and pathogenesis of myasthenia gravis is presented, with particular emphasis on the immunological aspects of the disease. Part 1, published in the January/February issue of MUSCLE & NERVE, dealt with the clinical and genetic features of myasthenia gravis which led to the autoimmune theory of the etiology of the disease. Part 2, which appears in this issue, provides a review of the dysfunction of physiology, pharmacology, and structure of the neuromuscular junction in myasthenia gravis, and the part played by the autoimmune process.

MUSCLE & NERVE 1:151-156 1978

MYASTHENIA GRAVIS: A PERSONAL VIEW OF PATHOGENESIS AND MECHANISM, PART 2

JOHN A. SIMPSON, MD

Turning now to the essential feature of myasthenia gravis (MG)—the muscular weakness that increases with prolonged or repeated muscular contraction and that is alleviated by rest—we must first recognize that all theories of disordered neuromuscular transmission are essentially model making. As I have pointed out before,40 we are all children of our age and we adopt the conceptual models appropriate to our time. Thus, in the first recognizable description of the disease (published in 1672 and quoted by Viets39), Thomas Willis concluded:

"Without doubt in these, although the Animal Spirits do alter a manner actuate and irradiate the whole nervous Stock, yet their numbers are so small, and in so few heaps, that when as many spirits ought to be heaped together somewhere in it for motion, there is great danger left presently in the neighbouring parts, their continuity should be broken. Wherefore, when the spirits inhabiting the Brain, are conscious of the debility of others disposed in the Members, they themselves refuse local motions, for that it would be too difficult a task to impose on their companions...; before noon the stock of the Spirits being spent, which had flowed into the Muscles, they are scarce able to move Hand or Foot."

This is a model based on the topical view of nervous activity, and it is clearly a presynaptic theory.

Two centuries passed before the magnificent contributions of Erb and Goldflam established the clinical picture.40 By that time the transient paralysis caused by curare was known, and many sources had reported finding no abnormalities on postmortem examination of patients who had died of MG. Oppenheim (1887)41 and Jolly (1895)42 recognized a resemblance between MG and curare poisoning and suggested using physostigmine for treatment, though this does not appear to have been tried until the famous case reported by Mary Walker (1934).43 This paper and the later one44 on the use of the physostigmine analog neostigmine coincided with the confirmation by Dale and Feldberg45 of the role of acetylcholine (ACh) in neuromuscular transmission. This was an era of rapid development in the study of biochemistry, and from that time all thinking turned toward an etiologic concept of a biochemical lesion causing a transmission block at the neuromuscular junction.

Over the next 25 years, rapid advances in neurophysiology and pharmacology suggested several mechanisms for such a block. This led to considerable disagreement. Most papers on the mechanism of MG started with a review of neuromuscular transmission...
and its blockade by pharmacologic means. In short, the models had become pharmacologic analogs. During that era, many older clinical observations were forgotten. The new theories tended to ignore the established fact that the thymus was invariably abnormal and that its removal could effect improvement in the myasthenic state. Those who recognized these facts were forced to postulate that the thymus was the source of a “myasthenic toxin” with curarelike properties—a concept perpetuated by the “thymitis” school and criticized above (see part 1). No satisfactory evidence for a circulating neuromuscular blocking substance in human myasthenia appeared until the recent reports of Toyka and colleagues. These have yet to be confirmed. The duration of neonatal myasthenia is much too long to attribute to placental passage of a substance with a molecular size resembling curare. Only the Mary Walker effect, examined below, is seriously offered as evidence of the presence of such a toxin in MG.

THE WALKER EFFECT

It has been stated by many authors since Laquer, Buzzard, and Walker that exercise of a major group of muscles in a myasthenic patient causes the release into the bloodstream of a substance that induces weakness of unexercised muscles. The procedure adopted by Walker and by subsequent researchers was to occlude the circulation in an upper limb while exercising the muscles of the forearm. They maintain that, in many cases, on releasing the constriction from the one part the clinical signs of myasthenia (e.g., ptosis) increase in other parts (the “Walker effect”).

Wilson and Stoner (1944) reported similar findings substantiated by cinematography, and others have made like claims, all of which I find unconvincing. The alteration in the palpebral fissure illustrated by Wilson and Stoner is not impressive. The latency of ptosis after release of the occluding cuff is recorded at 1.5 min, 10 sec–1 min, and 4–6 min. It is difficult to accept latencies of this order as being the result of the pharmacologic action of a substance passing from arm veins to the eyelid muscles. Since myasthenic ptosis may occur rapidly in situations of emotional stress or in bright light (as in an experimental situation with close-up photography), more convincing evidence is needed. It is unlikely that muscular activity produces a toxic substance proportional to the degree of contraction, such as lactic acid which might cause a reduction of serum calcium, since a blink will often restore the droopy eyelid to normal, presumably by posttetanic potentiation. My own experiments, and those of others, have led to considerable doubt about the reality of the Walker effect.

More serious consideration must be given to the experiments of Tsukiyama et al (1959), which used nerve stimulation distal to a cuff in one arm with EMG recording of the evoked muscular potential of the other arm. About 1 min after the cuff was released the test potential began to fall, reaching a minimum in 1–4 min with recovery in the next 10–15 min. The depression was reduced after administration of an anticholinesterase. If myasthenic “fatigue” were caused by a substance generated by motor nerve activity, or caused by a metabolite from contracting muscle, one would expect an escalating effect with continued stimulation. In fact, as shown below, the opposite happens. The greatest fall in amplitude of the action potentials takes place at the beginning of the decrement, and a subsequent plateau level is commonly established.

ELECTROPHYSIOLOGY

This is not the place to examine in detail the electrophysiologic and pharmacologic studies on which the rival models (discussed below) are based. These have been reviewed elsewhere. The essential facts will be outlined in association with the various models rather than in historical order.

First, are we justified in assuming that the defect is exclusively synaptotrophic? Jolly showed that the pathologic fatigability could be reproduced by faradic stimulation of the motor nerve while the “fatigued” muscle would still respond fully to locally applied galvanism. Later workers, using electrical recording of the antidromic action potential of the motor nerve, confirmed that the nerve continued to respond. Nevertheless, some involvement of muscle fibers is suggested by the reduction or abolition of the normal “staircase phenomenon” demonstrated by Slomiec et al. Ballantyne and Hanssen, using a computerized technique, have also found evidence for diminished conduction velocity of terminal motor nerve fibers and agree with others that there is significant reduction in the duration of motor unit potentials. But other physiologic evidence of denervation, such as fibrillation, is scanty.

Most of the electrophysiologic evidence for transmission failure is not very helpful in determining the mechanism involved. Conventional needle electromyography shows a progressive reduction in amplitude and fall out of motor unit activity, with development of synchronization and brief pauses followed by brief posttetanic facilitation. Some motor unit potentials become polyphasic, suggesting failure of single muscle fibers; yet sudden cessation of activity of whole units undoubtedly occurs, which could scarcely be the result of a lesion distal to the terminal arborization. Single fiber electromyography shows greater variability in the firing time relationships.
between muscle fibers belonging to the same motor unit ("jitter"), which increases during prolonged activity, and there is occasional blocking of single muscle fiber responses. Both of these defects can usually (but not invariably) be normalized by edrophonium. The EMG findings may be attributed to variability in the amplitude of endplate potentials, which has been demonstrated in human myasthenic muscle \(^{49}\) and which could be the result of any lesion that has this effect. Similarly, the decrementing mechanical response found by Jolly,\(^ {39}\) as well as the decrement of compound muscle action potential evoked by repetitive supramaximal nerve stimulation,\(^ {40}\) shows that the normal safety factor for transmission of repeated stimuli is missing from the myasthenic neuromuscular junction.

There are some clues to the site of the defect. Desmedt has stressed the "post-activation exhaustion" that follows a train of stimulation or a strong voluntary contraction and that slowly disappears with a half-time of 10–15 min.\(^ {12,13}\) Inasmuch as a similar phenomenon occurs in the cat poisoned with hemicholinium (a drug that acts mainly by inhibition of ACh synthesis), this model suggests a presynaptic lesion for myasthenic postactivation exhaustion. Desmedt considers this to be the cause of the clinical fatigability rather than the decrement during tetanization. As is the case with most pharmacologic probes, however, the action of hemicholinium is not exclusively presynaptic, and there is recent evidence that postactivation exhaustion is also caused by d-tubocurarine.\(^ {45}\)

If there is a biochemical defect, it must be in the synthesis or packaging of ACh or in its transfer to a readily releasable store,\(^ {13}\) rather than in its release from synaptic vesicles. A characteristic feature of the muscle action potential response to rapid (50/sec) trains of supramaximal nerve stimuli is that it is temporally facilitated after the initial decrement, and posttetanic facilitation is detectable after a single stimulus\(^ {52}\) or on continued stimulation after the muscle fibers have ceased to respond. The muscle potential evoked by the first stimulus of a train may be of lower amplitude than later potentials, so there must be some muscle fibers available for recruitment in the myasthenic; that is, there is sometimes a partial block to neuromuscular transmission of a single impulse. The degree of facilitation is highly variable; in some muscles it may be the dominant factor.\(^ {12,46}\)

Inasmuch as posttetanic transjunctional potentiation of this type is generally accepted as a prejunctional phenomenon, it seems unlikely that there can be a significant defect of ACh release in MG.\(^ {48}\) Therefore, I have drawn attention to the absence of facilitation in myasthenia which is associated with diseases of the lower motoneurons.\(^ {47}\) It is equally unlikely that denervation leading to secondary structural changes of the endplate would be a characteristic feature, as suggested by W. K. Engel.\(^ {51}\) Responses to veratrine, guanidine, and anticholinesterase drugs suggest that there is no significant deficiency of stored ACh in this disease. If Desmedt's interpretation of postactivation exhaustion is correct, it must apply to a "readily releasable fraction" rather than to the synthesis of "depot-ACh" or to the mechanism of release.

Support for Desmedt's interpretation came from a series of experiments summarized by Elmqqvist on biopsy specimens of intercostal muscle from myasthenic patients.\(^ {52}\) He reported that spontaneous miniature endplate potentials (MEPPs) were reduced in size. A general principle used in interpreting MEPP studies is that the frequency of the miniature potentials is usually controlled by the conditions of the presynaptic membrane, while their amplitude is usually controlled by the postsynaptic element.\(^ {53}\) These results should therefore, to a postsynaptic disorder in myasthenia. However, the Swedish researchers preferred to interpret them as indicating a lowered quantum content of ACh, by analogy with their findings with hemicholinium.\(^ {54}\) These findings were supported by studies of the depolarization of endplate regions of normal and myasthenic muscles by decamethonium and carbachol. In these studies, myasthenic endplates gave a normal response to iontophoretic microapplication of ACh. While I accept these data, I do not believe that the conclusions for a presynaptic mechanism ("small quanta") necessarily follow.\(^ {10,89}\) Recent evidence on autoimmune MG in the rat\(^ {60}\) supports my interpretation that the small MEPPs are caused by a decrease in the number of reacting postsynaptic ACh receptor (AChR) sites. Grob reported that spontaneous endplate activity in limb muscles of myasthenic patients was more difficult than normal to locate, but it did not differ significantly in amplitude, duration, or frequency.\(^ {24}\) He disagreed with Elmqqvist regarding the chemosensitivity of the endplates.

**PHARMACOLOGY**

Pharmacologic studies on myasthenic patients have in general indicated a postsynaptic disorder. Churchill-Davidson and Richardson showed that the neuromuscular blocking drug decamethonium did not have its normal depolarizing action in myasthenics.\(^ {5}\) Instead, it produced a curare-type competitive block, sometimes preceded by a brief depolarization block ("dual response"). Grob et al showed that the actions of ACh and choline were altered in a similar manner.\(^ {29}\) These findings point to a postjunctional change, prob-
ably involving receptor sites. Contradictory statements in the literature on the sensitivity of the endplate to ACh can be attributed to unrecognized dose-response relationships; there is little doubt that the myasthenic endplate shows diminished sensitivity to ACh. For a fuller review of this controversial subject, see Simpson. Increased sensitivity to curare and quinine accompanies all types of diminution of the safety factor for synaptic transmission and does not influence the argument. Nevertheless, it is important to note that hypersensitivity to \(\alpha\)-tubocurarine persists in biopsied myasthenic muscle after repeated washing, which is further evidence against a circulating curarelike substance.

MODELS OF NEUROMUSCULAR BLOCK

At this point it is necessary to draw attention to the nature of the arguments for the transmission defect in myasthenic muscle. The above limited review is sufficient to indicate that three main theories have emerged: (1) the circulating curarelike substance, (2) the presynaptic defect, and (3) the postsynaptic defect. Each of these has been constructed from a pharmacologic analogy. They are models. It is implicitly assumed (1) that all mammalian neuromuscular junctions show the same pharmacologic reactions, (2) that the pharmacology of the normal junction also is relevant for the pathologic junction, and (3) that there is no anatomic abnormality of the neuromuscular junctions. All these assumptions are probably false. In my opinion, the only logical conclusion that can be drawn from the reported findings is that there are both pre- and postsynaptic abnormalities but there is no circulating curarelike substance.

For many years, I have discussed the possibility that the abnormalities described could result from a geometric change in the junction’s structure, with the added possibility of receptor blockade by an antibody (a “personal” blocking substance transmissible only to one’s offspring). An analysis by Eccles and Jaeger of the factors determining efficiency of synaptic transmission showed that a flat ending is much less efficient than a knob-shaped one. The width of the synaptic cleft must be as narrow as possible, yet wide enough to allow transmitter substance to escape by diffusion. The subsynaptic membrane should provide many receptor areas close to the site of ACh release. This implies a folded subsynaptic membrane, but the folds must not be too deep or too shallow. Modification in each of these respects would account for the electrophysiologic and pharmacologic findings in MG. Therefore, I have suggested a possible mechanism for the small MEPP findings (and also for some apparent anomalies in the earlier papers from the Swedish group) that makes it unnecessary to interpret amplitude changes on a presynaptic basis (“small quanta”).

Blockade of AChRs in rats by the \(\alpha\)-toxin derived from the venom of the cobra Naja naja atra, described in part I, supports the concept that a reduction of available AChRs may play an important role in MG. The experimental findings include small-amplitude MEPPs and all the other electrophysiologic phenomena of human MG, including postactivation exhaustion and decremental response at slow rates of stimulation, with return to normal after some hours. Drachman et al now consider MG to be a receptor disorder. There is little doubt that the postsynaptic lesion is of major importance, but an additional presynaptic disorder cannot yet be excluded.

As pointed out above, the pharmacologic models are based on three unproved assumptions. We also have to account for the characteristic distribution of muscle susceptibility and for the relapsing and remitting clinical course. We now have evidence that the characteristic twitch responses of “white” and “red” muscles to nerve stimulation, and their different responses to drugs, correlate with the geometry of their neuromuscular junctions. The latter are modified part parastis with the twitch characteristics in nerve transposition experiments. Is there histologic evidence for abnormal endplate structure in human MG?

ENDPLATE STRUCTURE

It was generally accepted, especially during the “biochemical lesion” era, that there is no histologic abnormality of the neuromuscular junction in MG. The use of intravital staining with methylene blue showed otherwise. Mott and Barada used this method in one patient as early as 1923. They did not detect any abnormality, but contemporary critics have suggested that their diagnosis of MG was erroneous. Coers and Wool used intravital staining in addition to staining of the subneural apparatus for cholineserases (which, incidentally, is not present in excess). They demonstrated florid morphologic changes of the intramuscular nerve endings. They found that two types of abnormal endings occurred in myasthenic muscle. The “dystrophic” type, with increased branching of the terminal arborization and distribution of terminal knobs over a wider-than-normal area of muscle, was considered to be a reaction to muscle fiber disease and not specific for MG. The “dysplastic” type had few terminal knobs. These were arranged serially along a scanty number of terminal branches, and ended on a long endplate region running parallel to the muscle fiber. The dysplastic type, common in myasthenic
Myasthenia Gravis

replacement of
is the trophysiologic, and pharmacologic
remissions and this could suggest that the morphologic changes are correlated with the transmission failure. The recent work showing an inverse correlation between immune complexes on the postsynaptic membrane and the severity of myasthenia supports this point of view.

The light-microscopic and ultrastructural studies suggest that degeneration and repair of the neuromuscular junction occur simultaneously in MG. This could readily account for the presence of relapses and remissions in stage 1 of the natural history of the disease.

It has been my contention that AChR blockade with antibody, and structural changes resulting from cell-mediated immunologic damage of the neuromuscular junction, both pre- and postsynaptically, would adequately account for all known clinical, electrophysiologic, and pharmacologic phenomena of MG. The transmissible factor related to the thymus must cause the demonstrated geometric change of the endplate rather than a curarelike block. This may be an exaggeration of a normal process of remodeling or replacing of endplates, or of AChRs, for which there is some evidence in normal mammals. I have suggested that an immunologic mechanism might be used in the normal animal as a homeostatic device for detecting and removing effete and degenerating tissues. Possibly the thymus also plays a role in promoting regeneration. It does appear to have endocrine-type secretion granules in the Hassall epithelial cells, but their function is unknown.

My reason for ending with a speculation here is that if we could isolate the factor responsible for the active regenerative activity of damaged motor nerve terminals, we would perhaps have the ultimate therapy for myasthenia gravis. When the autoimmune hypothesis was first propounded, it was highly speculative; it has taken 15 years to find acceptable evidence for many points. I can do no better than to end with the same quotation from Hughlings Jackson:

The use of hypotheses is the method of science. To suppose we can make discoveries by the Baconian method is a delusion. A hypothesis or supposition is not a conclusion; it is only a starting point for methodical observation and experiment, the end of the journey being not to prove it but to disprove it.

REFERENCES

Myasthenia Gravis


The place of surgery in the treatment of myasthenia gravis

K. FRASER, J. A. SIMPSON AND J. CRAWFORD* 

SUMMARY

The clinical conditions, operative and postoperative management of 64 patients submitted to thymectomy from 1965 to 1976 have been reviewed retrospectively. The results of 11 thymectomies done before 1965 have been previously reported (Fraser, 1966). We consider that the duration of illness is a more important criterion than the age or sex of the patient or the distribution of weakness, and we recognize three stages (Simpson, 1974). Stage I, the active stage, is characterized by remissions and best response to thymectomy; it normally lasts for 5–7 years and is followed by stage 2, the inactive stage. In stage 2 there is less risk of death but few spontaneous remissions and less response to thymectomy. In stage 3, the burned-out stage, there may be steady improvement but thymectomy is without benefit and responsiveness to anticholinergic drugs becomes less.

It has been our policy to perform thymectomy as early as possible in stage 1. In addition, stage 2 patients have been advised operation if the response to anticholinesterase medication has been unsatisfactory. Although major improvement is not anticipated until 2–3 years after operation, we have felt justified in offering thymectomy because we have had no death as a direct result of the actual operative procedure. One patient died suddenly and unexpectedly on the tenth day postoperatively (1/7 per cent). At follow-up there were 6 late deaths. Of the 52 patients available for review, 92.3 per cent had no symptoms or only mild disability.

The role of the thymus in myasthenia gravis was first recognized by Weigert in 1901. In 1960 Simpson showed the immune nature of the disease. In 1912 Sauerbruch removed the first thymus in a patient who had hyperthyroidism associated with myasthenia gravis. The patient obtained marked improvement in the myasthenia gravis. The operation was done by the transcervical route (Schumacher, 1913). Apart from one thymectomy by Haberer in 1917, nothing further was heard of the operation until 1936, when Blalock successfully removed a thymic tumour in a patient with myasthenia gravis. Five years later he took the logical step and commenced removing the thymus even in the absence of tumour in an attempt to alleviate symptoms of the disease (Blalock et al., 1939; Blalock, 1944). In 1942 Keynes commenced thymectomy for myasthenia gravis in Britain, and published his first of several classic articles on this treatment in 1946. Great tribute is due to Keynes and other surgeons, especially Blalock, for their surgical skill and courage. Even in 1942 transthoracic operations were still very much in their infancy while operations within the mediastinum were excursions into territory as yet unexplored and unknown.

Patients and methods

Fifty of the patients were female and 14 were male. Age incidence was as set out in Table I. As one would expect, the majority were between the ages of 20 and 40, 37 patients being in this age group. The ages ranged from 2 to 66 years.

Eight patients had a clinical history of less than 6 months, 36 patients a history of 6 months to 2 years, 11 patients from 2 to 5 years and 9 patients had had symptoms for more than 5 years.

One patient had ocular signs only, 12 patients had a mild generalization weakness including ocular signs and 51 had a moderately severe degree of weakness and difficulty with deglutition and speech. Four cases were in stage 2 and 2 in stage 3; all others were in stage 1. The stage 3 patients were operated on as a last resort. One of them had rheumatoid arthritis, goitre, polythsemia and was positive for ANF. The other had systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Preoperative preparation

No patient in this series required artificial ventilation before operation. All patients had respiratory function tests. These tests were unhelpful as a guide to the postoperative management of the disease. Physiotherapy was carried out for 1 week before operation. Following Keynes' insistence, under no circumstances were enemas given for fear of the possible consequent sudden death that an enema may cause (Keynes, 1946).

Anaesthesia and operation

Anticholinesterase drug therapy was continued until the last dose due before operation, which was omitted (normally 3–4 h before operation).

Premedication was light—originally methaqualone and now nitrazepam given 2 h before surgery. The patient was confined to bed during that time.

Anaesthesia was induced originally with thiopentone, later replaced by propofol which in turn has been succeeded by Althesin. Intravenous induction was followed by nitrous oxide, oxygen and halothane under which orotracheal intubation was performed and intermittent positive respiration maintained using 1 per cent halothane (Crawford, 1971). A nasogastric tube was passed before transfer from the anaesthetic room to the operating theatre. Electrocardiogram, pulse and blood pressure monitoring facilities were used in the latter part of the series. An intravenous infusion was always instituted but it was never necessary to give more fluid than required to keep the vein open. After operation spontaneous ventilation was allowed to return, and with a tidal volume of 250–300 ml and a minute tidal volume of 4–5 l measured by a Wright's respirometer, the patient was allowed to leave the theatre.

Surgery was performed through a vertical midline incision and the sternum was split in the axis of the wound. This traditional approach is used for two reasons: first, thyamic tissue can be, and is not infrequently, found in the fatty tissue outwith the gland, and secondly because the authors agree with the Leading Article in the British Medical Journal which states that the surgeon can see what he is doing through this.

* K. Fraser and J. Crawford, Western Infirmary, Glasgow.

J. A. Simpson, Professor of Neurology, The Institute of Neurological Sciences, Southern General Hospital, Glasgow.
approach (Leading Article, 1975). All thymic tissue was removed and any portions of fat seen in the vicinity were also excised. Sometimes the dissection of the thymus caused the tearing of one and occasionally both pleurae; if this happened the pleural cavities were drained and the usual retrosternal drain was omitted.

Postoperative management

The most difficult period in the management of myasthenic patients is the 3 days after operation. We believe that we have successfully solved the major problems which are, (a) preservation of respiration and (b) regulation of anticholinesterase medication in a rapidly changing situation. A team approach is essential but we have found it advantageous to have a strict protocol to ensure that responsibilities are clearly defined so that anticholinesterase medication is prescribed with exact knowledge of the concurrent efforts of other members of the team. Decisions on drainage, fluid and electrolyte replacement and antibiotics were taken by the surgeon, those on ventilation and analgesia by the anaesthetist and on anticholinesterase medication only by the neurologist.

Drainage, fluid balance and antibiotics: When the pleurae were not opened the drainage was by a single retrosternal drain into a Redivue suction bottle. This drain was removed after 24-48 h. Drainage tubes within the thorax were removed after 36-48 h. Intravenous drips were discontinued after 24 h, fluid balance being mainly maintained by mouth. Antibiotics were not used prophylactically and, indeed, were rarely required for postoperative pneumonia. Absence of tracheostomy was double a factor in reducing the incidence of pulmonary and mediastinal infection. When necessary, the appropriate antibiotic was used with preference for penicillin and avoidance of antibiotics which have a neuromuscular blocking action (notably streptomycin and neomycin), which may be dangerous for myasthenic patients.

Ventilation and analgesia: Provided that the neuromuscular status of the patient is satisfactory at the time of discharge, it is not necessary to withhold anticholinesterase medication. A single dose of anticholinesterase medication should be given as soon as the patient can swallow and be transferred to the ward. The patient must be in the presence of a neurologist and the drug dosage carefully titrated to maintain satisfactory respiratory function. Thereafter, the drug dosage is to be determined by the ward neurologist. If he is not satisfied with the patient's response, the patient is to be transferred to theITUstaff for observation and further medication is to be prescribed. In the absence of a neurologist, the first dose of anticholinesterase medication is to be given in a hospital where the patient is to be transferred immediately to the ICU staff. The patient is to be observed closely for 6 h after the first dose of anticholinesterase medication, and the dosage will be determined by the ward neurologist. The subsequent dosage will be determined by titration with the ward neurologist. The dose of anticholinesterase medication is to be reduced by 50% if the patient shows a decrease in muscular power of 50% or more after the first dose.

Pathology of the thymus gland

At operation the thymus gland showed a wide range of variation. Some glands were largely made up of tissue similar to fatty tissue, frequently with complete separation of the two halves of the body. Eight glands were small and 1 was atrophic; 3 were made up of thick, dense tissue and 3 had recognizable germinal centres. One patient had sarcoidosis in the mediastinal lymph glands and intercostal muscle. The histological changes of the thymus in this series of cases have been reported by Vetters and Simpson (1974), who found that the frequency of germinal centres bore no correlation to the speed or degree of recovery following thymectomy. Eight patients were found to have thymic tumours, some of which had been suspected before operation. The largest of these tumours was 4 x 2 cm. The histological decision as to malignancy in thymic tumours was notoriously difficult and on occasion the surgeon may be the person most capable of deciding as to whether invasion

Anticholinesterase medication: There has been no change in the surgical management since the previous report by Fraser (1966). The good results of this further series are a measure of the success of our combined policy. The essential difference from that adopted elsewhere is the decided reduction in anticholinesterase medication almost unchanged, regarding the operation as an incident requiring the minimum of modification to dosage. In many centres it is the practice to withdraw all anticholinesterase drugs before operation and not to resume for at least 24 h after it, commonly for longer, and also to perform a tracheostomy as a routine. The rationale for that course of action is that it has been recognized for 20 years or more that the requirement for neostigmine or its equivalent often drops dramatically during the first 48 h after operation and that if this is not recognized the patient may be thrown into a cholinergic state with increased paralysy, pulmonary oedema and cardiac effects.

The method we use requires careful personal observation by an experienced observer. The patient is kept in a conscious patient in the recovery room. If there is significantly more myasthenic-type weakness than at the last preoperative examination, a full dose of pyridostigmine is given. Until normal swallowing power is restored, the crushed tablets are given by nasogastric tube in preference to parenteral routes. If this first examination shows no deterioration since going to theatre, the first dose is resumed until the second examination which is made after the interval previously required between doses, individually from 2 to 6 h. At that time the preoperative dosage is resumed, provided that no signs of overdose are present. The patient is then watched carefully by the attending staff and re-examined frequently by the neurologist during the first 3 days and then at intervals of not more than 12 h until recovery is well under way. Dosage is prescribed only as far ahead as the next anticipated visit. Meanwhile, any change in the patient's condition must be reported to the neurologist by telephone. No other attendant is empowered to alter the dosage or cholinergic medication. No attempt is made to achieve maximal control during this time. It is sufficient that breathing and swallowing should be adequate and if weakness is present that it should be of myasthenic and not of cholinergic type.

The indications for reducing or withholding a dose of pyridostigmine or for increasing the time between dosage are (a) evidence of overdosage, (b) no detectable increase of muscular power 1 h after a dose, (c) pupil diameter of 3 mm or less in normal ward lighting, (d) fasciculation of skeletal muscles (best seen in the less myasthenic muscles, usually in the legs). The importance of avoiding other drugs which constrict the pupils will be obvious. For similar reasons atropine and related drugs should be avoided unless there are special indications. Other muscarinic effects should not be seen. An electroencephalogram is rarely necessary and must be used with discretion, covered with atropine.

The gastric tube is removed as soon as tablets can be swallowed. On average patients were out of bed by the second day and ready for discharge home by 8-10 days after operation. By that time a new dosage regime of pyridostigmine had been established according to the principles outlined by Simpson (1971).
has in fact occurred. Six of these tumours showed 'surgical' invasion in that they were adherent to the pleura, in one case to the phrenic nerve and in another to the innominate vein. Despite this, 5 tumours were reported as non-malignant and the sixth as showing low grade malignancy. In one case tumour cells were seen outwith the capsule of the thymoma but were not invading the surrounding tissues. This patient is still alive 7½ years after the operation, although his condition is not improved to any extent.

Mortality
One death occurred on the tenth postoperative day in another hospital, the result of cardiac arrhythmia. This occurred in a 38-year-old woman who had had symptoms for 24 years in early stage I. In addition to myasthenia gravis she had rheumatoid arthritis, a non-toxic goitre and quite severe polycythemia (Simpson, 1976). That she was a poor operative risk was recognized and fully explained to her. As a precaution she was ventilated for 4 days in the intensive therapy unit, but she was well enough thereafter to return to the surgical ward and was discharged to the referring hospital on the eighth day. She collapsed suddenly the next evening with cardiac arrhythmia followed by cardiac arrest which could not be reverted. Autopsy was unhelpful as to the cause of death.

A further 6 patients died after recovering from the operation. One patient of 65, having a low grade malignant tumour, died unexpectedly at 2 months from acute bronchopneumonia. One patient (stage 3 before operation), who died after 2½ months, had concomitant rheumatoid arthritis and the thymic gland histology showed features of systemic lupus erythematosus. One of the patients having a thymic tumour died with a lung abscess at 1 year after operation. One death occurred after 2 years from a fulminating generalized infection, as reported by the general practitioner; this patient had had a simple thymoma removed. Another died from acute pancreatitis at 2½ years and 1 from generalized weakness after 3½ years.

Results
Of the surviving 57 patients, there is no follow-up for 2. A further 3 patients have an incomplete follow-up, 1 being satisfactory at 2 months, 1 at 4 months and 1 was fit to travel to Australia over 1 year after surgery.

Of the remainder at the time of review in late 1976, 26 patients had no symptoms and 22 patients had mild symptoms such as occasional weakness and/or diplopia; 9 of these stated that they were 'greatly improved' or 'incomparably better' since thymectomy. Eight patients had either given birth to a baby (1 had had 2 babies) or were pregnant at the time of review. Two patients were not improved and 2 patients were worse.

Of the 8 patients in whom a tumour was found and removed at thymectomy (8 per cent), 3 have only mild symptoms 3-7 years after surgery and 2 show no change at 7 and 7½ years. Three patients with tumours have died; 1 died unexpectedly at 2 months from pneumonia, 1 at 1 year from a lung abscess and the third at 2 years from a fulminating infection. One patient has not been traced.

This assessment (Table II) indicates the satisfactory clinical state of most patients after operation. As the severity of myasthenia before operation is not standardized, it may be preferable to use a grading based on the change of status when the follow-up state is compared with the preoperative condition. For this purpose, the cases are summarized in Table III according to the classification of Simpson (1958).

Category A: Full working life with no restriction. No neostigmine required. No subjective weakness. A small degree of permanent objective weakness, unrelieved by neostigmine, is permitted. The present state must represent a marked improvement from the original.

Category B: Able to lead a full life with only minor myasthenic symptoms, requiring no neostigmine or controlled by not more than 4 tablets (60 mg) daily. A significant improvement is required.

Category C: Full life with few restrictions but (a) demonstrable myasthenia not requiring neostigmine or (b) still requiring neostigmine but at least 40 per cent less than before and with improved response.

Category D: Must be (a) improved, but neostigmine requirements unchanged or increased; (b) unimproved, irrespective of neostigmine dosage or (c) worse.

It will be noted that the requirements for reduced dosage of neostigmine (pyridostigmine) loads the assessment in favour of 'no change', but those in categories A, B and C are all able to lead a full life. These are severe criteria, especially for cases on a low dosage preoperatively. Nevertheless, the results of the present series of 92-3 per cent having no symptoms or only mild disability are even better than those reported in 1958 by Simpson (Table III). This is attributable to the lower mortality in the present series. The poor long term prognosis for patients with thymoma is confirmed. For the non-tumour cases males have a surprising advantage, contrary to all previous reports.

Discussion
Myasthenic patients once controlled with anticholinesterase drugs should be offered thymectomy provided that they can be under the constant care of a neurologist, anaesthetist and a surgeon who have worked together in the treatment of this disease.

In 1958 Simpson showed that thymectomy in myasthenia gravis offered a great saving of life and a greater proportion of remissions. With personal attention to pre and postoperative control of anti-cholinesterase dosage, since 1956 no patient has failed to survive thymectomy, yet as late as 1971 a Leading Article in the British Medical Journal described thymectomy as having a relatively high morbidity and mortality due to the wide spontaneous fluctuation

<table>
<thead>
<tr>
<th>Years since operation</th>
<th>Non-myasth.</th>
<th>Mild</th>
<th>Unchanged</th>
<th>Worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-5</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5-10</td>
<td>12</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10+</td>
<td>1</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>23</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Both sexes</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-thym.</td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>7</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>18</td>
<td>2</td>
<td>35-7</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>—</td>
<td>14-3</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>1</td>
<td>12-5</td>
<td>3</td>
</tr>
<tr>
<td>Dead (all causes)</td>
<td>—</td>
<td>—</td>
<td>7-1</td>
<td>3</td>
</tr>
<tr>
<td>Not traced</td>
<td>3</td>
<td>—</td>
<td>5-4</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>10</td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Both sexes</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymoma</td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>7</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>18</td>
<td>2</td>
<td>35-7</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>—</td>
<td>14-3</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>1</td>
<td>12-5</td>
<td>3</td>
</tr>
<tr>
<td>Dead (all causes)</td>
<td>—</td>
<td>—</td>
<td>7-1</td>
<td>3</td>
</tr>
<tr>
<td>Not traced</td>
<td>3</td>
<td>—</td>
<td>5-4</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>10</td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>
in anticholinesterase requirements which may develop in the immediate postoperative phase. By 1975, however, Havard had stated that the risks of thymectomy were greatly reduced consequent on the advances in ventilation and better differentiation between myasthenic and cholinergic crises (Havard, 1975). Havard found complete remission or substantial improvement in 80 per cent of the cases without tumour: sometimes it takes 5 years for such improvement to occur. A similar figure is quoted by Edwards and Wilson (1972). Havard believes that thymectomy should be offered to most patients with myasthenia gravis other than those with only oculomotor symptoms. Thymomas should always be removed as thymectomy will prolong life and increase the rate of remission. Havard found that 85 per cent of tumour cases untreated were dead within 1 year (Havard, 1973), but Simpson found a mean survival (from onset of myasthenia to death) of 4–5 years in patients with thymomas (Simpson, 1976). With modern techniques and the better understanding of the constant supervision required postoperatively, the operation should present no great problem. The long term results show that 48 out of the 64 original cases (75 per cent) had either no disability or only minor symptoms when reviewed.

We attribute the excellent results in this series to two factors. First, improved postoperative management has eliminated deaths associated with the operation. The better long-term prognosis is, in our opinion, due to the policy of operation in stage 1 and, indeed, as early as practicable after the onset of symptoms. The number of thymic tumours, though representative (8 per cent of the total), is too small to assess, but it is encouraging that we have not experienced the mortality noted in the earlier series (i.e. 60 per cent in less than 3 years after operation).

We have found that preoperative lung function tests provide no reliable criteria for the type of respiratory support required in the postoperative phase, in contrast to the views expressed by Loach et al. (1975). The present authors aim to avoid laryngoectomy, and this procedure was required in only 3 patients. We believe this avoidance of laryngectomy and/or prolonged ventilation results from the very close neurological supervision in the immediate postoperative hours and early days to balance the drug therapy. We further believe that the standard surgical approach is necessary if all the thymic tissue is to be excised.

Addendum

Since this review was completed a further 4 females and 1 male patient have undergone thymectomy for myasthenia gravis. Only 1 required to be ventilated and this was on the fourth day and mainly owing to sputum retention. The time interval is too short for assessment of improvement.

Acknowledgements

Our thanks are due to Dr Ian Melville for permission to include some of his cases and to Dr Hugh Wishart for help with anaesthesia in some of the earlier cases of the series. We thank Mrs Isabel Gray for secretarial help.

References


Paper accepted 30.11.1977.
There are now a number of methods of treatment for myasthenia gravis (MG). The purpose of this review is to indicate that there is no "best" treatment - all may be required for different aspects of management. Details will be provided by other speakers. The first priority is to preserve life by controlling respiration if it is threatened, and to promote maximum power of muscles by raising the safety factor for neuromuscular transmission. Then the primary immunological disorder should be treated.

SAFETY FACTOR FOR NEUROMUSCULAR TRANSMISSION

The safety factor for transmission is raised by drugs which i) potentiate the production or release of ACh ii) sensitise the ACh receptors or amplify the muscle response iii) inhibit hydrolysis of ACh by cholinesterase. Drugs which lower the safety factor for transmission should be avoided. For references, see Simpson (1974) (18).

Potentiation of ACh release and sensitisation of ACh receptors.

4-aminopyridine chloride and guanidine hydrocholoride increase the release of ACh from motor nerve terminals and guanidine has some post-synaptic facilitatory action on neuromuscular transmission. Though effective in the carcinomatous myasthenic syndrome, they have no proven value in MG. Despite exceedingly weak anticholinesterase activity, adrenaline and many of its primary and tertiary amine analogues potentiate the transmitter process, probably by augmenting ACh output, but with a possible additional action on muscle membrane. The structure of catecholamines is appropriate for both facilitation and depression, dependent on dosage and on other drugs administered. Adrenaline is of no practical value in MG, but ephedrine, an inhibitor of the amine oxidase which oxidizes adrenaline, has been claimed to have an antimyasthenic action. An oral dose of 10-25 mg thrice daily is used. Large doses may aggravate the weakness. I have not been convinced that ephedrine has any useful clinical action on skeletal muscle in MG. Apparent clinical improvement may be due to bronchodilation (15), advantageous in a patient taking anticholinesterases. The role of ephedrine in MG is entirely adjuvant.
Veratrum alkaloids cause repetitive firing of nerve endings and of the stimulated muscle and hence 'amplify' the stimulus-response relationship. Thus veratrine and germine diacetate may temporarily increase the efficiency of neuromuscular transmission in MG but with impaired oxidative metabolism of muscle and nerve, and loss of intracellular K⁺, bronchiolar constriction and occasional cardiac arrhythmia. Veratrine causes hypotension but germine is claimed to be without this action. I have not used this group of drugs. Their value is certainly marginal, probably not persistent, and entirely inferior to anticholinesterases.

Anticholinesterase drugs

Inhibitors of endplate acetylcholinesterase prolong the occupancy of receptor sites by ACh, thus raising the safety factor for transmission. If carried to excess, so that ACh persists at receptor sites, the endplate remains depolarized or becomes desensitized ('cholinergic blockade'). The first effective anticholinesterase was phystostigmine, a carbamate ester and tertiary amine. Another is galanthamine, from the Bulgarian snowdrop. They cross the blood-brain barrier and act on central synapses, unlike the quaternary ammonium compounds neostigmine etc. which have replaced phystostigmine. Some hydroxyanilinium salts or esters also have anticholinesterase activity, notably edrophonium. All of these drugs probably also act on the motor nerve terminals and perhaps directly on the post-synaptic membrane, but it is generally accepted that the anticholinesterase activity is the more important clinically.

Edrophonium, administered intravenously (2-10 mg), has a peak action too brief for treatment of MG and it is used to confirm the diagnosis of MG or to differentiate between underdosage and overdosage of anticholinesterases. It is a very reliable test agent, but the test should not be repeated without awareness that residual activity can be demonstrated electrophysiologically for at least 30 min after i.v. injection.

Neostigmine should rarely be given intravenously as bradycardia may be dangerous but may be given by subcutaneous or intramuscular injection (1 mg parenteral is approximately equivalent to 15 mg orally). Although absorption is incomplete and irregular, it is rapid and neostigmine should normally be given by mouth. Crushed tablets by gastric tube are preferable to injection if dysphagia prevents normal administration. Following a dose there is a surge of muscular power for 30-60 minutes followed by continued activity at a lower level for 2-6 hr. Then strength is lost rapidly, causing difficulty in adjusting timing of dosage. Most myasthenics prefer pyridostigmine for this reason, but in some cases the 'boost' effect of neostigmine is valuable if the tablets are given 30 min before a meal or in anticipation of a special effort.

Pyridostigmine does not have the same peak effect and its plateau of activity is very little longer than neostigmine but it wanes more slowly, allowing a sustained blood level to be achieved by judiciously timed dosage. The 60 mg tablet is approximately equivalent to 15 mg neostigmine.
The useful duration of activity of both neostigmine and pyridostigmine varies individually from 1-8 hours or more. For effective treatment, spacing of dosage should first be established by having the patient keep a written record of the effect of one tablet. This is repeated 4 or more times, with a gap of at least 1 hr after power has relapsed to pre-dosage level. The interval being established, dosages are then timed so that one is taken 30 min before each major meal. Each dose is then increased by ½-tablet increments until maximum improvement occurs. The amount of each dose may be the same, or adjusted according to the activity of the succeeding epoch. In some patients uninterrupted sleep may be preferred, others require regular dosage, day and night.

The most difficult decision is the dose required by a particular patient. In many cases there is no stage of full restoration of neuromuscular transmission, with normal strength, between myasthenic weakness and "cholinergic block" due to overdosage. Furthermore, as the safety factor for transmission is not equally reduced in all muscles the drug requirements of some muscles will represent an overdose for others. In stage 2 or 3 myasthenia, fasciculation of lower limb muscles is common when facial and bulbar muscles are still underdosed. Fasciculation in stage 1 or immediately after thymectomy is not desirable. With suitable precautions for ventilatory control, the endrophonium test is valuable in assessing dose levels, particular attention being paid to chewing, swallowing and breathing. When in doubt, it is always safer to have the patient slightly underdosed. If muscarinic effects are prominent it is customary to use atropine or propantheline. I prefer to avoid them, if possible, because the most reliable indicator of an early cholinergic state is the pupil size, and this guide is lost if anticholinergic drugs are used. The pupil diameter should not be less than 3 mm in normal room lighting.

Ambenonium has a slightly longer action than pyridostigmine and central actions are more common but as its muscarinic side effects are less prominent it may be difficult to detect the onset of cholinergic crisis. A 25 mg tablet is about equivalent to 15 mg neostigmine. I do not support the demand for long acting drugs. All of them have a gradual cumulative effect so that a patient may seem to be correctly dosed on discharge from hospital but return with overdosage signs in 2-3 weeks. This is a serious disadvantage of bis-neostigmine compounds such as distigmin (5 mg). My experience agrees with Pateisky et al (1955) (13). Organophosphorus compounds, which are potent anticholinesterases, are no longer used because of the cumulative effects and because they have more action on central synapses than the quaternary ammonium compounds. Cumulative effects go pass<sup>2</sup> passu with long duration activity. A short acting drug is much safer when used at near-toxic levels of dosage.

Potassium and aldosterone inhibitors

Potassium is used as an adjuvant in MG but I am not convinced that it is beneficial. It may cause nausea and diarrhoea.
resembling cholinergic crisis. Spironolactone, given to conserve potassium, is of no proven value though it gives a sensation of well being. Provision of potassium to counteract loss of intracellular potassium during steroid therapy is quite another matter and its use is rational.

Drugs which lower the safety factor

These should only be used if the indication is clamant, as in infection which is life threatening, or in treatment of associated epilepsy with phenytoin.

Inhibition of ACh production or release

A number of antibiotics have this action; they include streptomycin, dihydrostreptomycin, neomycin, kanamycin, gentamycin, viomycin, bacitracin, polymyxin A and B, and colistin, especially with renal insufficiency. Low ionized serum calcium may be implicated in a presynaptic action (20).

Reduction of stimulus/response relationship

Membrane stabilizers, hydantoinates, quinine, quinidine, and procainamide are, in principle, harmful but rarely cause significant deterioration in MG.

Block of ACh receptors

Relaxation for surgery is complicated by the myasthenic's sensitivity to neuromuscular blocking drugs such as curare, suxamethonium etc, and the anomalous responses which are dose-dependent and vary in different muscles. It is best to use curare; despite increased sensitivity, the mode of action is unchanged and neostigmine is still its antidote.

Drugs which may be dangerous

Enemas may cause sudden death in myasthenics. The mechanism is unknown; stretching of a bowel rendered tonic by anticholinesterases may be postulated. Corticosteroids, ACTH and thyroxine may cause temporary deterioration. Respiratory depressants, including morphine and sedatives, must be used with care, but diazepam is relatively safe. A myasthenic syndrome may be caused by pencillamine but there is no evidence that it aggravates spontaneous MG.

ANTI-IMMUNOLOGICAL THERAPY

The highest treatable level in the chain of pathogenesis leading from the unknown precipitating factor, acting on a genetically susceptible individual, to the disordered motor end-plate is the immunological lesion(s) long proposed and recently accepted, and unquestionably involving the thymus gland. In principle, the autoimmune disorder may respond to i) removal or
suppression of the thymus ii) ultra-thymic immunosuppression iii) removal of antibodies and immuno-aggressive cells.

Removal or suppression of thymus

Controversy about the value of thymectomy was resolved by the analysis of Simpson (1958) (17) which showed a clear benefit in favour of thymectomy, regardless of age or sex of the patient, provided the disease was still in what I later termed 'the active stage' and that there was no thymic tumour. The operation is now entirely safe regardless of the surgical method and the favourable results have improved further with operation at an early stage (4,17). The contrary view of Papatestas et al (1971) (11), was later reversed (12). Even in the active stage, thymectomy is not a cure. Rather it appears to cause a "shift to the left" (towards remission) and the further course becomes more benign, as is increasingly evident with passage of time. Recovery processes appear to be potentiated. Presumably the stage of abnormal immunological tolerance is at first thymic dependent but later becomes autonomous. Regardless of the reasons, the clinical lesson is clear. Thymectomy is beneficial (and safer) if the operation is carried out before these secondary changes take place, and it should be advised as an early treatment (17), not postponed until there is "increasing severity of the myasthenia despite adequate attempts to control symptoms by drug therapy" (14).

It is still controversial whether the pathology of the thymus, at least so far as germinal centres are concerned, is important. In my cases (19), the presence of large numbers of germinal centres has not been associated with a better response to thymectomy, contrary to the opinion of some authors but supported by the extensive New York experience (1).

It is, unfortunately, impossible to aggregate the reports on thymectomy because of differing indications for surgery (notably duration of illness) and methods of assessment (post-operative group vs change of status), but it is now widely accepted that early operation leads to progressive improvement, of unpredictable degree, best evident in the 2nd or 3rd year but sometimes immediate.

Alternative means of suppressing the thymus have not yet been shown to be so effective. Carotid sinus denervation, claimed to cause adrenocortical hypertrophy and thymic atrophy, has been abandoned. Radiotherapy as a method of destroying thymic function is less certain than thymectomy. Pre-operative radiotherapy has been advised before removal of a thymoma (5) and also for non-tumour cases (14,16). I do not consider that the additional benefit is proven and have had no cause to regret abandoning radiotherapy 20 years ago. It may be considered for the rare patient who is "unfit" for surgery, but it should be stressed that the myasthenic state may be temporarily exacerbated.

Ultra-thymic immunosuppression

Early reports on treatment with non-steroid immunosuppressive
drugs were not impressive but merit serious consideration because of the possibility of response in those cases where the autoimmune process is no longer thymus-dependent. Matell et al (1976) (7) support Mertens et al (1969) (8) that there is gradual improvement maximal after 6-15 months, with azathioprine, even after unsuccessful thymectomy or steroids and they have shown a decrease in receptor antibody titre in the serum especially in patients with thymoma (6). Further studies are necessary since the duration of illness may be less relevant for ultra-thymic immunosuppression than for thymectomy.

Corticosteroids are assumed to modify MG by their immunosuppressive action but additional actions on the safety factor for transmission and endplate remodelling are possible. Early reports of "rebound" remission following ACTH, after initial deterioration, were not followed up because of early fatalities until we learned to cope with crisis situations in MG and intensive therapy units became commonplace. It was then possible to use larger dosage of ACTH or prednisone with striking benefit (9). The first week deterioration is associated with a rise of receptor antibody preceding a sustained fall (6). Any corticosteroid or timing regime may be used (2) at any clinical stage, including the most advanced. Serum concentration of AChR antibody decreases (6) and anticholinesterase dosage may be reduced progressively (but not abruptly withdrawn). A common regime is prednisone 100 mg daily or on alternate days for a month or more. Results of treatment are impressive but the side effects and hazards are not negligible (2).

Removal of antibodies and immuno-aggressive cells

Thoracic duct lymph drainage, with removal of 0.5-2 litres daily to a total of 4-56 litres causes improvement within 48 hr. Although myasthenia increases again in a few days, all 20 patients reported by Matell et al (1976) (7) remained greatly improved after more than a year, though the long-term effect might be due to additional immunosuppressive therapy. Serum AChR antibody concentration decreased by 10-60%, and this correlated well with clinical improvement (6). About 10% of the total blood lymphocytes were removed but as they reacted normally to mitogenic stimulation there is no evidence that cell depletion is important. Furthermore, retransfusion of homologous cell-free lymph or an immunoglobulin-containing fraction of lymph worsened the myasthenic symptoms. That the anti-AChR antibody is the important factor is shown by the similar response to plasmapheresis.

Plasmapheresis (plasma exchange) effectively reduces serum anti-AChR antibody with a progressive improvement in strength. Newsom-Davis et al (1978) (10) reported good results in 7 of 8 cases, the exception being congenital myasthenia without detectable elevation of anti-AChR antibody. All but one case was already receiving prednisone and all had had thymectomy. They used up to 9 daily plasma exchanges, usually of 2 litres each, for 10-12 days
and followed this with cyclophosphamide or azathioprine in 3. Muscle power increased after a lag of 2 days. Dau et al (1977) (3) treated 8 cases with good effect. Muscular strength improved during the first exchange. This is also our experience. We have treated 20 cases, with average of six 4 litre exchanges; 3 relapsed in 3-40 weeks but one remitted with a second course. Seventeen patients have not relapsed in a year's follow up. They were started, after the 3rd exchange, on prednisolone 100 mg and azathioprine 150 mg/day for 4 weeks, tailing off during the next 6 weeks. Susceptibility to infection is increased, causing death in 1 case, as also noted with lymph duct drainage (7). It is too early to define the role of antibody depletion in the long term management of MG. If it merely removed an immuno-pharmacological block the role would be restricted to management of myasthenic crisis or for preparation before thymectomy and would not benefit the transmission failure due to morphological changes at endplates. If, as seems possible (10) it also permits synthesis of new receptors, it would be advisable to combine antibody depletion with long-term immunosuppression and this is now our standard practice.

HAZARDS OF TREATMENT

No treatment is free from possible dangers. Signs of overdosage with anticholinesterase drugs are now well recognised and readily avoided. Particular care is required in the first two days after thymectomy (4). Prolonged use of these drugs may damage the motor endplates but this is a small risk and one which must be accepted.

The potential dangers of long term steroids are well known. Although said to be negligible with alternate-day steroid administration, steroids should not yet be considered to be the optimal treatment for MG. With modern management thymectomy is safe, especially if carried out at an early stage by a team experienced in the post-operative care (4). Lymph duct drainage and plasmapheresis cause short term liability to infection, and occasional syncope. They are relatively safe, but for repeated plasmapheresis it is necessary to make an arteriovenous fistula or insert a shunt. Immunosuppressive drugs other than steroids are potentially dangerous to the haemopoietic system, but the risk appears to be less than anticipated.

SUMMARY

Rational treatment of MG requires judicious application of three principles i) immediate and continued use of drugs to raise the safety factor for neuromuscular transmission. Pyridostigmine is the most generally useful and long acting drugs are dangerous, ii) plasmapheresis followed by long term immunosuppression and/or steroids gives temporary relief and promotes endplate regeneration iii) early thymectomy is the only proven method of permanently lowering the autoimmune response. Rationally, the best treatment of
all would be to identify and remove the primary cause, possibly a virus, and to promote regeneration of endplate receptors. These must be the goals of future research.

REFERENCES

Myasthenia Gravis: Treatment Principles

Dr. John A. Simpson, Department of Neurology, Glasgow University and Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland

There is no "best" treatment for myasthenia gravis (MG). However, there are now a number of treatment methods, all of which may be required for different aspects of management. The first priorities are to preserve life by controlling respiration if it is threatened and to promote maximum power of muscles by raising the safety factor for neuromuscular transmission. Then the primary immunological disorder should be treated.

Safety Factor for Neuromuscular Transmission

The safety factor for neuromuscular transmission is raised by drugs that:

- potentiate the production or release of acetylcholine (ACh);
- sensitize the ACh receptors or amplify muscle response; and
- inhibit hydrolysis of ACh by cholinesterase.

Drugs that lower the safety factor for transmission should be avoided.

Guaniidine hydrochloride and 4-aminoypyridine hydrochloride, which increase the release of ACh from motor nerve terminals, have no proven value in the treatment of MG, although they are effective in the carcinomatous myasthenic syndrome. Catecholamines can both facilitate and depress neurotransmission, depending on dosage and concurrent medications. Adrenaline is of no practical value in treating MG, but ephedrine has been claimed to have an antmyasthenic action. Despite exceedingly weak anticholinesterase activity, adrenaline and many of its primary and tertiary amine analogs potentiate the transmission process. Large doses of ephedrine may aggravate weakness, however, and it may not have any useful clinical action on skeletal muscle, the apparent improvement being due to bronchodilation.

Veratrum alkaloids, such as veratrine and germaine dicholate, amplify the response of muscle to neural stimulation and, therefore, increase the efficiency of neuromuscular transmission in MG. However, they also impair muscle and nerve metabolism. Their value is marginal, probably not persistent, and entirely inferior to anticholinesterases.

Anticholinesterase Drugs

Anticholinesterase drugs raise the safety factor for transmission by prolonging the time during which ACh can occupy receptor sites. If the time of occupancy is excessive, however, the endplate becomes desensitized ("cholinergic blockade"). The first effective anticholinesterases were physostigmine and galanthamine, which cross the blood-brain barrier to act on central synapses. The quaternary ammonium compounds in use today, such as neostigmine, probably act on the motor nerve terminals and perhaps the post-synaptic membrane. Some hydroxyanilinium salts and esters, such as edrophonium, also have anticholinesterase activity.

Edrophonium has a peak action too brief for use in treatment of MG. It is, however, a very reliable test agent for confirming the diagnosis of MG and for differentiating between overdosage of anticholinesterases.

Neostigmine should be given by mouth, as it is absorbed rapidly. Crushed tablets administered by gastric tube are preferable to injection when dysphagia precludes normal administration. Neostigmine induces a surge of muscular power that tapers over two to six hours, after which time strength is lost rapidly, making the timing of dosage difficult.

Pyridostigmine does not have the same peak effect and its plateau of activity is only slightly longer than neostigmine. It wanes more slowly, however, allowing attainment of a sustained blood level through judiciously timed doses. For this reason most myasthenics prefer pyridostigmine, although the "boost" effect of neostigmine is valuable before meals or in anticipation of a special effort. Anbenonium has a slightly longer action than pyridostigmine built may be difficult to detect the onset of cholinergic crisis with this drug.

The most difficult decision to be made is what dosage to give a particular patient. The useful duration of activity of both neostigmine and pyridostigmine varies between one and eight hours among individuals. In many patients there is no stage of full restoration of neuromuscular transmission between myasthenic weakness and cholinergic block.

Furthermore, the drug requirement for some muscles will represent an overdose or underdose for others. When in doubt, it is always safe to have the patient slightly underdosed, and the most reliable indicator of an early cholinergic state is pupil size.

Long acting drugs have a gradual cumulative effect so that a patient who may seem to be correctly dosed initially will develop overdosage signs in a few weeks. When using nontoxic dosage levels, therefore, it is much safer to use a short-acting drug.

Potassium, which is sometimes used as adjuvant therapy in MG, may cause nausea and diarrhea that resemble cholinergic crisis. Nor is spironolactone, given to conserve potassium, of proven value, although it contributes to a sense of well-being.

Drugs that lower the safety factor should only be used if the indication is clamant, as in life-threatening infection or in treating concurrent epilepsy with phenytoin.

Drugs and Procedures that Reduce Safety Factor

Medications and procedures that can prove harmful to the MG patient by reducing the safety factor include:

- antibiotics that inhibit ACh production or release, such as streptomycin, dihydrostreptomycin, neomycin, kanamycin, gentamycin, viomycin, bacitracin, polymyxins A, polymyxin B and colistin;
- drugs that reduce the response of muscle to stimulus, such as membrane stabilizers, hydantoinates, quinine, quinidine and procainamide;
- drugs that block ACh receptors, such as those used to provide muscle relaxation during surgery, and of which curare is still preferred;
- respiratory depressants including morphine and sedatives, although diazepam is relatively safe;
- hormones such as corticosteroids, ACTH and thyroxine, which cause temporary deterioration; and
-
• enemas, which may cause sudden death in myasthenics through an as yet unknown mechanism.

The highest treatable level in the chain of pathogenesis leading from the unknown precipitating factor acting on a genetically susceptible individual to the disordered motor endplate is the immunological lesion that unquestionably involves the thymus gland. In principle, the autoimmune disorder may respond to:

- removal or suppression of the thymus;
- ultrathymic immunsuppression; and
- removal of antibodies and immmuno-aggressive cells.

Thymectomy—
“Clearly Beneficial”

Regardless of the age and sex of the patient, thymectomy is clearly beneficial when the disease is in the early ‘active’ stage and there is no thymic tumor. Thymectomy is not a cure, however; rather, it shifts the progress of the disease towards remission and the further course becomes increasingly benign. Presumably the abnormal immunologic tolerance is at first thymus-dependent but later becomes autonomous. Whatever the reason, thymectomy is safe and beneficial and should not be postponed until drug therapy becomes ineffective. Early operation leads to progressive improvement of unpredictable degree that is best evident in the second or third year thereafter, but is sometimes immediate.

Alternative means of thymic suppression have not been as effective. Carotid sinus denervation has been abandoned and radiotherapy is certainly less sure than thymectomy. Radiotherapy may be considered for the rare patient who is ‘unfit’ for surgery, but it should be stressed that the myasthenic state may be temporarily exacerbated.

Early reports on treatment with nonsteroid immunosuppressive drugs, while not impressive, merit further consideration because of the possibility of response in those cases where the autoimmune process is no longer thymus-dependent. The duration of illness may be less relevant when considering ultra-thymic immunosuppression than thymectomy, however.

Corticosteroids are assumed to modify MG by their immunosuppressive action, but no evidence on the safety factor for transmission and endplate remodelling are postulated. Once the ability to handle MG crisis situations evolved, it became possible to use large doses of ACTH or prednisone with striking benefit. Although the results of corticosteroid treatment are impressive, however, the side effects and hazards of corticosteroid therapy are not negligible.

It is too early to define the role of antibody depletion in the long term management of MG. Because it seems to permit the synthesis of new ACh receptors, it is advisable to combine antibody depletion with long term immunosuppression and this is becoming standard practice. Thoracic duct lymph drainage leads to rapid improvement which can be maintained, perhaps with additional immunosuppressive therapy. Plasmapheresis (plasma exchange) is also effective in reducing serum anti-ACh antibody and produces a progressive improvement in strength. Susceptibility to infection, however, increases with antibody depletion.

Rational treatment of MG requires judicial application of three principles:

- immediate and continued use of drugs to raise the safety factor for neuromuscular transmission, for which purpose pyridostigmine is usually best and long acting drugs dangerous;
- plasmapheresis followed by long term immunosuppression and steroids given temporary relief and promotes endplate regeneration; and
- early thymectomy is the only proven method of permanently lowering the autoimmune response.

The best treatment of all would be to identify and remove the primary cause of the disease, possibly viral, and to promote regeneration of endplate receptors. These must be the goals of future research.

References:
PLASMA-EXCHANGE COMBINED WITH IMMUNOSUPPRESSIVE THERAPY IN MYASTHENIA GRAVIS

P. O. Behan  R. A. Shakir  J. A. Simpson
Department of Neurology, Institute of Neurological Sciences, Glasgow
A. K. Burnett
Department of Haematology, Glasgow Royal Infirmary
T. L. Allan  G. Haase
Department of Haematology, Stobhill Hospital, Glasgow

Summary

Twenty-one patients with myasthenia gravis underwent a course of plasma exchange combined with immunosuppressive therapy. In fifteen there was dramatic clinical improvement which has been maintained for periods up to 19 months. Nine of these patients now take no anticholinesterase drugs. Six patients had a recurrence 3-9 months after the first course but in the three given a second course remissions were again obtained.

Introduction

Myasthenia gravis is characterised by fatiguable muscle weakness. Autoimmune mechanisms are clearly involved in its pathogenesis: it is associated with other autoimmune diseases, certain HLA antigens are over-represented, various autoantibodies are found in high frequency, T-cell function is impaired, thymectomy and immunosuppressive and steroid therapy have beneficial effects; and more than 70% of patients have histological abnormalities in the thymus gland. More than 90% of patients with the disease have antibodies to acetylcholine receptor protein (AChR) in significant titre. Similar antibodies are found in experimental autoimmune myasthenia gravis, a disease which can be passively transferred to normal animals by infusion of antibodies from affected donors or patients with myasthenia gravis.

Because of these studies, small numbers of patients have already been treated by plasma-exchange and there is evidence of short-term clinical remission. We report here our observations on a large series of patients with myasthenia gravis treated by plasma-exchange combined with immunosuppression.

Patients and Methods

Thirteen women and eight men aged 27-75 (mean 52) with undisputed myasthenia gravis were studied. All patients had the disease confirmed by a classical clinical history, demonstrable fatiguable weakness, a positive response to edrophonium hydrochloride, and a decrementing response to repetitive supramaximal nerve stimulation. Nineteen patients had the generalised form of the disease and in two only the eye muscles were clinically affected (duration 1-29 yr, mean 9). Seventeen patients had had a thymectomy. Six had associated disorders including rheumatoid arthritis (two), asthma (two), hypothyroidism (one), and epilepsy (one). All patients were on anticholinesterase therapy. Five were also on steroids (30-60 mg prednisolone per day) before plasma exchange, and three of these five had received in addition, in the preceding 6 months, a 3-month course of azathioprine (150 mg daily) without showing clinical improvement.

Plasma-exchange

16-32 l of plasma were exchanged on continuous-flow cell separators over 2-3 weeks; one patient had 14 4-l exchanges in two separate fortnightly periods. Another two patients had two further courses of exchange, with immunosuppression, after recurrences. Replacement fluid in all cases was plasma protein fraction.

Immunosuppression

At the end of the first week—i.e., after the third plasma-exchange—each patient was placed on prednisolone (100 mg daily) and azathioprine (150 mg daily). The azathioprine dose was maintained for 3 months but the steroids were gradually reduced over this period.

Clinical Assessment

Patients were graded before and immediately after treatment and 4 months later. An objective assessment of the severity of myasthenia was obtained from: (1) the longest time that outstretched arms could be held horizontally; (2) the duration of maintained upward gaze without blinking; (3) measurements of peak expiratory flow-rate and lung vital capacity. A clinical grade was then assigned: 0 (excellent); 1 (mild symproms); 2 (moderate symptoms); 3 (moderate severe disease); or 4 (severe disease requiring ventilation).

Follow-up

The duration of follow-up was 3-19 months (mean 9).

Results

All patients showed undisputed clinical improvement at the end of treatment (see table). Indeed, after only three exchanges most claimed some improvement which was often confirmed by the examiner. The two patients with pure ocular myasthenia (12 and 13) had complete resolution of signs and symptoms. Improvement after exchange was also seen as a reduction in clinical grade and confirmed by reduction in the requirement for anticholinesterase drugs after plasmapheresis.

Patients continued to improve with time (see table), so that on outpatient monthly review most patients were...
found to need less drug treatment as time passed; eleven had stopped all drugs 3-6 months after treatment.

The main complications were related to the exchange technique. Repeat exchange was difficult in several patient who had inaccessible veins or in whom the necessity for inserting Scriber shunts or making arterio-
venous fistule produced thromboses. A few patients had sweating and fainting attacks; in two, these were severe enough to cause postponement of the procedure. Fourteen patients stated that within ten days of the plasma-
exchange they had symptoms of a "flu-like" illness, characterised by sore throat, muscle aches, and a mild fever. Viral studies were negative. Other complications were more serious: one patient (no. 1) who had been on a respirator for severe myasthenia gravis associated with a thymoma for several years, had a good clinical response to plasma-exchange but total marrow failure and fatal septicemia then developed. In another patient ster-
od myopathy developed but improved when steroids were stopped. In general, however, the procedure was tolerated well.

There was recurrence in six patients at 3, 4, 7, 8, 8, and 9 months. Recurrent disease was usually more severe. It was precipitated by an upper respiratory tract infection and had a rapid onset. Three of these patients had a further course of plasma-exchange and recovery again was excellent in all three; but one patient (no. 9) relapsed again after 6 months. The other three patients, because of age and the state of their veins, were not given another course.

Discussion

The mainstay of therapy for patients with myasthenia gravis has been the anticholinesterase drugs, which improve neuromuscular transmission. Another form of therapy is thymectomy although how this brings about improvement is not fully understood: it is clear that some immunological reaction occurs but its precise nature is undefined. Success with prednisolone and other immuno-suppressive therapy has also been claimed in a certain proportion of cases. Despite these therapies many patients continue to deteriorate. Of our twenty-
one patients, five made no response to steroids and three of these obtained no benefit from combined steroids and azathioprine. All these therapies also have complications and side-effects, and thymectomy and immuno-
suppressive therapy, for example, may be slow to take effect; they are thus not ideal therapy for the seriously ill patient. Furthermore, steroids may precipitate severe myasthenia gravis with respiratory failure in an other-
wise mild myasthenic.

The usefulness of plasma-exchange in other condi-
tions has been reviewed. It was used first to remove the immunoglobulins in macroglobulinemia and multiple myeloma but is now valuable in the removal of excess of other plasma factors, as in the homozygous familial hypercholesterolemias, and in diseases mediated by immune complexes (variants of polyarteritis nodosa and systemic lupus erythematosus) or by autoantibody, such as Goodpasture's syndrome.

Plasma-exchange has two advantages in the treatment of myasthenia gravis: first, it works quickly and is therefore useful in treating severe, fulminating disease which is causing respiratory embarrassment and in preparing ill and weak patients for thymectomy; second, it is easy to do and its effects, when combined with immunosuppres-
sion, may last for long periods, as we observed. We com-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of disease (yr)</th>
<th>Years since thymectomy</th>
<th>Before plasma exchange</th>
<th>4 months after plasma-exchange</th>
<th>Drugs (mg/day)</th>
<th>Clinical grade</th>
<th>Drugs (mg/day)</th>
<th>Clinical grade</th>
<th>Duration of improvement (months)</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (M, 61)</td>
<td>18</td>
<td>18</td>
<td>4</td>
<td>1080</td>
<td>40</td>
<td>1</td>
<td>120</td>
<td>20</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>2 (M, 51)</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>480</td>
<td>40</td>
<td>1</td>
<td>120</td>
<td>20</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>3 (F, 30)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>360</td>
<td>40</td>
<td>0</td>
<td>240</td>
<td>180</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>4 (F, 26)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>360</td>
<td>40</td>
<td>0</td>
<td>240</td>
<td>180</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>5 (F, 43)</td>
<td>14</td>
<td>14</td>
<td>1</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>6 (F, 64)</td>
<td>29</td>
<td>29</td>
<td>1</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>7 (F, 66)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>8 (F, 46)</td>
<td>20</td>
<td>7</td>
<td>2</td>
<td>120</td>
<td>120</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>9 (F, 68)</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>240</td>
<td>240</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>10 (M, 31)</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>360</td>
<td>360</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>11 (M, 46)</td>
<td>20</td>
<td>18</td>
<td>3</td>
<td>1020</td>
<td>1020</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>12 (M, 46)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>240</td>
<td>240</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>13 (M, 66)</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>14 (F, 56)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>15 (M, 36)</td>
<td>14</td>
<td>7</td>
<td>3</td>
<td>1200</td>
<td>1200</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>16 (F, 34)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>180</td>
<td>180</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>17 (F, 52)</td>
<td>20</td>
<td>18</td>
<td>3</td>
<td>1020</td>
<td>1020</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>18 (F, 47)</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>480</td>
<td>480</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>19 (M, 62)</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>720</td>
<td>720</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>20 (F, 28)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>600</td>
<td>600</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>21 (F, 75)</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>480</td>
<td>480</td>
<td>0</td>
<td>120</td>
<td>120</td>
<td>4</td>
<td>+</td>
</tr>
</tbody>
</table>

Drugs: Pyr=pyridostigmine; Ster=prednisolone.
*150 mg azathioprine daily.
ideal for the rare cases of myasthenic crisis or even cholinergic crisis, in which the anticholinesterases could be removed.

The technique's disadvantages are its cost and the possibility that in some patients clinical improvement may be only short-lived. Our patients showed complications, most of them related to technique, but there may be others: reactions to foreign products, air embolism, infection, and hemorrhage. Hypotension, sweating attacks, and odd pains such as cramps and lumbago-like sensations were also noted by some patients. Fourteen patients complained of a "flu-like" illness. Although no virus could be identified, it is possible that in patients known to have T-cell defects and gross immunological abnormalities, the removal of immunoglobulins together with the initiation of immunosuppression, may either activate a latent virus or increase susceptibility to infection. One of our patients had myasthenia gravis secondary to a thymoma and had been on a respirator for five years, having failed to respond to other forms of therapy including anticholinesterases, thymectomy, and steroids. Overwhelming sepsis, particularly of the lungs and severe sepsis, developed after plasma-exchange. Whether the marrow failure was secondary to infection, or infection complicated the marrow failure secondary to immunosuppression, is a moot point. The thymoma may have contributed to marrow failure since marrow suppressing factors are found in patients with thymoma. The other patient with a serious complication (steroid myopathy) recovered rapidly on withdrawal of steroids.

Our results confirm those in two small groups of patients. Improvement was reported in two patients but not in a patient who had congenital myasthenia gravis. This patient, however, only had four exchanges, which in view of our experience may not have been sufficient. These patients were treated with steroids, cyclophosphamide and azathioprine, started five days after the last exchange and continued for six weeks, followed by a maintenance dose of azathioprine. In a more detailed study, all five patients improved on plasma-exchange combined with prednisone and azathioprine therapy. Ten exchanges were required for improvement. We found major improvement after six, and three of the patients required additional plasmaexchanges to maintain improvement.

The case-history of patient 19 suggests that insufficient plasma-exchange may not improve clinical deterioration. This patient had severe disease but on admission negative findings were obtained with edrophonium hydrochloride and electrophysiological testing. After six exchanges he showed no clinical improvement but the edrophonium test became positive. Complete respiratory failure then developed, which needed a tracheostomy and assisted ventilation. A further course of plasma-exchange, however, brought about an excellent response with continuing improvement. Now, a year later, no drugs are needed and he is well.

There was "no cumulative long-term benefit" in a smaller series of patients than ours (seven), who were treated by immunosuppression and intermittent courses of plasma-exchange. The differences may have arisen because we exchanged a larger volume of plasma and started the immunosuppressive regime at the time of plasma-exchange.

Comparison can be made between our results and those in Goodpasture's syndrome, in which autoantibodies also circulate. Plasma-exchange improves Goodpasture's syndrome and changes its natural history. In myasthenia gravis the post-synaptic endplate is in a state of constant damage and it is conceivable that when aggressive autoimmune factors have been removed, repair will be facilitated. In theory, therefore, the abrogation of the autoimmune response may induce a permanent remission. The mechanism of action of plasma-exchange in myasthenia gravis and the role of the serum factors removed will be discussed elsewhere.

The results of serial immunological studies including acetylcholine receptor antibody titres, complement component determinations, and lymphocyte transformation will also be reported separately. This study strongly suggests that plasma exchange combined with immunosuppression can bring about dramatic and sustained improvement of myasthenia gravis and may alter its natural history.

This work was supported by The Muscular Dystrophy Association of Great Britain.

Requests for reprints should be addressed to P.O.B.  

REFERENCES

Chapter Sixteen

ACETYLCHOLINE RECEPTOR ANTIBODY TITRES IN MYASTHENIA GRAVIS

T Barkas, R Harrison, G G Lunt, F A Stephenson, P O Behan and J A Simpson

One of us first suggested in 1960 that myasthenia gravis was an autoimmune disease [1]. The suggestion was based on the association between myasthenia and other disorders subsequently shown to be autoimmune. The hypothesis was that a breakdown of immunological tolerance in myasthenic patients led to the development of antibodies directed against end plate receptors of skeletal muscle. It is only in the last four years, however, that the presence of such antibodies has been demonstrated in the sera of patients suffering from myasthenia gravis [2-4].

The end plate receptor of skeletal muscle is a nicotinic acetylcholine receptor and the study of its immunology has been facilitated by a combination of two discoveries. First, it was found that the electric organ of various species of electric fish contained a rich source of nicotinic acetylcholine receptors. These are pharmacologically identical to the receptor of the mammalian neuromuscular junction [5]. In contrast to a muscle fibre, which has a single synapse containing approximately $4 \times 10^7$ acetylcholine receptors [6], a single electroplax cell from the electric organ of the eel, _Electrophorus electricus_, contains some $2 \times 10^{11}$ receptors [7]. The cells of the electric ray _Torpedo_ contain even higher concentrations of receptor [8]. Secondly, certain snake venom $\alpha$-toxins have been found to bind with high affinity and great specificity to the acetylcholine binding site of these receptors [9]. The $\alpha$-toxins have accordingly been used not only to purify the receptor, using affinity chromatography, but also, after radioactive labelling, to monitor the purification procedure in terms of acetylcholine binding sites. In this way the isolation of milligram quantities of receptor protein has been achieved [10] and the techniques so developed have been applied to the very much less readily available mammalian receptor [11]. In 1972, Patrick and Lindstrom [12] injected purified eel acetylcholine receptor into rabbits which subsequently developed myasthenia-like signs. The occurrence of this experimental model, experimental autoimmune myasthenia gravis (EAMG), suggested
that an immune response to foreign (eel) acetylcholine receptor could be directed against self-receptor so causing the observed myasthenic signs. This clearly supported the earlier suggestion [1] of an autoimmune basis for myasthenia gravis and caused an explosion of scientific interest in this disease.

The availability of purified eel receptor that could be radioactively labelled with $^{125}$I-α--bungarotoxin (the α--toxin from the Taiwan banded krait) led to the development of an assay for eel acetylcholine receptor antibodies in the sera of experimental animals with EAMG [13]. The assay involved precipitation of antigen-antibody complexes with sheep (or goat) anti-rabbit IgG. We have recently found that protein A from the cell walls of Staphylococcus aureus is equally effective in precipitating the initial antigen-antibody complex [14]. Use of the above assays has shown that levels of circulating antibodies are closely related to clinical signs of weakness in EAMG [4,15,16].

Lindstrom [17] modified the assay for acetylcholine receptor antibodies to detect autologous antibodies in the sera of patients with myasthenia gravis. In the absence of purified human acetylcholine receptor he exploited the specificity of α--bungarotoxin for the acetylcholine-binding sites of the receptor by using the α--toxin to label directly the receptor in a detergent extract of whole human muscle without the need for purification. The detergent extract containing radio-labelled receptor was then allowed to react directly with human serum, and specific antigen-antibody complexes were precipitated with sheep anti-human IgG. In this way human acetylcholine receptor antibodies were detected in the sera of over 90 per cent of myasthenic patients [17,18].

We have assayed human acetylcholine receptor antibodies in 39 myasthenic patients using a modification [19] of the Lindstrom assay. Patients were classified according to severity of both cranial and generalised symptoms on a scale of 0 to 3, where 3 represents the most severely affected state. We find that the means of antibody titres show poor correlation with clinical signs. This is in agreement with the results of Lindstrom et al [4] and Ito et al [20]. The lack of correlation in our data can be largely attributed to the occurrence of occasional extreme titres in all groups of patients. Median titres accordingly show a much better correlation with severity of signs (Table I). The unexplained exceptionally high and low titres preclude unequivocal statements concerning the role of acetylcholine receptor antibodies in myasthenia gravis.

The most dramatic evidence for the involvement of a humoral factor in the aetiology of this disease is the striking clinical improvement in myasthenic patients following plasma-exchange [21,22]. We have monitored the serum levels of human acetylcholine receptor antibodies in eight myasthenic patients during the course of a series of plasma-exchanges. In each exchange four litres of the patient’s plasma were replaced and the process repeated on up to six occasions at two to four day intervals. After the third plasma exchange each patient received 100 mg of prednisolone and 150 mg azathioprine for one month. These drugs were then reduced gradually over the next month.

170
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Antibody Titre $10^{-10} M$ (α-bungarotoxin binding sites)</th>
<th>Clinical State</th>
<th>1st Exchange</th>
<th>2nd Exchange</th>
<th>3rd Exchange</th>
<th>4th Exchange</th>
<th>5th Exchange</th>
<th>6th Exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cranial</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>1</td>
<td>52</td>
<td>F</td>
<td></td>
<td>3</td>
<td>25.7</td>
<td>7.1</td>
<td>16.3</td>
<td>3.0</td>
<td>6.1</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>F</td>
<td></td>
<td>3</td>
<td>37.8</td>
<td>22.7</td>
<td>22.8</td>
<td>2.9</td>
<td>5.6</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td></td>
<td>3</td>
<td>29.8</td>
<td>19.6</td>
<td>25.9</td>
<td>5.5</td>
<td>28.3</td>
<td>10.2</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>F</td>
<td></td>
<td>3</td>
<td>9.6</td>
<td>1.4</td>
<td>0.8</td>
<td>7.8</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>F</td>
<td></td>
<td>3</td>
<td>46.8</td>
<td>8.6</td>
<td>7.1</td>
<td>3.2</td>
<td>44.1</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td></td>
<td>2</td>
<td>14.2</td>
<td>10.8</td>
<td>19.0</td>
<td>11.8</td>
<td>12.0</td>
<td>5.2</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>F</td>
<td></td>
<td>3</td>
<td>1.4</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>F</td>
<td></td>
<td>2</td>
<td>0.3</td>
<td>2.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**TABLE I Antibody Titres of Sera from Myasthenic Patients Undergoing Successive Plasmaphereses**
Seven out of eight patients showed apparently complete remission over time periods of up to six months after the series of plasma-exchanges [23]. Serum samples were taken immediately before and after each plasma exchange and were assayed for acetylcholine receptor antibodies as previously described. Patients 1 to 5 showed similar behaviour in that their antibody titres fell after each exchange but then rose again, not quite, in general, attaining the previous higher value. The overall trend was accordingly down, and all patients showed marked clinical improvement [23]. Patient 6 showed an apparently similar trend except that antibody titres surprisingly rose after each of the fifth and sixth plasma-exchanges and, more importantly, the patient showed little clinical improvement. This patient did not respond to 10 mg of edrophonium chloride given intravenously and had no clinical improvement when treated with up to 900 mg of pyridostigmine daily. Prednisolone given in doses from 60 to 100 mg daily for two months was likewise without any improvement. However, after plasma exchange he showed marked and exquisite sensitivity to edrophonium and to other anticholinesterases. Patients 7 and 8, on the other hand, both achieved complete remission of symptoms after five plasma exchanges, behaviour difficult to explain in terms of their antibody titres which, both initially and throughout, differed little from zero.

Serum from myasthenic patients has been shown to block the electrophysiological response to iontophoretically-applied acetylcholine in cultured human [24], rat [25] and chicken [26] muscle cells. In the case of chicken cells, some myasthenic sera that gave greater than 90 per cent block of acetylcholine-induced response nonetheless had human acetylcholine receptor antibody titres that did not differ significantly from zero.

It is clear from the results described here that, whereas some humoral factor is involved in the aetiology of myasthenia gravis, its effect is not fully reflected in serum human acetylcholine receptor antibody titres assayed by the method of Lindstrom. This method determines antibodies directed at all antigenic sites other than the acetylcholine binding site. It may be that assays of antibodies directed specifically against the acetylcholine binding site of the receptor would give a better correlation with clinical symptoms. This has been found by Zurn and Fulpius [27] to be the case in a rabbit injected with purified Torpedo receptor although Lindstrom [28] dismisses the importance of such antibodies. It is also possible that antibodies directed against other antigenic sites exposed on the membrane-bound receptor could block neuromuscular transmission. The Lindstrom assay includes all such subpopulations and specific assays might prove to be more significant. A recent report from Sobel et al [29] shows that the ionophore responsible for ion-translocation in the post-synaptic membrane is a protein distinct from the acetylcholine receptor protein. Antibodies directed against the ionophore protein could also block neuromuscular transmission and it is doubtful whether such antibodies would be detected in the Lindstrom assay.

Whereas it appears that acetylcholine receptor antibody titres may be useful
TABLE II Antibody Titres of Sera from 39 Myasthenic Patients

<table>
<thead>
<tr>
<th>Clinical State</th>
<th>Antibody Titre $10^{-10}_M$ (a-bungarotoxin binding sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Cranial</td>
<td>General</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

in following the progress of individual patients during therapy the significance of such titres as currently assayed is not clear and development of assays for specific antibody subpopulations may provide a better aid in understanding and monitoring myasthenia gravis.

Acknowledgements

We wish to thank the Medical Research Council, the Science Research Council and the Muscular Dystrophy Group of Great Britain for support of this work.

References

6 Fambrough, DM, Drachman, DB and Salyamurti, S (1973) Science, 182, 293
7 Karlin, A (1974) Life Sciences, 14, 1385
12 Patrick, J and Lindstrom, J (1973) Science, 180, 871

173
18 Lindstrom, JM, Seybold, NE, Lennon, V, Whittingham, S and Duane, D (1976) Neurology, 26, 1054
19 Barkas, T, Harrison, R, Lunt, GG and Stephenson, FA. In preparation
23 Behan, PO, Simpson, JA, Cummings, R, MacDonald, GA. In preparation
26 Harvey, AL, Robertson, G, Barkas, T, Harrison, R, Lunt, GG, Stephenson, FA, Campbell, M and Teague, R. In preparation

174
Eighth Symposium on Current Research in Muscular Dystrophy and Allied Neuromuscular Diseases

held at
The University of Newcastle Upon Tyne
3-5 January 1980

ABSTRACTS OF COMMUNICATIONS
Anti-acetylcholine receptor antibody titres in the sera of myasthenic patients treated with plasma-exchange combined with immunosuppressive therapy.
B.Carter, R.Harrison, G.G.Iunt, P.O.Behan and J.A. Simpson

Myasthenia gravis is now generally accepted to be an autoimmune disease in which circulating antibodies specific for the acetylcholine receptor (AChR) play an important role. Plasma-exchange coupled with immunosuppressive therapy has accordingly been used in treatment of the disease with long term benefit to the patient. The clinical effects of extensive plasma-exchange coupled with a 3-month course of immunosuppression have recently been described (Behan et al. 1979) in the cases of 21 myasthenic patients. We now report serial assays of anti-AChR antibodies in the sera of 19 of the above patients observed over follow-up periods of up to 18 months.

During the periods of observation all 19 patients showed anti-AChR antibody titres that were significantly elevated relative to those of normal controls although there was little correlation between titre and clinical state across the range of patients.

The anti-AChR antibody titre of a given patient followed a standard pattern during each series of plasma-exchanges. Each exchange resulted in a sharp fall in titre which rose before the next exchange, but which, after 3 exchanges, generally remained well below the initial value. After the third exchange the average titre (of 22 courses of plasma-exchange) was 22% of the pre-exchange level while after the complete series of exchanges (average 5.8 exchanges per course) the corresponding figure was 16%.

Following a series of plasma exchanges, the anti-AChR antibody titres remained below pre-exchange levels for the total period of post-exchange observation in 13 out of the 18 patients followed. [One patient developed total sepsicaemia 4 months after completion of exchange (Behan et al., 1979)]. In these cases the general pattern was that of a sharp rise in titre during the first month after a series of exchanges followed by a gradual fall and stabilization of titres over the ensuing months.

In 7 instances, recurrence of symptoms occurred and in 6 of these cases relapse was shown to be associated with a rise in anti-AChR antibody titre, supporting the observation (Newsom-Davis et al, 1979) that an inverse relationship generally exists between clinical state and anti-AChR antibody titres after plasma-exchange.


Anti-acetylcholine receptor antibody titres in the sera of myasthenia patients treated with plasma exchange combined with immunosuppressive therapy

BARBARA CARTER, ROGER HARRISON, GEORGE G LUNT, PETER O BEHAN, AND JOHN A SIMPSON

From the Department of Biochemistry, University of Bath, Bath, and Department of Neurology, Institute of Neurological Sciences, Glasgow.

Summary

Anti-acetylcholine receptor antibody titres have been monitored in the sera of 19 myasthenic patients treated with plasma exchange combined with a three month period of immunosuppressive therapy. In general the post-exchange titres stabilised at below pre-exchange levels for prolonged periods which were associated with clinical improvement. In seven instances recurrence of symptoms occurred and in six of these cases relapse was shown to be associated with a rise in anti-acetylcholine receptor antibody titre.

Myasthenia gravis is now generally accepted to be an autoimmune disease in which circulating antibodies specific for the acetylcholine receptor (AChR) play an important role.1 Plasma exchange has been successfully used in the treatment of Goodpasture's syndrome,2 an autoimmune disease in which antibodies are formed against glomerular and pulmonary basement membranes3 and reports have appeared of its application to myasthenic patients.4,5 These papers describe the results of plasma-exchange used in conjunction with immunosuppressive therapy in the treatment of limited numbers (maximum seven) of myasthenic patients, and serial assays of circulating anti-AChR antibodies over periods of up to 12 months are reported.6 Nineteen myasthenic patients have now been treated by plasma-exchange in combination with a three month period of immunosuppression and the details of their clinical condition throughout an 18 month follow-up period have been reported.7 The anti-AChR antibody titres of these patients were assayed over the same period and these results are discussed in the present paper.

Patients and methods

Patients

Nineteen patients with undisputed myasthenia gravis were studied. All patients had the disease confirmed by a classical clinical history, demonstrable fatiguable weakness, a positive response to edrophonium hydrochloride and a decrementing response to repetitive supramaximal nerve stimulation. There were 12 females and seven males, whose ages ranged from 26 to 75 with a mean of 51 years. Seventeen patients had the generalised form of the disease, whilst two had only the eye muscles clinically affected. Myasthenia had been present from 2-29 years with a mean duration of 10 years. Sixteen patients had had a thymectomy previously.

All patients were on anticholinesterase therapy. Five were also on steroids (30-60 mg prednisolone per day) prior to plasma exchange, and three of these five patients had received, in the preceding six months, a three-month course of azathioprine (150 mg daily), in addition to their steroids, without showing any clinical improvement.

Plasma exchange Patients had from 16-32 litres of plasma exchanged on continuous flow cell separators over a period of two to three weeks. Replacement fluid in all cases was plasma protein fraction.

Immunosuppression At the end of the first week of plasma exchange, that is, after the third exchange, each patient was placed on prednisolone (100 mg daily) and azathioprine (150 mg daily). The azathioprine dose was maintained for three months but the steroids were gradually reduced over this period. Patients undergoing a single course of plasma exchange were not treated with
immunosuppressive drugs beyond this point, whereas those undergoing a second course of plasma exchange were treated with drugs over a further three month period exactly as described above.

Clinical assessment Patients were graded before treatment and four months later. An objective assessment of the severity of myasthenia was obtained using: 1 the maximum time that outstretched arms could be held horizontally; 2 measurements of lung vital capacity (table).

\textsuperscript{131}I-Iodination of a-bungarotoxin a-Bungarotoxin from \textit{Bungarus multicinctus} was purchased from Boehringer, Mannheim as a lyophilised powder (1 mg) which was reconstituted in distilled water (2 ml) and stored at \(-20^\circ\text{C}\) prior to use. The stock solution (20 ml) was added to \textsuperscript{131}Iodine (100 mCi/ml). The Radiochemical Centre, Amersham (10 \mu l), 0.5% w/v Chloramine T in 0.01 M-potassium phosphate buffer, pH 7.5, (10 \mu l) and 0.05 M-potassium phosphate buffer, pH 7.5 (10 \mu l). The mixture was stirred for 1 min after which were added 0.016%/ sodium metabisulphite in 0.01 M-potassium phosphate buffer, pH 7.5 (0.75 ml) and 1% /KI in 0.01 M potassium phosphate-buffer, pH 7.5 (0.2 ml) giving a final volume of 1 ml. The solution was added to a column (27 cm\times 1 cm) of Sephadex G-25, previously allowed to swell overnight in 0.05 M-potassium phosphate buffer, pH 7.5, and equilibrated in 0.01 M potassium phosphate buffer, pH 7.5, containing 1% bovine serum albumin) and eluted with 0.01 M potassium phosphate buffer, pH 7.5, containing 1% bovine serum albumin. Fractions (1 ml) were collected and samples (5 \mu l) counted on a \(\gamma\)-counter. The most active fractions of the first, protein-containing peak of radioactivity were combined and the specific activity of the pooled fractions (the "stock" solution) was calculated assuming total recovery of protein and relating this to acid-precipitable counts. The "stock" solution was diluted 100 times in assay buffer (0.01 M-potassium phosphate, pH 7.4, containing 0.01 M-Na\(_2\)PO\(_4\), 0.1% /+, bovine serum albumin and 1% v/v Triton X-100) to give the "working" solution used in determination of AChR activity and in the radioimmunoassay.

Preparation of AChR from human skeletal muscle Human skeletal muscle was obtained from amputated legs at the time of operation and either used immediately or stored at \(-20^\circ\text{C}\). The chopped muscle was homogenised (1 min) in 4 volumes of phosphate-buffered saline containing 0.01 M-potassium phosphate, 0.1 M-NaCl and 0.01 M-Na\(_2\)PO\(_4\), and then centrifuged at 20,000 \(g\) for 1 h at \(+4^\circ\text{C}\). The pellet was resuspended in 2 volumes of phosphate-buffered saline, as above but with the addition of 2%, v/v Triton X-100, stirred for 16 h at \(+4^\circ\text{C}\) and centrifuged at 20,000 \(g\) for 1 h at \(+4^\circ\text{C}\). The resulting supernatant was filtered through glass wool to remove lipid particles and stored at \(+4^\circ\text{C}\).

The concentration of AChR in the receptor preparation was determined by the binding of \textsuperscript{125}I-a-bungarotoxin. The receptor extract

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Duration of disease (yr)</th>
<th>Years since thymectomy</th>
<th>Vital capacity (litres)</th>
<th>Duration of outstretched arms (seconds)</th>
<th>Drugs per mg/day</th>
<th>Vital capacity (litres)</th>
<th>Duration of outstretched arms (seconds)</th>
<th>Drugs mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>M</td>
<td>61</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>1080</td>
<td>40</td>
<td>3.2</td>
<td>56</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>51</td>
<td>6</td>
<td>1</td>
<td>2.5</td>
<td>400</td>
<td>3.5</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>30</td>
<td>2</td>
<td>2</td>
<td>3.0</td>
<td>360</td>
<td>40</td>
<td>3.0</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>26</td>
<td>3</td>
<td>3</td>
<td>2.8</td>
<td>720</td>
<td>3.5</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>43</td>
<td>8</td>
<td>2</td>
<td>2.8</td>
<td>360</td>
<td>3.5</td>
<td>156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>64</td>
<td>29</td>
<td>4</td>
<td>2.8</td>
<td>420</td>
<td>3.4</td>
<td>140</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>66</td>
<td>4</td>
<td>3</td>
<td>3.0</td>
<td>780</td>
<td>3.5</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>44</td>
<td>7</td>
<td>2</td>
<td>2.6</td>
<td>120</td>
<td>3.3</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9*</td>
<td>M</td>
<td>68</td>
<td>10</td>
<td>4</td>
<td>2.4</td>
<td>720</td>
<td>3.0</td>
<td>91</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>46</td>
<td>20</td>
<td>18</td>
<td>2.5</td>
<td>1020</td>
<td>3.5</td>
<td>101</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>11†</td>
<td>M</td>
<td>46</td>
<td>5</td>
<td>2</td>
<td>3.5</td>
<td>240</td>
<td>3.5</td>
<td>not impaired</td>
<td>not impaired</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>66</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>720</td>
<td>3.4</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>56</td>
<td>2</td>
<td>—</td>
<td>3.2</td>
<td>720</td>
<td>3.5</td>
<td>117</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>14*</td>
<td>M</td>
<td>36</td>
<td>10</td>
<td>7</td>
<td>3.0</td>
<td>1200</td>
<td>40</td>
<td>3.5</td>
<td>110</td>
<td>360</td>
</tr>
<tr>
<td>15†</td>
<td>F</td>
<td>52</td>
<td>18</td>
<td>3</td>
<td>3.5</td>
<td>1020</td>
<td>3.5</td>
<td>not impaired</td>
<td>not impaired</td>
<td>720</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>47</td>
<td>16</td>
<td>10</td>
<td>2.2</td>
<td>480</td>
<td>3.5</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>62</td>
<td>5</td>
<td>3</td>
<td>2.4</td>
<td>720</td>
<td>3.4</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>26</td>
<td>3</td>
<td>3</td>
<td>2.6</td>
<td>600</td>
<td>3.5</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>75</td>
<td>5</td>
<td>2</td>
<td>2.1</td>
<td>480</td>
<td>3.5</td>
<td>173</td>
<td>240</td>
<td></td>
</tr>
</tbody>
</table>

* thymoma; † focal myasthenia only; ‡plus 150 mg azathioprine daily. pyr. = pyridostigmine; ster. = prednisolone.
Anti-acetylcholine receptor antibody titres in the sera of myasthenia patients

(100 μl) was mixed with 125I-α-bungarotoxin solution (see above) (50 μl), saturated (NH₄)₂SO₄ solution (104 μl) and assay buffer, (100 μl), and the mixture was incubated for 16 h at +4°C. The resulting precipitate was collected on glass fibre filters (Whatman Ltd., Maidstone, U.K.) washed with 30% \( \frac{\text{aq}}{\text{aq}} \) aqueous (NH₄)₂SO₄ solution and counted in a γ-counter. The assay was repeated as above except that the assay buffer additionally contained 0-1 mM-curare and the counts so obtained were subtracted to give specific binding. Anti-AChR antibody assay Anti-AChR antibodies in the sera of myasthenic patients were assayed by a modification of the assay described by Lindstrom.⁸

AChR extract (0-9 ml, 1-5-20×10⁻¹⁰ M) was mixed with assay buffer (100 μl) and ¹²⁵I-α-bungarotoxin “working” solution and incubated for 6-8 h at +4°C. Test serum (5 μl) was added to the mixture which was then allowed to stand at +4°C for 16 h before goat anti(human IgG) antiserum was added to precipitate the ¹²⁵I-α-bungarotoxin–AChR–antibody complex. The supernatant was removed after centrifugation (3000 × g, 10 min) and the pellet was washed with 0-01 M-potassium phosphate buffer, pH 7-0, containing 0-85%, \( \frac{\text{aq}}{\text{aq}} \) NaCl and 0-01 M-Na₂SO₄ and counted in a γ-counter. Correction for non-specifically-bound ¹²⁵I-α-bungarotoxin was made by carrying out parallel assays in which the AChR extract was mixed with 0-1 mM-curare prior to addition of ¹²⁵I-α-bungarotoxin. The counts so obtained were subtracted from those in the assay described above, giving specific binding which was expressed as moles ¹²⁵I-α-bungarotoxin binding sites per litre of serum.

In general, series of serum samples from an individual patient were assayed using a single AChR extract. However comparability between extracts was always checked by repeat assays on standard samples of myasthenic sera.

Serum samples taken in Glasgow were immediately frozen at −20°C and were maintained frozen until their assay, which was performed at Bath.

Results

Nineteen myasthenic patients (table) were plasma-exchanged over a period of two to three weeks, after the first week of which an immunosuppressive regimen was initiated and subsequently maintained for three months (see the Patients and Methods section). Anti-AChR antibody titre in the sera of these patients were periodically assayed for up to 18 months (mean 12 months) following plasma exchange, during which time five patients (cases 3, 6, 7, 9) were treated with a second course of plasma exchange and immunosuppression. The clinical states of these patients have been fully described in a previous paper.

During the periods of observation all 19 patients showed anti-AChR antibody titres (fig 1) that were significantly elevated (3-284×10⁻¹⁰ M α-bungarotoxin sites) relative to those of normal controls (0-6×10⁻¹⁰ M sites, SEM, 0-1, n=20).

The titre of each patient was measured during the course of plasma-exchange and a typical pattern of values is shown in fig 2. Not surprisingly each exchange resulted in a sharp fall in anti-AChR antibody titre which rose before the next exchange, but which after three exchanges generally remained well below the initial value. After the third exchange the average titre of 22 courses of plasma-exchange was 22% of the pre-exchange level while after the complete series of exchanges (average 5-8 exchanges per course) it was 16%.

Following a series of plasma exchanges, the anti-AChR antibody titres remained below pre-exchange levels for the total period of post-exchange observation (between 6 and 18 months) in 13 out of the 18 patients followed. One patient (case 1) died 4 months after completion of exchange. In these cases the general pattern was that of a sharp rise in titre during the first month after a series of exchanges followed by a gradual fall and stabilisation of titres over the ensuing months (fig 1). Of the five patients (cases 3, 5, 11, 17, 19) who showed elevated post-exchange anti-AChR antibody titres (that is above pre-exchange levels), three (cases 3, 17, 19) suffered a recurrence of myasthenic symptoms coincident with their elevated titres (fig 1b (c)). In addition four further patients (cases 6, 7, 9, 14) underwent relapses. In three of these (cases 6, 7, 14) the recurrence of symptoms was also associated with a peak of anti-AChR-antibody titre although in these cases pre-exchange levels were not surpassed. The remaining patient (case 9) suffered two relapses (fig 1(d)) which were not shown to coincide with elevated titres.

It is noteworthy that of the three patients with thymoma, all had relatively high pre-exchange anti-AChR antibody titres and two (cases 9 and 14) suffered subsequent relapses. The third (case 1) died of total marrow failure and septicemia.⁸ The numbers are clearly too small, however, to attach particular significance to this at the present stage.
Barbara Carter, Roger Harrison, George G Lunt, Peter O Behan, and John A Simpson

Fig 1 Anti-AChR antibody titres of myasthenic patients monitored over periods of time that include at least one plasma-exchange.
(a) • —•, Case 1: △ — △, Case 4; ■ —■, Case 14; ○—○, Case 16: △ — △, Case 18.
(b) ● —●, Case 9; △ — △, Case 17; ■ —■, Case 19.
(c) ● —●, Case 7; △ — △, Case 12; ■ —■, Case 13; ○—○, Case 15.
(d) ● —●, Case 2; △ — △, Case 6; ■ —■, Case 10.
(e) ● —●, Case 3; △ — △, Case 5; ■ —■, Case 8; ○—○, Case 11.
Dotted lines thus: . . . 5 . . . indicate a series of plasma-exchanges; the number of individual exchanges being shown in the circle. © indicates recurrence of myasthenic symptoms.

Discussion

Our observation of elevated anti-AChR antibody titres in all the myasthenic patients described in this paper is consistent with previous figures of around 90% obtained from large samples of myasthenics in a range of clinical states. Although correlation of antibody titre with severity of disease is generally poor, it is true that the clinical state of a given patient is often reflected in his anti-AChR titre over a period of time and the patients chosen for plasma-exchange contain many whose clinical condition, in spite of conventional treatment, has continued to deteriorate. The probability of elevated titres in these patients might accordingly be expected
Anti-acetylcholine receptor antibody titres in the sera of myasthenia patients

to be somewhat higher than that of a random sample.

The lack of good correlation between anti-AChR antibody titre and clinical state across a range of patients is well illustrated by the present data. Thus the pre-exchange titres range from 0.8 to 284×10^10 M α-bungarotoxin-binding sites of which one of the highest (205×10^10 M sites) was shown by a patient (case 4) with relatively good physical performance (table) whereas a pre-exchange titre of only 2.8×10^10 M α-bungarotoxin binding sites was obtained from one (case 3) of the four patients confined to a respirator.

The pattern of anti-AChR antibody titres shown in fig 2 is typical of that obtained during the course of each series of plasma exchanges in the present study and is similar to others reported previously.32

The combination of plasma-exchange with a three month course of immunosuppression in the present study led to a marked clinical improvement2 which was maintained for up to 18 months (mean 12.5 months) in 11 out of 19 patients. A further four patients had a second plasma-exchange following recurrence of symptoms (after 4, 8, 10 and 10 months respectively). After this their second exchange their clinical state improved and was maintained for 13, 9, 10, and 2 months respectively.8 In 13 out of these 15 recovered patients their sustained clinical improvement was associated with anti-AChR antibody titres that apparently remained below the pre-exchange levels throughout the post-exchange period. Conversely, of the seven patients who suffered recurrence of myasthenic symptoms (cases 3, 6, 7, 9, 14, 17, 19), six (cases 3, 6, 7, 14, 17, 19) showed a worsening of their clinical condition that was accompanied by an elevation in anti-AChR antibody titre, although in only three of these cases (cases 3, 17, 19) was the pre-exchange level surpassed. Our results clearly support earlier conclusions, based on smaller numbers of patients treated with extended immunosuppression,5 7 33 that an inverse relationship generally exists between clinical state and anti-AChR antibody titres after plasma-exchange, although we have observed both elevation of titre without obvious relapse (cases 5, 11) and relapse without observed rise in titre (case 9; in this case, however, a transient rise in titre could well have been missed).

The fact that anti-AChR antibody titres generally correspond to clinical condition when serially assayed in a single patient and yet not when compared across a range of patients, sug-

ests either that individuals have widely different threshold values for antibody-initiated impairment of function or that anti-AChR antibodies as presently assayed are not the primary agents in the pathobiology of myasthenia gravis. They could merely reflect variations in a more basic pathogenic factor which could, for instance, simply be a particular sub-population of AChR antibodies. Heterogeneity of anti-AChR antibodies in myasthenic sera has been demonstrated by several combinations of differential assay methods (Mittag et al.34), but no sub-population whose titres show close correlation with disease state has yet been identified.

Newson-Davis et al.7 have compared the long term reductions in anti-AChR antibody titre brought about in 7 patients receiving plasma-exchange, in combination with extended periods of immunosuppression, with the reductions produced in 6 patients treated by immunosuppression alone. They found that the percentage decrease in AChR antibody titre was not significantly different in the two groups of treated patients and concluded that plasma-exchange is of use only in the short term control of severe myasthenic symptoms. Our presently described use of plasma exchange combined with a three month period of immunosuppression has led to dramatic clinical improvement in most of the 19 patients so treated and the general reduction of anti-AChR antibody titres over a long period is comparable with that obtained by Newson-Davis and his colleagues. The evidence7 that a more extensive series of plasma exchanges can lead to greater chances of long term improvement is of interest in view of the anti-AChR antibody titre profiles observed during the course of a single series of plasma exchanges. As plasma-exchanges beyond the third appear to lead to relatively little further reduction in anti-AChR antibody titre, it is possible that the reduction in titre per se is of less long term relevance than the repeated stimulation of the immune system in the presence of anti-metabolites. Thus it may be, as suggested by Dau et al.,5 that azathioprine mediates a semi-selective cytotoxic action on specific lymphocytes proliferating in response to removal of anti-AChR antibodies from the circulation. The continued monitoring of the patients treated in the present study may well serve to clarify this issue. Meanwhile the demonstrated efficacy of plasma-exchange combined with a limited period of immunosuppression in bringing about sustained clinical improvement together with depressed anti-AChR antibody levels serve to recommend
its use as a viable treatment of severe myasthenia gravis.

We are grateful to the Muscular Dystrophy Group of Great Britain for support.

References

18. Weinberg CB, Hall ZW. Antibodies from patients with myasthenia gravis recognise determinants unique to extrajunctional acetylcholine receptors. Proc Nat Acad Sci USA 1979; 76:504-8.
Myasthenia Gravis

JOHN A. SIMPSON

Most physicians, and certainly all neurologists, would recognise a 'typical' myasthenic facies. The asymmetrical ptosis, and squint associated with weakness of eye closure (a valuable sign), facial weakness, and a tendency to use the hand to support the jaw and head, all add up to an unmistakable picture. The diagnosis is also clear if the patient complains of limb weakness, especially shoulder girdle, which increases with effort and is relieved by a short rest. Unfortunately many patients do not mention that weakness is worse in the evening than in the morning, though they immediately recognise this when asked about it—that is when the doctor already suspects the diagnosis. In fact, this is not the typical picture. It is much more difficult to diagnose when presented with a young healthy looking girl complaining of double vision, loss of voice or severe muscular weakness occurring intermittently and in whom the history points to a clear relation between symptoms and an emotional disturbance, a common precipitating factor. Rapid routine examination of motor function may not reveal any loss of power. It is necessary to fatigue the muscles complained of. Not surprisingly, the initial diagnosis is usually hysterical paralysis or (because of the relapsing-remitting course) multiple sclerosis.

If the diagnosis of myasthenia gravis is considered, the diagnosis is easy. A simple fatigue test is usually sufficient and it may be supplemented by the edrophonium (Tensilon) test. Although often said to be misleading, I find it a very reliable test if carried out correctly and if only objective signs are used. So called 'false positive' results are due to accepting the patient's subjective assessment which is frequently unreliable. Electromyography is unnecessary except for cases in remission at the time of investigation. All the methods used, including the various modifications of the Harvey-Masland test, curarisation, and even single-fibre electromyography may be normal at this time. If positive, they show only that there is diminution of the safety factor which is normally present for neuromuscular transmission, i.e. the surplus of acetylcholine, released by maximal nerve stimulation, over the amount required to depolarise all the ACh receptors at the endplates. The recently detected antibody against ACh receptor is highly specific but may be undetectable by present techniques in some patient with unuestionable myasthenia gravis.

Myasthenia gravis affects people of all ages, sex and race. Onset is most common in the third decade of life and is then much more common in women. Late onset myasthenia is more equally

Professor of Neurology, University of Glasgow,
From the Institute of Neurological Sciences,
Southern General Hospital, Glasgow, Scotland.

Based on Chandy Oration delivered at the Annual Conference of the Neurological Society of India, Calcutta, December 1980.
distributed between the sexes and indeed becomes more common in the male. As the genetic background and thymic pathology are different in late onset disease there are many who consider that there are a number of different but related diseases. This may be so, but the data may also be interpreted as indicating that different risk factors become dominant at different times of life. The recognition of the rare congenital form of myasthenia was an early pointer to a genetic factor but its validity is in dispute as current research is disclosing that many (though probably not all) of these cases have a different defect of neuromuscular transmission and no ant-AChR antibody. They must not be confused with neonatal myasthenia gravis. In this disorder the baby born to a myasthenic mother (never a myasthenic father) has neostigminesensitive myasthenia for the first few weeks of life and then recovers. This happens in 1 in 7 live births to myasthenic mothers and was one of the clues leading to the original autoimmune hypothesis of myasthenia gravis since it indicates passage of a large molecule blocking substance through the placenta. Equally significant was my observation of a familial linkage between myasthenia gravis and disorders of the thyroid and other autoimmune diseases. This fact, coupled with the evidence that myasthenia is rarely found in identical twins, suggested that there is an inherited factor which permits development of autoimmune diseases, including myasthenia gravis. According to present idea this is likely to be an immunoreactive (Ir) gene. We cannot detect this in human chromosomes but can identify nearby genes on the sixth chromosome controlling responses to leukocyte antigens (HLA). It is generally agreed that the linked HLA antigens A1, B8, Dw3 are important in the younger myasthenics but not obligatory. (In Japan the linkage is with B12 but I understand that Indians resemble Europeans). It is controversial whether late onset and thymoma-related myasthenia exhibit different linkages but, as I have already said, this would not necessarily imply different disease processes. They could be linked to risk factors at different ages and to the body’s reaction to them, including the severity and distribution of the disease.

The subdivision of myasthenia into Osserman groups implies that these have different courses or response to treatment. I find this of very limited value but instead have stressed three clinical stages. Stage 1, the 'active' stage is the period in which remissions can be expected but in which most of the deaths occur. It is the stage in which the further course can be modified by thymectomy. In Stage 2, there is still active myasthenia as assessed by response to anticholinesterase drugs and presence of humoral antibodies but the risk of death is much lower and the possibility of satisfactory remission is low. Clearly, it is not justifiable to base treatment on the expectation of spontaneous remission, but equally it may not be justifiable to employ therapies which are dangerous either immediately or in the long term (and this includes steroids). Some patients eventually reach a third stage with neuro-muscular damage which cannot be corrected by anticholinesterases but with less severe physical disability. Immunological reactivity is much less than in the first two stages but, perhaps surprisingly, striking improvement may still occur with steroid therapy. There is no doubt that steroids
are immunosuppressive, but I question whether this is the sole or even the most important action in myasthenia gravis. Recent work in my laboratory supports the concept that steroids may promote regeneration of receptors or endplates. To interpret these clinical facts we must now turn to the pathology and pathogenesis.

Pathology

Since the beginning of this century it has been known that some patients with myasthenia gravis have a thymic tumour, usually noninvasive (thymoma) but this accounts for only 15% of cases. Norris9 drew attention to a high incidence of lymphoid “hyperplasia” of the thymus and Sloan16 stressed the importance of ‘germinal centres’ in the medullary portion of the glands. It is less widely known that many glands are atrophic, especially in late onset myasthenia and that the beneficial effect of thymectomy is apparently not related to the histological type12. In the long term, the prognosis is worse for patients with a thymoma, even after surgical removal, but the short term results of thymectomy may be equally good. Prior to 1961 thymus was considered to be a ductless gland but it seemed to me, in postulating an autoimmune disease, that the germinal centres indicated immunological activity, a point of view which is now orthodox.

The other feature recognised for many years is the lymphorrhage in muscle. It is inconstant and possibly transitory. Its perivenular position also suggested an immunological reaction though not at the neuromuscular junction.5 We now know that both thymic germinal centres and lymphorrhages in muscle are not unique to myasthenia gravis but they do appear to be markers of immunological activity.

To account for the natural history, neonatal myasthenia, thymic and muscle pathology and some related diseases of other tissues which will be described later, I formulated an autoimmune hypothesis in the 1950s and published this in 1960.8

Pathogenesis

At that time the popular theories of myasthenia gravis were either that the thymus produced a “curare-like substance” or that the neuromuscular disorder was a form of thyrotoxic myopathy, because of a recognised correlation with thyrotoxicosis. The latter possibility was excluded by the experimental studies of Engel1 and my finding that myasthenia was correlated with all non-tumour types of thyroid disease. The possible role of a thymic toxin persists to this day.

Soon after Simpson9, Strauss et al11 reported that serum from myasthenic patients contained a complement-fixing antibody against the myosin of skeletal muscle (“anti-striational antibody”). It later turned out that some of the reported findings were non-specific but antibody against the A-band of muscle was highly correlated with thymoma (irrespective of whether this is associated with myasthenia or not). Indeed this is now the best method for detecting the presence of a thymoma though it is usually visible on a chest radiograph. When it was then shown that the same antibody would fix to myoid cells in the thymus, some workers postulated that the initial immunological attack was on these myoid cells and only secondarily on skeletal muscles with which they share antigens. It should be noted that this is an antibody against a constituent of

47
the muscle fibre, not against the endplate. For the next 12 years all efforts to find an acetylcholine receptor (AChR) antibody were fruitless and the tide of opinion turned against all autoimmune theories. (Readers requiring detailed references should consult Simpson 1977, 1978). In 1973 Patrick and Lindstrom were investigating the protein of AChR by raising antibody against receptor protein purified from the electric organ of the electric eel, which is a homologue of the endplate of muscle. They noticed that the animals used to produce the antibody became weak with an illness like myasthenia gravis. This confirmed the feasibility of the mechanism I had proposed, namely that antibody against AChR could block neuromuscular transmission. Immediately a range of animals with "experimental autoimmune myasthenia gravis" became available. The other important technical advances since 1960 were the evidence that spontaneous miniature endplate potentials are reduced in size at myasthenic endplates (which provided an electrophysiological method for detecting endplate damage even in animals without clinical weakness) and the recognition of the specific affinity between α-bungarotoxin (a snake venom) and AChR which gave a method for identifying ACh receptors histologically (by labelling the toxin with an isotope or immunoperoxidase) and for identifying the presence of anti-AChR substances in blood by showing they could prevent α-bungarotoxin from reaching receptors. This led to the development of a number of assay procedures and the eventual identification of anti-receptor antibodies. Finally Engel et al convincingly demonstrated the presence of IgG (and the C₃ component of complement at the postsynaptic membranes of human myasthenic neuromuscular junctions and gave ultra-microscopic evidence of destruction of a proportion of the ACh receptors. The post-junctional lesion is now considered to be the cause of the lowered safety factor for neuromuscular transmission. At the ultramicroscopic level, it now appears that antibody IgG combines with sites on two adjacent ionophores of the postsynaptic surface, rather than with the actual receptor site, and this causes a conformational change which prevents the pores from opening. In addition, along with complement the IgG causes lysis of receptors. It seems likely that there are a number of subtypes of anti-AChR antibody and only one of these may be significant in pathogenesis. This might account for the fact that the antibody titre assessed by contemporary techniques has a poor correlation with the severity of the myasthenia (as judged by Osserman type) though it fluctuates in parallel with changes of severity in each subject. (A race is now on to detect a more specific sub-type of antibody and to raise an anti-idiotype antibody against it, which would have great possibilities for the treatment of myasthenia gravis).

The critical question is, what causes this immunological attack on the endplate? Some workers still favour the idea of initial attack on the thymus which is presumed to share antigens with skeletal muscle. Clearly this would only be tenable if there is AChR protein in the thymus. This is at present controversial: some workers believe there is but others do not. Personally, I do not regard this is a vital matter to decide, for the following reason.
MYASTHENIA GRAVIS

Associated disorders

I have already indicated that the most important reason for conceiving the autoimmune hypothesis was my observation that myasthenia gravis is linked (probably genetically) with a number of diseases: thyrotoxicosis, non-toxic goitre, Hashimoto's disease, primary myxoedema, pernicious anaemia, "rheumatoid" arthritis, epilepsy, pemphigus, juvenile onset diabetes and other diseases which we now recognise as having an immunological component. Clearly, if the thymic myoid cell genesis of myasthenia is correct, it is necessary either to have primitive thyroid, gastric, joint, skin and islet cells in the thymus (for which there is no evidence) or postulate an entirely separate pathogenesis for these associated autoimmune diseases. I simply do not believe that Nature works that way. It is much more likely that there is a multiple but not generalised breakdown of immunological tolerance for "self" constituents and therefore that the thymus plays some role in establishing tolerance.

We have learned a lot about immunological mechanisms in the last decade, but it would be arrogant to consider that we have a complete picture. Something is missing, and the clues are present in clinical studies. A plausible hypothesis which has a vogue at present is that immunological attack on "self" antigens is prevented by suppressor T-cells from the thymus and so autoimmune disease may represent relative failure of suppressor cells. Against this, I have drawn attention to sudden flare up of other autoimmune diseases soon after effective thymectomy for myasthenia gravis and this suggests that suppressor cell were active before thymectomy. This is not an appropriate occasion to go further into the immunological problems of myasthenia gravis. I refer those interested to a recent review. In these 20 years a hypothesis has become a theory and then accepted dogma as theoretical biology and technological developments have caught up with conclusions derived from clinical observations. Where there is still an incompatibility, let us not assume too hastily that the clinical observations are wrong. Time after time it has been shown that awkward clinical findings become explicable as scientific knowledge advances. Where they don't fit, it is likely to be the theory that is wrong. There is still an important role for the observant clinician.

REFERENCES

8. Simpson, J.A. History and current concepts of the autoimmune nature of myas-


Clinical Constraints to Pathogenesis Models of Myasthenia Gravis

John A. Simpson

"The weakness and fatigability characteristic of skeletal muscles in patients with myasthenia gravis (MG) is now known to result from impaired neuromuscular transmission due to an autoimmune response to skeletal muscle acetylcholine receptors (AChR)." Few will now dispute this conclusion. It is unnecessary to review the evidence on experimental autoimmune myasthenia gravis (EAMG). The demonstration by Engel et al. (1977) that IgG and the C3 component of complement are present as immune complexes bound to the postsynaptic membrane of the neuromuscular junctions in human MG is convincing support for the Simpson (1960) hypothesis, and it can scarcely be doubted that the IgG demonstrated there is anti-AChR antibody, though this has not been proven directly. In addition, these authors have shown that the amplitude of the miniature endplate potentials is directly correlated with the length of the postsynaptic membrane binding immune complexes, and the abundance of immune complexes decreases with increasing severity of the disease. These observations point to a progressive loss of immunoreactive acetylcholine receptor sites as the disease advances.

The conclusion of Engel et al. (1977) that the presence of IgG with C3 component of complement would, if followed by sequential activation of C5-C9, lead to lytic destruction of the postsynaptic membrane is indisputable and would be in harmony with earlier evidence for loss of binding sites for α-bungarotoxin at myasthenic endplates (see review by Simpson). Engel and his colleagues rightly point out that this does not exclude other effects of anti-AChR antibody. There is functional evidence that anti-AChR antibody increases the degradation of junctional (and extrajunctional) receptors of muscle and induces modulation of AChR without involving complement.

An immunopharmacological block, as suggested by Simpson (1960), remains a possibility. This concept might be supported by the rapid beneficial effects observed in experimental autoimmune myasthenia gravis.
effect of plasma exchange, but not necessarily so, as the regeneration of receptors is probably very rapid and there is usually a time lag of two days or more before muscle power is restored. Myasthenic symptoms are reported to relapse promptly after retransfusion of homologous cell-free lymph to patients who have benefited from lymph duct drainage. The blocking effect of serum from MG patients on cultured chick muscle end-plates is evident within 30 minutes. A recent report from Japanese workers states that serum from myasthenic patients reduces the MEPP amplitude of rat endplates and that this effect can be reversed by washing with a control solution.

Passive transfer of anti-AChR antibody and immune complexes from MG patients would help to differentiate between the three main possibilities: i) lysis of postsynaptic membrane, ii) IgG-induced modulation of AChR and iii) immunopharmacological blockade. Passive transfer of myasthenia from man to mouse by human myasthenic serum has been obtained by Toyka et al. (1975). Our failure to repeat their results may have several explanations. It may be necessary to use animals of specific H2 haplotype but we think this is unlikely as we have induced EAMG in numerous strains of rats. It may be that passive transfer requires previous reduction of the safety factor for neuromuscular transmission. Unquestionably, the presence of circulating anti-AChR globulin is not necessarily associated with detectable neuromuscular transmission failure as it has been detected in the serum of apparently normal babies born to myasthenic mothers and in a healthy parent or sibling of a myasthenic patient.

It may be concluded that anti-AChR antibody is necessary for the myasthenic state in human MG and EAMG but that its presence does not necessarily lead to the clinical syndrome. The nature of the damage it causes to the receptor membrane is rapidly being disclosed, but at this point it is important to notice that the present evidence indicates that antibody against human AChR (as an example of a syngeneic antigen) may not be immunogenic, unlike antibody to allogeneic antigen, or the syngeneic antigen may become immunogenic if the extraction procedure causes a conformational change (Table 1).

From the clinical point of view, a more important consideration is the cause of the immunological reaction in human MG. In the animal model the immunogenic stimulus is an allogeneic antigen such as Torpedo AChR. The absence of a response to syngeneic AChR makes it unlikely that the autoimmune reaction is a response to the release of a sequestered antigen, even from receptors in the thymus, unless the receptors there or in muscle
Table 1. Sequestered antigens.

<table>
<thead>
<tr>
<th>Source of AChR</th>
<th>Immunogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denervated muscle</td>
<td></td>
</tr>
<tr>
<td>Syngeneic AChR</td>
<td></td>
</tr>
<tr>
<td>Syngeneic AChR + detergent etc</td>
<td>+</td>
</tr>
<tr>
<td>Allogeneic AChR</td>
<td>+</td>
</tr>
<tr>
<td>Allogeneic AChR denatured</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions:
1) Exposure of sequestered antigen
2) Conformational change of antigen

have undergone a conformational change. This is a tenable hypothesis since a virus could be responsible.

I feel that the sequestered antigen theory is unlikely for many reasons, not least that major reasons for introducing an immunological theory of MG were the recognition of linkage with disorders of other organs, later shown to be autoimmune diseases and of anti-myosin activity of myasthenic sera. A sequestered antigen theory of autoimmunity would require ad hoc explanations for each disease in the cluster. The same consideration argues against a forbidden clone theory since it would presuppose a number of "forbidden" mutant clones. If a mutant lymphocyte carrying "new" or "depressed" antigens is allowed to survive to attack "self" antigens, it would be expected that the resulting disease would be progressive, whereas autoimmune diseases, including MG, show remissions and are commonly self-limiting. Furthermore, it is implicit in this concept that anti-AChR antibody is always pathogenic. On the contrary, as pointed out above, it has been found in considerable titer in the serum of clinically normal babies born to myasthenic women and in low titers in a parent or sibling of a patient with MG. (Very low titer assays in normal subjects are difficult to interpret but might be physiological, as part of the mechanism for removing effete tissue). These are not conclusive arguments but they are against the forbidden clone theory (Table 2).

It seems more probable that autoimmune disease is due to faulty control of immunological mechanisms. I have argued the general case for this elsewhere and will now examine some possible lesions in the light of observations on myasthenia gravis. The sequestered antigen and forbidden clone theories have the underlying assumption that the immunological system as a whole is normal but that it is either exposed to an unfamiliar antigen or has mutant lymphocytes with cell-bound antigens which are not destroyed and which are allowed to proliferate to attack normal tissue.
Table 2. Forbidden mutant clone.

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive disease</td>
<td>Remissions, Self-limiting</td>
</tr>
<tr>
<td>Anti-AChR antibody</td>
<td>Anti-AChR in unaffected babies</td>
</tr>
<tr>
<td>always pathogenic</td>
<td>relatives</td>
</tr>
<tr>
<td></td>
<td>Low titer antibody in normal</td>
</tr>
<tr>
<td></td>
<td>subjects (?)</td>
</tr>
</tbody>
</table>

Conclusion: Improbable hypothesis

So far as MG is concerned there is evidence against the assumption that homeostasis is normal (Table 3). Immunodeficiency has been demonstrated by Dawkins et al. (1976) and Simpson et al. (1976), and the clustering of autoimmune diseases suggests that immunological homeostasis is defective.

Table 3. Sequestered antigen and forbidden clone theories.

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal immunological system</td>
<td>Immunodeficiency</td>
</tr>
<tr>
<td>Monoclonal response</td>
<td>Autoimmunity clusters</td>
</tr>
</tbody>
</table>

Conclusion: Defective immunological homeostasis

Faulty immunological control could be due to failure to recognize antigens (Table 4) or to poor suppressor cell activity (Table 5). There is no experimental evidence to indicate recognition failure. Defective suppressor cell activity is an attractive hypothesis but it is then difficult to account for the beneficial effect of thymectomy. Furthermore, Simpson et al. (1976) pointed out that autoimmune disease may flare up soon after thymectomy (see later paper). Is there a missing link in our present ideas about immunology? A useful model to explore would be a "self identification

Table 4. Recognition failure.

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not antigen specific</td>
<td>Commonly one antigen</td>
</tr>
<tr>
<td>Permanent defect</td>
<td>Intermittent, Self-limiting</td>
</tr>
<tr>
<td>Poor response to challenge</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Conclusion: No evidence of failure to recognize antigens
Table 5. Poor suppressor cell activity.

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto-aggressive lymphocytes</td>
<td>Minimal CMI</td>
</tr>
<tr>
<td>Auto-aggressive antibody</td>
<td>Poor correlation between anti-AChR and MG status</td>
</tr>
<tr>
<td>Not antigen specific</td>
<td>Autoimmunity clusters</td>
</tr>
<tr>
<td>Thymectomy should not release from suppressor effect</td>
<td>Post-thymectomy Hashimoto</td>
</tr>
<tr>
<td></td>
<td>Pemphigus, Ulcerative colitis, (S.L.E. ?)</td>
</tr>
</tbody>
</table>

Conclusions:
1) Poor suppressor cell activity not the sole disorder. Sensitized T-cells required.
2) Suppressor cell activity is actively inhibited by thymus.

system,” which would inhibit the normal responses to an antigen. Auto-aggression would then result from deficiency of that system.

The concept of autoimmune diseases as immunological deficiency states has been ably argued by Fudenberg (1968). Table 6 lists some of the abnormalities predictable from the concept, and it is undoubtedly compatible with the observations on MG. We cannot at present identify immunoreactive (Ir) genes in man, but the HLA locus on the sixth chromosome is believed to be close to the Ir locus. Along with others, we have shown that the HLA-A1-B8-Dw3 haplotype is common in myasthenics but that it is not obligatory. It is shared with other disorders associated with anergy and immunodeficiency. In principle, it is possible that selective deficiency may be genetically determined. Fudenberg (1968) has pointed out that this would allow persistence of microorganisms which are normally handled readily by the immune system, leaving them free to attack tissues for which they have tropism.

There is little evidence for viral persistence in MG. Tindall *et al.* (1978)

Table 6. Breakdown of immunological tolerance.

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defective tolerance to self-antigens</td>
<td>Autoimmunity clusters</td>
</tr>
<tr>
<td>Thymoma (?)</td>
<td>+</td>
</tr>
<tr>
<td>Reticulo-endothelial tumours</td>
<td>+</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>+</td>
</tr>
<tr>
<td>Ir genes — general</td>
<td>HLA — A1 — B8 — Dw3</td>
</tr>
<tr>
<td>— specific</td>
<td>Mouse C57</td>
</tr>
<tr>
<td>Virus persistence</td>
<td>CMV</td>
</tr>
<tr>
<td>Drug induced breakdown</td>
<td>Penicillamine</td>
</tr>
<tr>
<td>Virus induced breakdown</td>
<td>β-adrenergic blockers</td>
</tr>
<tr>
<td></td>
<td>???</td>
</tr>
</tbody>
</table>
recently reported elevated titers of complement fixing antibody to cytomegalovirus (CMV) in myasthenics not treated with thymectomy or steroids. If confirmed, this is likely to be due to immunodeficiency rather than a cause of it. Tindall et al. (1978) proposed that CMV protein may be incorporated into membranes with subsequent induction of anti-AChR antibody. The criticism of the single antigen instructive theory made above and of all "thymitis" theories applies to this one. Nevertheless, a viral cause for defective thymic function is possible if there is a single type of controlling cell, such as a secretor of thymosin. The role suggested for a thymic hormone by these authors is fully compatible with the ideas developed in this paper if we allow that "unregulated" B-cells may produce an excess of antibody. But this is probably not the whole story since the work of Plescia et al. (1976) suggests that there may be at least two fractions with thymosin like activity, one being immunoenhancing, the other immunosuppressive. Surely this line of research is at least as exciting as the work on the endplate, and potentially more important for the cure of myasthenia gravis.

REFERENCES


Discussion

Dr. Lennon: In association with Drs. Lindström and Seybold we demonstrated that syngeneic muscle AChR is myasthenogenic in rats (J. Exp. Med., 1976). The receptor, was solubilized in non-ionic Triton X-100 detergent and purified by alpha-neurotoxin affinity chromatography and was injected with adjuvants. Furthermore, extensively denatured AChR (Torpedo) sub-
units, prepared in the ionic detergent SDS, have been reported by Lindström and co-workers to be weakly autoimmunogenic in rats.

Dr. Engel: I very much enjoyed your talk and the precise logic with which you analyze complex problems. The question was raised by you whether immunoglobulin G localized at the MG endplate is directed against the receptor. There are two lines of arguments for this: (1) the IgG is localized on terminal expansions of junctional folds where the receptor is known to be localized; and (2) after passive transfer of antireceptor antibodies from affected to normal rats, the antibody is again localized on the terminal expansions of the junctional folds.

Dr. Simpson: I am pretty sure that you are right.

Dr. Appel: Could you speculate on etiological factors which explain age and sex differences?

Dr. Simpson: No, I have no appropriate answer for that one. I don't think the hormonal factor is playing an important role in producing MG, and contraceptive pills, which are recently regarded as a possible factor, may not contribute to MG.

Dr. Lennon: The lupus-like disease which occurs spontaneously in New Zealand Black mice has been reported by Tallal and his associates to be explicitly dependent on female sex hormones. Males have a much lower incidence of the immune complex complications and survive longer than females. However, castrated male mice treated with female sex hormones have a similar incidence and degree of SLE-type pathology as females, and, conversely, castrated female mice treated with androgens are protected from the immunopathologic complications.

Dr. Takamori: What is the role of lytic component of complement in the process of myasthenia gravis? I also wonder how much the immunopharmacologic action of the antibodies contributes to the defect. Finally, do you agree with the possible role of the thymus in antibody production?

Dr. Simpson: I have made no personal studies on the role of complement at the endplate although we have studied serum complement and immune complexes (Behan and Behan, 1979). I refer the question to Dr. Engel. The concept of immunopharmacologic block was conceived at a time when our knowledge was limited and it was not known that AChR regenerated so rapidly (or at all!). It is still possible, but it is becoming an unnecessary idea which may eventually be discarded. AChR may be present in thymus (controversial) but antigenic stimulation of that site is not essential for autoimmunity. There are many examples of MG originating years after thymectomy.

Dr. Engel: As regards to the role of complement in the mouse-passive transfer model, the disease in mice is rather mild and usually subclinical. Further, Toyka has shown that the endplate maintains its structural integrity in these mice. Since complement mediated destruction of the endplate is not a feature of this model, it is not surprising that C5-deficient mice do not differ
from the non-CS deficient mice in their response to human antireceptor antibody. We believe that complement-mediated lysis of the postsynaptic membrane plays an important role in the pathogenesis of human MG.

**Dr. Simpson:** I do not know about the mouse. Our passive transfer studies (human MG) were on the rat. We used diaphragm MEPP recording but could not find significant evidence of passive MG.

**Dr. Kawanami:** We found AChR protein in fetal calf thymus. The cellular immunity to this protein in MG was studied by the leukocyte migration inhibition test. The results will be presented tomorrow. I have some questions pertaining to what you said about AChR protein in thymus and that it has nothing to do with the pathogenesis of MG. Before such a discussion, we need additional studies on the protein, its specificity and immunogenicity.

**Dr. Simpson:** I accept that there are myoid cells in the thymus. It is not agreed (e.g. by Dr. Appel) that there are ACh receptors. Even if we accept them, I cannot agree that there is an antigenic stimulus arising from the receptors. This could indicate an instruction theory of immunogenesis which I have criticized in my paper. For instance, it would be necessary to postulate similar thymic analogs for thyroid, joint, gastric and other tissues. There is no evidence that they exist.

**Dr. Appel:** We could not find AChR in the human thymus. We found no cross reactivity with muscarinic AChR reactivity.

**Dr. Lennon:** T-lymphocytes have been reported to have muscarinic-type AChR on their surface membranes. I am not aware of any evidence for their antigenic cross reactivity with nicotinic AChR. Might I ask Dr. Appel what criteria would satisfy him that the AChR in thymus are nicotinic and of skeletal muscle type other than the association of a pharmacologically specific alpha-bungarotoxin binding on a molecule which is precipitable by antibodies to ACh receptors?

**Dr. Appel:** The subunits to which toxin is binding range around 38,000, or even as high as 45,000. I don’t know if these have been demonstrated yet.
The thymus was formerly regarded as an endocrine gland involved in growth and development, and it may be appropriate to draw attention to the possibility that its role may be more than the immunological one which has dominated thinking since 1961.13

The concept that the thymus produces a curare-like substance is also obsolete23 though a variant survives in the thymin (thymopoietin) postulated in the thymitis theory of G. Goldstein.32 Despite a considerable volume of work from that author and his collaborators, most independent investigators have failed to reproduce their results.43 Minor changes in neuromuscular transmission in experimental animals may result from myositis as in certain myasthenic syndromes in the human. Major objections to any theory requiring a neuromuscular blocking substance produced by the thymus are that a) thymectomy does not cure myasthenia gravis immediately, and b) myasthenia gravis may occur many years after apparently complete thymectomy.54 Nevertheless there is no doubt that the thymus is involved in the pathogenesis of myasthenia gravis and that thymectomy influences the further course of the disorder, and few would now disagree that the mechanism involved is immunological. Experimental myasthenia gravis bypasses the thymus by introducing a high concentration of AChR antigen to the animal. It is not initially an auto-immune disease but autoantibodies appear after the first stage, and the term experimental autoimmune myasthenia gravis (EAMG) has been widely accepted. There is evidence that the reactivity of the experimental animal is influenced by the thymus and the disease is difficult to produce in thymectomized animals.65 Clearly the thymus influences the degree of immunological response to self or foreign antigen but does not itself produce anti-AChR antibodies or a curare-like substance.

Thymus in myasthenia gravis

There are pathological changes in 70–80 percent of patients with myas-
thenia gravis. The most consistent finding is lymphoid hyperplasia of the cortex and medulla with T lymphocytes in both parts, not mainly in the cortex as in normal subjects.\textsuperscript{7} But in older patients the gland may show apparently normal involution. Lymphoid hyperplasia is commonly, but not invariably, associated with numerous germinal centers in the medulla.\textsuperscript{8} Their significance is uncertain as the prevalence of germinal centers in the thymus bears no clear relationship to the duration or severity of myasthenia gravis or to the clinical response to thymectomy.\textsuperscript{9,10} Indeed, our studies showed a tendency for patients with relatively unreactive glands to obtain a better result from thymectomy. The only consistent finding by Vetters and Simpson (1974)\textsuperscript{10} was a relative decrease in the percentage of the thymus area occupied by cortex and a relative increase in the amount of medulla compared to the data of Hammar (1929)\textsuperscript{11} for non-myasthenic subjects who had died suddenly. This increase could be due to stress rather than to medullary hyperplasia. Thymic hyperplasia may, in fact, be a myth as the data of Castleman and Norris (1949)\textsuperscript{9} show that it is more common for myasthenic thymus glands to weigh less than average, compared with normal subjects of the same age. We have not been impressed with histological evidence of "thymitis" and have drawn attention to similar changes in other disorders.\textsuperscript{16} It would therefore be necessary to postulate that only one disease with thymic germinal center formation and lymphoid follicles causes excessive release of "thymin" while others, histologically identical, do not. A necessary conclusion from the Goldstein hypothesis is that thymectomy should cure myasthenia gravis within weeks and that the disease should not occur in a thymectomized subject. But the facts are otherwise.

**Myoid cell antibody**

Clinical constraints also argue strongly against another concept which is widely discussed. Accepting that the pathogenesis of myasthenia gravis is immunological, many authors have attempted to account for the development of anti-muscle antibodies by postulating that the primary antigen is the myoid cell which may be found in the thymus.\textsuperscript{12} Note that this presupposes an instructive theory of antibody production, and it would then be logically necessary either to have similar anlages of other organs within the thymus to account for the other autoimmune diseases in myasthenic patients or to have ad hoc mechanisms for each. The myoid cell hypothesis would only be tenable if acetylcholine receptors were in the thymus\textsuperscript{13,14} but this is still disputed, especially in human thymus.\textsuperscript{15} The subject has been reviewed by Vincent et al. (1979).\textsuperscript{16} If the initial antigenic stimulus is
in the thymus it is difficult to account for those cases of myasthenia gravis appearing many years after thymectomy, unless the disease is present in a subclinical form or some residual or "accessory" thymic tissue remains. Both are possible but without supporting evidence at present.

Since my objections to the myositis and myoid cell theories are based on the clinical constraints, it is now necessary to examine the evidence from human disease for clues to the role of the thymus.

Thymectomy

Although a small number of thymectomies had been performed previously for myasthenia gravis, the modern history of this operation begins with the work of Blalock (1941)\(^{17}\) and other American surgeons. In the following decade these workers were progressively disappointed with the results and Eaton and Clagett (1950)\(^{18}\) concluded that "at present thymectomy in the treatment of myasthenia gravis is recommended by us because of the potentially malignant character of the thymomas and not because of anticipated improvement in the myasthenia gravis." During the same period, in London, Sir Geoffrey Keynes had been reporting good results but stressed the importance of early operation and that results were best in non-thymoma cases.\(^{19}\) An independent review by Simpson (1958)\(^{20}\) confirmed his claims and showed that the American results were similar if cases with thymic tumors were evaluated separately, and further that operation appeared to arrest progression of the disease, so that statistical differences from nonoperated cases did not appear until 2-3 years after operation. These conclusions have stood the test of time although it is only fairly recently that American surgeons have agreed that the results of surgery are best with early operation and that the later benign course is increasingly evident with the passage of time.\(^{21,22}\) To be effective, thymectomy must be carried out during the "active stage" (stage 1 of Simpson, 1969a).\(^{23}\) With a population largely consisting of stage 1 patients, more than 90% of patients are relieved of all symptoms or have only mild disability.\(^{24}\) Although the long-term prognosis for patients with thymoma remains poor, the initial response may be just as satisfactory as with non-thymoma cases.

Thymectomy is undoubtedly effective in stage 1 patients. However, the response of individual patients is unpredictable. It may be immediate or delayed. After removal of the gland a major relapse is rare but it does occur, and attention has already been drawn to the onset of clinical myasthenia years after removal of a thymoma. If the results of thymectomy in a large series are scrutinized it appears that the operation does not "cure"
the disease but promotes a shift towards normality. This is best seen if cases are classified according to change of status\textsuperscript{20} rather than to disability categories as in papers from New York and elsewhere. I interpret these results to mean that thymectomy reduces immunological reactivity but does not arrest it. In other words, the role of the thymus in immunology is homeostatic.

**Helper and suppressor cells**

A possible mechanism for controlling T-and B-cell function would be a balance between helper and suppressor cells. A relative deficiency of thymic suppressor cells may be postulated in myasthenia gravis. Mischak \textit{et al.} (1979)\textsuperscript{23} have recently published evidence for reduced mitogen-induced suppressor cell activity and others have shown immunodeficiency in myasthenic patients.\textsuperscript{26,27} Once again there are clinical observations which make it difficult to accept that general suppressor activity is depressed in myasthenic patients. In the first place it is difficult to understand how thymectomy could improve this situation. Furthermore, Simpson \textit{et al.} (1976)\textsuperscript{27} have pointed out that autoimmune disease may flare up soon after thymectomy. This has been reported for systemic lupus erythematosus and chronic ulcerative colitis,\textsuperscript{28,29} Hashimoto's disease,\textsuperscript{30} and pemphigus vulgaris.\textsuperscript{31,32} It appears improbable that suppressor cells were inactive prior to thymectomy unless part of a general immunological deficiency (see earlier paper).\textsuperscript{27}

**Immunological surveillance and cancer**

The familial and individual clustering of autoimmune diseases, including myasthenia gravis, and the responses to thymectomy would be compatible with the idea that the thymus is responsible for immunological surveillance, regulating the types (and amounts) of tissue permitted to grow and persist in the body. If this system constituted a natural defence against cancer, as suggested by Thomas (1959)\textsuperscript{33} it might be supposed that thymectomy might increase the risk of cancer. The hypothesis was examined by Vessey and Doll (1972).\textsuperscript{34} Their study, which included many patients studied by me, provided no evidence for an increased risk of neoplastic diseases, and my continuing experience supports their conclusion. On the contrary, Papatestas \textit{et al.} (1971)\textsuperscript{35} have noted a three-fold increase over the expected incidence of extra-thymic neoplasms in patients with myasthenia gravis who have not had a thymectomy, while following thymectomy the incidence returned to expected levels. One is led to the strange conclusion that immunological surveillance, if such be the mechanism, is
better without the thymus, at least in myasthenic patients. A logical conclusion is that immunological surveillance is primarily an extra-thymic function. A "hyperplastic" or neoplastic thymus reduces the capacity to distinguish self from non-self.

Hormonal function of the thymus

It is entirely possible that the thymus has other hormonal functions. A review of pre-1960 experimental studies on the thymus suggests that the differentiation of the immunologically competent cells and antibodies may be a survival of a wider action on tissue differentiation in the fetus. Szent-Gyorgi et al. (1962) claimed to have isolated a growth-promoting factor (promine) and a growth-inhibiting factor (retine) from calf thymus which could play a part in the regulation of breakdown and repair of organs showing a regular turnover of cells. They were concerned particularly with carcinogenesis. The implications for myasthenia gravis are clear but unfortunately the validity of the early reports is uncertain.

There is histological evidence for a secretory activity of the thymus and functional studies since Miller (1961) indicate that the gland produces one or more chemical substances which influence lymphocyte production or activity. Currently the most interesting work is that of A. L. Goldstein which indicates that there may be a thymic hormone, thymosin, which influences precursor T-cells, possibly via an adenylate cyclase-dependent process. Goldstein et al. (1976) suggest that genetic factors and/or viral infection may lead to deficiency of suppressor or regulatory T-cells which in turn remove the mechanisms controlling B-cell function (including formation of autoantibodies). Further speculation is unjustified until this work is verified in other laboratories, but at this stage it appears to me to be compatible with all that we know about myasthenia gravis, including the clinical constraints outlined in this and the previous paper.

REFERENCES

Discussion

Dr. Appel: How common is pemphigus and Hashimoto’s disease following thymectomy? It is rare in our series.

Dr. Simpson: The number of cases of autoimmune diseases flaring up after thymectomy is certainly small and not statistically significant—but biologically significant nevertheless!
Pathogenesis and treatment of myasthenia gravis

Sir,—The review article by Drs Glenis K Scadding and C W H Havard (17 October, p 1008) on myasthenia gravis is an excellent summary of present ideas on pathogenesis and treatment. I particularly endorse the conservative advice on the use of corticosteroids. The initial deterioration during steroid treatment is sufficient to justify hospital admission during the first two to three weeks of this form of therapy. However, I do not consider that the effectiveness of anticholinesterases is enhanced during this period. On the contrary, many patients require temporary increase in dosage levels of pyridostigmine. Our electrophysiological studies indicate a double action of corticosteroids at the neuromuscular junction in experimental myasthenia gravis. A short-lasting reduction in prejunctional acetylcholine stores precedes a more sustained increase in amplitude of miniature endplate potentials of a subpopulation of endplates (postsynaptic).

The review repeats a frequent misquotation of my 1960 paper. The autoimmune hypothesis was not formulated on account of the high incidence of other autoimmune phenomena. I drew attention to several previously unrecognised clinical correlations but at that date few of them were recognised as immunological. Many other clinical and pathological factors were involved in the hypothesis, which led to the concept of a "multisystem" disorder resembling systemic lupus erythematosus. I mention this because it leads to a different theory of autoimmunity, which could not be accounted for by reaction to acetylcholine receptors within the thymus. I have set out the argument in some detail elsewhere.

General belief that there is now a role for the thymus in pathogenesis has led to acceptance of thymectomy, though the evidence for its value and for the delayed benefit was already clear in 1958 from the experience of Sir Geoffrey Keynes and other pioneers. We also aim to remove the gland completely, but where is the evidence that later deterioration, if it occurs, is due to regrowth of the thymus?

We have demonstrated the feasibility of preparing heterologous anti-idiotypic antisera to anticholinesterase antibodies but at present the blocking effect of the antiserum is restricted almost totally to the original inducing antibodies. If it proves necessary to prepare individual antisera for each patient, the method would be unlikely to have a practical application.

I congratulate Drs Scadding and Havard on an excellent condensation of a rapidly advancing subject. We seem to be approaching a complete understanding of the pathogenesis of myasthenia gravis. But there's still a lot of excitement in it.

J A Simpson

Institute of Neurological Sciences,
Southern General Hospital,
Glasgow G51 4TF

4 Simpson JA. Brain 1959;82:122-44.
Myasthenia gravis

JOHN A. SIMPSON, M.D., F.R.C.P., F.R.C.P. (ED.), F.R.C.P. (GLAS.), F.R.S. (EDIN.)
Professor of Neurology, University of Glasgow; Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow

Myasthenia gravis is rare. A population of 100,000 will produce a new case only once in two to three years but recognition is important as the disease can be treated and is otherwise crippling or fatal. Myasthenia gravis most commonly affects young women, but it may occur in either sex at any time from infancy to old age; it has a striking tendency to fluctuate in severity. Remission of months or years is common in the initial stage but so infrequent in later stages that it is unwise to plan treatment in the expectation of spontaneous remission.

Clinical picture
The diagnosis of myasthenia gravis should be considered in any patient with variable muscular weakness especially if power is completely restored by resting. Weakness means demonstrable loss of muscular power; ‘tiredness’ is not a symptom of myasthenia, though it may accompany it, and should not be used as a guide to dosage.

Non-specific infections or emotional stress precipitate some of the relapses. Once established, however, symptoms are increased by exercising the affected (‘fatigable’) muscles and relieved promptly by rest. Weakness is likely to be greater after midday, but this is not invariably.

Early manifestations are drooping of one or both eyelids, double vision and weakness of neck flexion. In advanced cases, the patient suffers from weakness of the shoulder muscles (inability to hold the arms outstretched for one minute) followed by weakness of the face and tongue with difficulties of speech and swallowing and, in severe cases, dyspnoea. Any skeletal muscle may be involved.

Autoimmune correlations
Myasthenia gravis is an autoimmune disease. Younger patients commonly have an HLA-B8 gene and personal or familial linkage with other autoimmune diseases (thyroid diseases, pernicious anaemia, rheumatoid arthritis, diabetes mellitus and pemphigus are the most common). Onset in later life is associated with a thymic tumour (thymoma), but in these cases the autoimmune correlations are slightly different: the HLA linkage is different and, unlike the disease in younger patients, it is more common in men. In both types humoral antibodies against the acetylcholine receptors of skeletal muscle can be measured in the blood—except in some patients who have only extraocular muscle involvement. This is the most definitive diagnostic test: others such as electro-myographic evidence of reduced neuromuscular transmission and brief remission after injection of edrophonium (Tensilon) are non-specific. Their interpretation is difficult and the practitioner is advised to refer the patient with suspected myasthenia gravis to a centre with adequate experience.

Differential diagnosis
The differential diagnosis includes consideration of multiple sclerosis, motor neurone disease, Parkinsonism, peripheral neuropathy, thyrotoxic eye disease and some rare causes of muscular weakness. The most frequent mistake is to attribute the emotion-precipitated paralysis with full remissions to hysteria. Physical signs may be absent until the muscles complained of have been exercised. Myasthenic syndromes occur also as congenital myasthenia (a different disease without immunological cause), in adults with latent carcinoma (especially of the lung) and in some rare immunological disorders.

Pathology
Transmission of motor commands, from nervous system to skeletal muscle, requires liberation of acetylcholine from motor nerve terminals into the synaptic cleft of the endplate of each muscle fibre where it attaches to specialized acetylcholine receptors causing the generation of an endplate...
potential. If this potential reaches an adequate voltage, the action potential mechanism of the muscle is triggered and the fibre contracts. A surplus of acetylcholine and acetylcholine receptors constitutes a 'safety factor' so that transmission is preserved despite decreasing output by the nerve terminals during maintained effort. To respond to each action potential in the motor nerve, each jet of acetylcholine must be rapidly destroyed by an enzyme (acetylcholinesterase) in the endplate. In myasthenia gravis a high proportion of the receptors is damaged or blocked by antibody directed against part of the receptor, reducing the safety factor and eventually preventing neuromuscular transmission in an increasing proportion of the endplates in some or all skeletal muscles. When very severe, the muscle is deprived of all motor innervation and may become atrophic. This is rarely detectable clinically except in the tongue in which a rather typical 'triple grooving' may be seen, but the loss of receptors may be such that full transmission cannot be restored even with optimal dosage of an anticholinesterase drug (fig. 1).

Pathogenesis
The exact nature of the immunological damage is still uncertain. Clinical severity correlates poorly with titre of antibody in the plasma. The antibody-antigen reaction at the endplate requires complement—plasma complement fractions are reduced during a relapse and immune complexes can be found in the plasma.

It is not known how the autoimmune reaction is initiated. An animal model (experimental autoimmune myasthenia gravis) can be produced by inoculating many mammalian species with acetylcholine receptors from endplates of other species (including man) or from the electric organ (biologically similar to motor endplates) of certain fish. Myasthenia can be transferred passively from an affected animal to another—either of the same or of a different species—and it is likely that similar passive transfer accounts for the rare neonatal myasthenia which occurs in one in seven children born to myasthenic mothers. These passively transferred types of the disease are self-limiting. (The baby becomes normal in six to ten weeks if treated with anticholinesterase drugs until the condition disappears.)

Although it uses the same antibody-acetylcholine receptor mechanism, the neonatal type is not autoimmune and neither is the initial stage of the animal model. We do not understand what leads the animal to produce antibodies against its own acetylcholine receptors and this aspect is still obscure in the natural myasthenia gravis of man. Recently, certain inducers have been recognized, notably penicillamine and some beta-adrenergic blockers, which reduce the natural immunological tolerance against 'self' proteins.

The proximate mechanisms for recognizing 'foreign' and 'self' proteins and for producing antibody against them in appropriate amounts are in the mononuclear cells of the reticuloendothelial system—notably the blood macrophages and lymphocytes but also similar cells in the tissues of the body. Macrophages remove effete or damaged cells and present their proteins to cells of the lymphocyte series. Some of these, the B-lymphocytes of the bone marrow, elaborate immunoglobulin 'antibody' which then participates in the destruction of cells bearing the appropriate antigen.

There is a hierarchy of lymphocytes. The B-cells are 'instructed' by T-lymphocytes. These are specialized cells originating in the thymus gland and contain sub-populations described as 'helper' and 'suppressor' cells (among others) which promote or suppress B-cell production of antibody. In myasthenia gravis this cellular component of the immunological reaction is presumably aberrant. The evidence now points to an immune deficiency rather than the 'auto-aggressive' mechanism of the earlier hypotheses about autoimmunity. Current research on subpopulations of lymphocytes should clarify this important matter.

The thymus
Since an autoimmune mechanism for myasthenia gravis was originally proposed it has become increasingly certain that the thymus plays an important regulatory role in immunological tolerance. Pathological changes are found in the thymus in most patients but we are still baffled as to what
feature is significant. Around 10 to 15% of patients have a thymoma (a tumour which is usually encapsulated but sometimes locally invasive). The others are widely believed to have a ‘hypoergic’ thymus although in fact the gland is rarely larger than normal for the patient’s age and in older patients it is atrophic in up to 30% of cases. In all three types (atrophic glands, large or ‘hypoergic’ glands and thymomas) the medulla commonly has an excess of germinal centres. This is not invariable and they are not exclusive to myasthenia gravis. Furthermore the clinical response to thymectomy appears to bear little or no relationship to the histology of the gland, except that late recurrence of myasthenia is more common in patients who have had a thymoma. Despite the lack of satisfactory evidence for the belief, recurrence some years after thymectomy is widely attributed to growth of thymic remnants left in situ at operation. Early failure of the operation is blamed on extra-thymic survival of clones of T-cells occurring in stage 2 of the disease (seven to ten years after onset). Thymic hormones regulating the T-cell population have been identified and may play a significant role, but it remains difficult to understand how an immunodeficient state with presumed defective suppressor cell function can be improved by removing the controlling organ. An essential part of the jigsaw is still missing and the unquestionable role of emotional factors cannot be given a rational explanation.

**Treatment**

The first priority is to preserve life by controlling respiration if it is threatened (a dyspnæic myasthenic patient should be sent to hospital for early admission), and to promote maximum power in the muscles by raising the safety factor for neuromuscular transmission. The primary immunological disorder should then be treated.

**Neuromuscular transmission**

The safety factor for neuromuscular transmission can be raised by drugs which (1) potentiate the production or release of acetylcholine (for example 4-aminopyridine, guanidine, ephedrine), (2) sensitize the acetylcholine receptors or amplify the muscle response (for example veratrum alkaloids, germine, potassium) or (3) inhibit hydrolysis of acetylcholine by cholinesterase. In practice only the anticholinesterase drugs are worth using. Ephedrine is virtually useless except as a euphoriant and bronchodilator—actions admittedly sometimes helpful even though not antimyasthenic. Potassium and potassium-retaining steroids are also useless and aggravate the bowel effects of anticholinesterases.

Of the many anticholinesterase drugs, use should be restricted to pyridostigmine (Mestinon) and neostigmine (Prostigmin). Longer-acting drugs (for example, bis-neostigmine compounds (Ubretid), physostigmine and organic phosphorus compounds) are dangerous since they are either cumulative or cross the blood-brain barrier to cause cerebral side effects.

Before discussing the use of the preferred anticholinesterases it is necessary to warn against substances that further lower the safety factor. These include aminoglycoside antibiotics (streptomycin, dihydrostreptomycin, neomycin, kanamycin, gentamicin, viomycin, bacitracin, polymyxin A and B, and colistin, especially in the presence of renal insufficiency) and membrane stabilizers (phenytoin, quinine, quinidine, procainamide). Low serum levels of ionized calcium also reduce the safety factor. One must, however, be sensible about this situation. These drugs should obviously be avoided if there are alternatives but, if there is a threat of rapid deterioration, they may be used provided the doctor is ready to increase the dose of pyridostigmine should the weakness increase. Obviously, too, neuromuscular relaxant drugs required by the anaesthetist also lower the safety factor. The damaged endplate responds anomalously to many of these. Despite markedly increased sensitivity to curare, it is best to use D-tubocurarine if relaxation is required during surgery as its mode of action is unchanged and neostigmine is still its antidote. Respiratory depressants must be used with care although diazepam is relatively safe if sedation is required. Penicillamine and some beta-blockers induce myasthenia but there is no evidence that they aggravate spontaneous myasthenia gravis. Deterioration with corticosteroids is discussed below. A warning must also be given about enemas. Although commonly and successfully used (in ignorance of the danger) there are reports of sudden death following an enema. The mechanism is unknown, but I have postulated a vagal reflex due to stretching a bowel rendered tonic by anticholinesterases.

Anticholinesterase drugs should not be given parenterally in domiciliary practice. If unable to swallow a tablet, the patient needs nasogastric intubation and can be given crushed tablets by that route. Neostigmine (15mg tablet) produces a surge of muscular power for 30 to 60 minutes followed by continued activity at a lower level for a time personal to each patient (2 to 6 hour). Then strength is lost rapidly. Since it is difficult to time the dosage to avoid this let-down, most patients prefer pyridostigmine though the neostigmine surge may be valuable if given 30 minutes before a meal or in anticipation of a special effort. Pyridostigmine (60mg tablet) has about the same plateau
level of action. Its peak effect is less and the plateau lasts slightly longer than that of neostigmine but its effect wanes more slowly, allowing a sustained blood level to be achieved by judiciously timed dosage (fig. 2).

Establishment of dosage.—First establish the timing by giving a single tablet. The patient should then keep a written record of the effect every half hour until weakness has returned to the pre-dosage level. Wait for one hour then repeat four or more times, with a gap of at least one hour between each trial. The interval being established, dosages are then timed so that one is taken 30 minutes before each major meal. Each dosage is then increased by half-tablet increments until the maximum improvement is achieved and may be of the same amount or adjusted according to the activity planned for the succeeding period. Some patients prefer to sleep uninterrupted, others require regular dosage day and night.

The temptation to increase the dosage so long as weakness persists must be resisted. In many cases, because of the receptor deficit described above, myasthenic weakness passes straight into a different type of neuromuscular block caused by overdosage ('cholinergic crisis') with no intervening stage of normal strength. Even at best, abnormal 'fatigability' can be demonstrated on clinical testing, with or without a home-made tension recorder (fig. 1). As the safety factor is not equally reduced in all muscles it is possible to overdose the less damaged (including the respiratory muscles) when others are still underdosed (for instance, diplopia is still present). An early sign of overdosage, fasciculation, is permissible in the spared muscles (often below the knees), but it is most important that the dose is correct for the respiratory and bulbar muscles. Watch the pupils—constriction to a diameter of 3mm or less in normal room lighting indicates overdosage. For this reason it is best to avoid giving atropine or propantheline unless the patient is suffering from severe diarrhoea. A short-acting drug (neostigmine) is safer when used at near-toxic levels of dosage—never in this circumstance use the type that have a longer action than pyridostigmine. It is always safer to have the patient slightly underdosed. Animal experiments suggest that dosage over a long term may damage neuromuscular junctions. There is no satisfactory evidence that this applies to man—and there is little alternative anyway—but I feel that the long-term results are best in those patients who consciously try to reduce the dosage to the minimum required for reasonable living.

Anti-immunological therapy

If the optimum dosage and timing of pyridostigmine does not restore strength to an acceptable level, temporary improvement is often obtained from plasma exchange, designed to remove circulating anti-acetylcholine receptor antibodies and immune complexes and replace them with fresh immunoglobulin. We have seen some prolonged remissions, but a respite of only a few weeks is more usual. Plasma exchange should be reserved for emergencies owing to expense, technical problems and occasional complications. Immunological protection against infection is also lowered, but it may buy time to enable other slower anti-immunological treatment to work.

Immunosuppression with azathioprine is being increasingly advocated. It produces gradual improvement over a period of one to two years and although there is no immediate benefit the hazards appear to be less than anticipated. Corticosteroids, too, are commonly used; however, their immunosuppressive action is no quicker than that of azathioprine and the long-term hazards are so great as to cause concern about widespread use in young
people, particularly if continued for many years. An immediate effect occurs before the immunosuppression (at least as judged by antibody titres); ACTH, cortisone or prednisolone given by many different dosage regimens often produces a marked deterioration of muscular power during the first ten days, but this is followed by a remarkable and sustained improvement (particularly in the extraocular muscles which commonly become unresponsive to anticholinesterase). Experimental studies in my laboratory indicate that this is due to a biphasic action on the neuromuscular junction, and is probably not an immunological reaction. If it is decided to use steroids, the patient is best admitted to a hospital with facilities for passive ventilation until the phase of improvement. High dosage (up to 100mg prednisolone) given on alternate days to spare the patient's own adrenal gland, is a favourite regimen. After one month the dosage is reduced gradually, but it is usually necessary to continue with 10mg or thereabouts daily or on alternate days for a year or more.

**Thymectomy**

Space does not permit a considered discussion of thymectomy. In summary, my practice is to advise the operation (by whatever route enables the surgeon to remove the whole (sic) gland) to every myasthenic with evidence of myasthenic weakness beyond the extraocular muscles, unless the myasthenia has been present for more than ten years and is stable. A deteriorating patient has nothing to lose because the operation has no mortality if certain precautions are adopted (Fraser et al., 1978). For the first 48 hours a striking remission may require considerable reduction in the dose of pyridostigmine. However, immediate benefit, if it occurs, is temporary. The major advantage is gained two to three years after operation when the further course of the illness is much more benign than without thymectomy. This is true even for atrophic and thymomatous glands, but the poorer long-term prognosis of the thymomas has already been mentioned above.

It has been impossible to give full references to all points discussed. Forgive me therefore for listing two personal papers in which further details may be sought (Simpson, 1978, 1981).

See General Practice Comment (p.1189).

**References**


PRELIMINARY COMMUNICATION

EXPERIMENTAL MYASTHENIA GRAVIS IS INHIBITED BY RECEPTOR-ANTI-RECEPTOR COMPLEXES

T. BARKAS and J. A. SIMPSON

Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF
(Received 3 July 1981)

SUMMARY Experimental autoimmune myasthenia gravis (EAMG) induced in rabbits by immunisation with purified nicotinic acetylcholine receptor from Torpedo marmorata is a highly reproducible model for the human disease.

Pretreatment of experimental animals with immune complexes containing receptor and anti-receptor antibodies suppressed the subsequent induction of EAMG. Animals were protected from the normal severe paralysis. Moreover, antibody levels were reduced and synthesis of antibody rapidly terminated. Possible mechanisms are discussed.

INTRODUCTION

The human neuromuscular disorder, myasthenia gravis, is now clearly characterised as an autoimmune disease in which a major self-antigen is the nicotinic acetylcholine receptor. Antibodies to the receptor can be detected in the serum of 90% of patients with myasthenia gravis (1). Experimental models of the disease can be readily induced in a number of species by immunisation with the pharmacologically similar receptor purified from the electric organs of fish such as Torpedo (2). The availability of pure antigen and good models of myasthenia make this a useful system for studying control of autoimmunity. One possible approach to specific immunotherapy is the production of anti-idiotypic antisera directed at the anti-receptor antibodies. Klaus (3,4) has reported that antigen–antibody complexes are highly potent immunogens and has also demonstrated that such complexes can be used to generate anti-idiotypic antisera (5,6). In the present report, experimental animals were pretreated with complexes of Torpedo receptor (treated so as not to induce EAMG) and anti-receptor antibodies before immunisation with untreated Torpedo receptor alone. Pretreated animals were found to be protected from subsequent induction of EAMG.

MATERIALS AND METHODS

Preparation of Nicotinic Acetylcholine Receptor and Antiserum

Nicotinic acetylcholine receptor (specific activity 5,000 pmoles/mg) from Torpedo marmorata was prepared (7) and rabbit antiserum to the purified receptor were raised as described previously (8).

Measurement of Receptor Levels, Antibody Titre and Immune Complexes

α-bungarotoxin (Boehringer) was iodinated by the method of Lindstrom et al. (9) to a specific activity of 100 Ci/m mole. Receptor activity was measured essentially as described by Schmidt and Raftery (10).

Anti-receptor antibody titres were determined as described previously (8). Immune complexes were assayed by polyethylene glycol precipitation and binding to Staphylococcus aureus (9).

Protein levels were determined by the modified Lowry method of Dubley and Grieve (11).

Preparation of Complexes

As in the normal purification procedure for the receptor, a 200 ml crude Triton extract of Torpedo electric organs (100 g) was prepared.

100 ml of this (50,000 pmoles) was mixed with 20 ml of Sepharose-immobilised α-cobratoxin for 2 hr at 20°C. The beads were then washed repeatedly with buffers containing 1 M sodium chloride, 9.686 pmoles of receptor were bound under these conditions. 20 ml of heat inactivated rabbit anti-Torpedo receptor antiserum was added and mixed for 2 hr at 20°C; then the beads were washed. This amount of serum was in excess of that required to saturate the bound receptor. 22,200 pmoles antibody being offered and 13,108 pmoles being bound. The total cycle of receptor and antibody addition was then repeated, 9,964 pmoles of receptor and 18,730 pmoles of antibody being bound. This second cycle was performed so as to include any antibodies against the α-toxin binding site of the receptor, the rationale being that saturating levels of the first antibody leave free antigen-binding sites available. These can then bind the second batch of receptor via an antigenic site rather than a toxin binding site, leaving the toxin-binding site accessible to the second batch of antisemum. Anti-toxin site antibodies have been successfully affinity purified by this process (Barkas, Gairns, Kerr, Coggins and Simpson, in preparation).

Weakly bound material was eluted by brief incubation with ammonia, under conditions (0.2 M, 1 hr at 4°C) which were shown not markedly to affect the activity of toxin-bound receptor. Complexes were then eluted with carbachol (1 M, 16 hr at 4°C) and dialysed against phosphate-buffered saline, pH 7.2 (PBS). The antibody-bound receptor was separated from a small amount of non-antibody bound protein by chromatography on protein A-Sepharose (Pharmacia). Bound material was eluted with 1 M acetic acid, neutralised and dialysed against PBS.
**Experimental Rabbits**

NZW female rabbits aged 24-36 weeks were used throughout.

(a) **Acid treated receptor compared with untreated receptor**

One batch of Torpedo receptor was used. A portion was dialysed against 1 M acetic acid for 1 hr at 4°C, neutralised and dialysed against PBS. Protein concentrations of untreated and treated receptor were identical. Rabbits were immunised intramuscularly with 100 μg aliquots of the appropriate receptor at three weekly intervals, initially in 1 ml of Freund's complete adjuvant (CFA) then in incomplete (IFA).

(b) **Complex pretreated with adjacent pretreated**

One group of rabbits was pretreated with three injections of 30 μg of complexes given intramuscularly initially in 1 ml of CFA then in IFA. Control rabbits received buffer-adjuvant mixtures.

Ten weeks later, both groups were tested normally for the indication of EAMG.

**Gel-filtration of Radiolabelled Complexes**

Complexes were iodinated by the method of Urbania et al. (12), to a specific activity of 200 μCi/μg and subjected to gel filtration on an 8 x 2 cm column of Ultragel ACA34 in PBS. Iodinated complexes were also treated with an equal volume of 6 M potassium thiocyanate in PBS at 20°C for 30 min, then gel filtered in PBS. 2 ml fractions were collected and 100 μl counted for radioactivity. An equal volume of PBS (0.5% BSA) was added, followed by two volumes of 20% (w/v) sulphosalicylic acid. After 30 min at 20°C, the tubes were centrifuged at 1500g for 10 min, the supernatants aspirated and the pellets counted. Monomeric and dimeric IgG were obtained for a Cohn fraction IV preparation of human IgG (West of Scotland Blood Transfusion Service).

**Preparation of Mouse IgG. Raising of Antiserum and Iodination of Mouse IgG**

Mouse IgG was purified by protein A chromatography and gel filtration on Ultragel ACA34. Antiserum to mouse IgG was raised in rabbits by intramuscular injection of two doses of 100 μg IgG at three-week intervals, the first being in complete Freund's adjuvant and the second in incomplete adjuvant. Mouse IgG was radiolabelled with I125 by the method of McConkey and Dixon (13).

**RESULTS**

**EAMG in Rabbits**

In experiments totalling approximately 40 animals, EAMG in rabbits has proved to be a highly reproducible system. 100 μg (1 ml) of purified Torpedo receptor in complete Freund's adjuvant is given intramuscularly at a single site. Twenty-one days later an identical dose in incomplete adjuvant is also given intramuscularly. Six-seven days later, weakness of the hind limbs is detected which progresses rapidly (within 24 hr) to total paralysis of the rabbit, such that it cannot right itself if placed on its back.

**Preparation of Complexes**

As described above, immune complexes containing receptor and anti-receptor antibodies were eluted from an α-toxin column using carbachol which breaks the bond between α-toxin and receptor leaving the receptor-antibody bond intact. The complexes were then bound to protein A-Sepharose to remove traces of non-immunoglobulin-bound material, and eluted with acetic acid. Although acetic acid can readily denature free receptor (see below), minimal dissociation of the complexes was observed. This could be demonstrated by reapplying the eluted material to protein A-Sepharose, only traces of protein not being bound. Complexes can be demonstrated in the material eluted from protein A (table 1). The majority of the material was of higher molecular weight than IgG and could be dissociated by potassium thiocyanate (fig 1).

Recovery of protein, toxin binding activity and antibody activity is shown in Table 1.

**Immunisation of Rabbits with Acid-treated Receptor**

Receptor purified in the absence of antibody and dialysed against 1 M acetic acid for one hour, then neutralised, was found to lose most of its toxin-binding ability (1-8-5-0% remaining). The treated receptor did not form a precipitin band with antiserum to the native receptor indicating that the antigenicity had been profoundly altered. Rabbits immunised with acid-treated receptor did not develop symptoms of EAMG even after three injections whereas rabbits immunised at the same time with the same batch of receptor not subjected to acid

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Recovery of protein, toxin binding and antibody activities during the preparation of receptor-antibody complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>Protein (μg)</td>
</tr>
<tr>
<td>α-Toxin bound material</td>
<td>7.5*</td>
</tr>
<tr>
<td>Ammonia eluate</td>
<td>1.4</td>
</tr>
<tr>
<td>(α-toxin-Sepharose)</td>
<td>2.3</td>
</tr>
<tr>
<td>Carbachol eluate</td>
<td>1.8</td>
</tr>
<tr>
<td>(α-toxin-Sepharose)</td>
<td></td>
</tr>
<tr>
<td>Acetic acid eluate</td>
<td></td>
</tr>
<tr>
<td>(protein A-Sepharose)</td>
<td></td>
</tr>
</tbody>
</table>

*Calculated from the difference between the starting material and that not bound to the affinity resin. Antibody measurements denote available receptor-binding sites, and were determined using Torpedo receptor labelled with I125-α-toxin and Staphylococcus aureus as precipitant. Complexes were detected by the PEG-S. aureus method.

224 BARKAS AND SIMPSON
treatment became paralysed after two injections. The sera from the group of rabbits receiving acid treated receptor did not form precipitin bands with either treated or untreated receptor, and contained very low levels of antibody reactive with the native receptor (166–355 nM receptor binding sites compared with 2,000–5,000 nM in the rabbits injected with untreated receptor). However, after a single injection of native receptor, the rabbits pretreated with acid-denatured receptor rapidly developed paralysis.

**Immunisation of Rabbits with Complexes**

Three rabbits were immunised three times with 80 μg of complexes and control rabbits with adjuvant alone (see Table 2). Two complex-immunised animals were bled at regular intervals, all were monitored for symptoms of weakness. No symptoms of weakness were observed, although low levels of anti-receptor antibodies were generated (Table 2), indicating the presence of antigenic sites on the complexes injected. Ten weeks later, both groups were tested for susceptibility to EAMG. Rabbits pretreated with adjuvant alone became, as usual, totally paralysed 7–8 days after the second injection of receptor. In marked contrast, no symptoms at all were observed in the complex-pretreated animals. Two weeks later, a third injection was given. Two (rabbits 1 and 3) again displayed no symptoms. The other (rabbit 2) developed transient mild weakness of the hind limbs. Symptoms at their worst were that the animal was disinclined to move from a sitting position but could easily do so, although with a hunched appearance. These symptoms persisted for two weeks, again unlike the rapid deterioration (24–48 hr to total paralysis) of normal EAMG. A further two injections of receptor produced no symptoms whatsoever in the rabbits. Antibody titres were measured in two rabbits (1 and 2). These are shown in Table 2. Antibody titres in the adjuvant pretreated controls were 2,000–15,000 nM. The results show a marked suppression of the antibody response in the complex-pretreated animals. The antibody titre was also noted to fall rapidly within one week in contrast to the expected prolonged response in rabbits to other antigens and that observed with the response to the antigen, mouse IgG.

**Table 2 Antibody titres in complex-pretreated rabbits**

<table>
<thead>
<tr>
<th>Immunogen and number of injections</th>
<th>Time of testing after immunogen (days)</th>
<th>Antibody titre (nM receptor binding sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rabbit 1</td>
</tr>
<tr>
<td>Complexes (2)</td>
<td>7</td>
<td>42.4</td>
</tr>
<tr>
<td>Complexes (3)</td>
<td>7</td>
<td>36.2</td>
</tr>
<tr>
<td>Receptor (2)</td>
<td>7</td>
<td>274</td>
</tr>
<tr>
<td>Receptor (2)</td>
<td>14</td>
<td>274</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>7</td>
<td>909</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>11</td>
<td>700</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>14</td>
<td>583</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>18</td>
<td>359</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>21</td>
<td>280</td>
</tr>
<tr>
<td>Receptor (3)</td>
<td>25</td>
<td>221</td>
</tr>
<tr>
<td>Receptor (4)</td>
<td>4</td>
<td>205</td>
</tr>
<tr>
<td>Receptor (4)</td>
<td>7</td>
<td>305</td>
</tr>
<tr>
<td>Receptor (4)</td>
<td>11</td>
<td>205</td>
</tr>
<tr>
<td>Receptor (5)</td>
<td>13</td>
<td>942</td>
</tr>
</tbody>
</table>

Complexes (80 μg) were injected intramuscularly in a final volume of 1 ml of CFA. After three weeks, a further 80 μg was given in IFA, followed by a similar dose 10 days later. After 10 weeks, receptor (100 μg) was injected in CFA followed in three weeks’ time by a further 100 μg of receptor in IFA. Blood samples were collected for a period of four weeks. A further 100 μg of receptor was injected followed two weeks later by a similar dose.

*Appearance of clinical weakness.
Characterisation of the Complexes

Gel filtration studies using iodinated complexes (fig. 1) suggest that the complexes are of relatively low molecular weight, eluting between monomeric and dimeric IgG. The upper trace suggests that most of the iodinated material is of higher molecular weight than IgG. This material is reduced in size by treatment with thiocyanate. The peak remaining after thiocyanate treatment is probably a micelle of iodinated Triton X100 (14) with an effective molecular weight of 90,000. This is supported by the acid precipitable counts in each fraction (lower trace). Untreated material peaks well in advance of monomeric IgG, whereas the thiocyanate treated material peaks with the IgG marker. No low molecular weight acid precipitable counts are detectable suggesting that the antigen is of too low a molecular weight to precipitate.

The complexes were precipitated with polyethylene glycol and bound to *S. aureus* (9) then were characterised by their ability to bind free radiolabelled toxin or toxin-labelled receptor. As shown in Table 3, binding of labelled receptor was observed, indicating the presence of free antigen-binding sites on the complexes. Binding of free toxin was negligible, indicating that the toxin-binding site of the receptor in the complexes had been either removed by the acetic acid treatment (as for free receptor) or occluded by antibody, presumably the former as this occurred only on elution from protein A (see table 1).

Table 3  Binding of radiolabelled toxin or receptor-toxin to PEG-precipitated complexes

<table>
<thead>
<tr>
<th>Fraction from protein</th>
<th>Binding of test probes to PEG-precipitated and <em>S. aureus</em>-bound complexes (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein A</td>
</tr>
<tr>
<td>Non-bound</td>
<td>0</td>
</tr>
<tr>
<td>Acetic acid eluate</td>
<td>18,844</td>
</tr>
<tr>
<td>PBS eluate</td>
<td>5,099</td>
</tr>
<tr>
<td>Total CPM added</td>
<td>70,142</td>
</tr>
</tbody>
</table>

After dialysis against PBS, the carbacho! eluate from the x-toxin column was applied to a 5 ml column of protein A-Sepharose. Non-bound material was eluted using PBS. The resin was then treated sequentially with 1M acetic acid and PBS. The acetic acid fraction was neutralised and dialysed against PBS.

Immune complexes were detected by the PEG-*S. aureus* method. 100 μl aliquots of sample or PBS alone were added to 200 μl PBS, 100 μl normal rabbit serum, 8 μl 500 mM EDTA and 2 ml 6% PEG 6000. The precipitated complexes were bound to *S. aureus* and detected using radiolabelled protein A (0.5 pmole). After counting, 1.25 x-toxin (0.1 pmoles, 0.17 μCi) was added and mixed for 90 min at 20°C. The *S. aureus* were again washed and the bound radiolabel measured. Finally receptor-toxin (0.05 pmole) was added and mixed for 90 min at 20°C. The *S. aureus* were then washed and bound receptor measured.

(ReM-receptor) (15). However, in contrast to the work with ReM-receptor, very little antibody reactive with native receptor was produced. Moreover, animals pretreated with three injections of acid-treated receptor alone were not protected from subsequent induction of EAMG. A third possibility is that the complexes stimulate the generation of anti-idiotypic antibodies as shown by Klaus (5). Complexes formed in antibody excess were found to be especially effective in this respect. The type of complex used in the present work has not been completely defined. It is clearly different from the classical large “antibody-excess” complex because of its low molecular weight. However, the acetylcholine receptor in its native form, consisting of five individual components (16), has been reported to bind no more than four Fab molecules (17). As the complexes detected here could not possibly contain the whole receptor molecule (effective molecular weight on gel filtration of 350,000 daltons), it is feasible that we are dealing with a complex containing one antibody molecule and a subunit of the receptor. A single subunit, especially after acid-treatment, might only contain one antigenic site, thus explaining the small size of the complex. The material is, however, similar to the classical antibody-excess complex in having free antigen-binding sites available (table 3).

Whatever the mechanism, the end result appears to be a rapid termination of antibody synthesis (table 2). A rapid fall in antibody levels could be explained if circulating anti-Torpedo antibodies bound to the rabbit receptor. This would be followed by degradation of the complex and renewed synthesis of receptor (2). This,
however, is unlikely to be the explanation as Torpedo and mammalian receptors cross-react only slightly (18).

Present studies are directed towards the characterisation of the complexes and a comparison of the effectiveness of free antibody and immune complexes of defined structure to suppress the induction of, or ongoing, EAMG. This is more readily studied in the mouse model which is less acute in onset and more akin to the human disease (19). Effects on the ongoing disease are relevant to human myasthenia as low levels of complexes have been detected in the sera of 40% of myasthenic patients (20), and it is intriguing to speculate that the relapses and remissions seen might be related to the production of such complexes. Finally, treatment with preformed defined complexes might have therapeutic potential in myasthenia gravis.

ACKNOWLEDGEMENTS

We thank Mrs. J. Gains and Mr. I. MacDonald for technical assistance, and Mrs. M. McColl for secretarial assistance. This work was supported by the Muscular Dystrophy Group of Great Britain.

REFERENCES

Lack of inter-animal cross-reaction of anti-acetylcholine receptor antibodies at the receptor-binding site as demonstrated by heterologous anti-idiotype antisera: implications for immunotherapy of myasthenia gravis

T. BARKAS & J. A. SIMPSON Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland

(Accepted for publication 10 July 1981)

SUMMARY

Anti-idiotype antisera were raised in rabbits by immunization with purified sheep anti Torpedo receptor antibodies. The antisera were able specifically to block the binding of receptor to the inducing antibodies but not anti-Torpedo antibodies from other animals of the same, or other, species. Rabbits producing the anti-idiotype sera were not protected from experimental autoimmune myasthenia gravis (EAMG). The implications of these observations for the potential use of anti-idiotype antisera in the treatment of myasthenia gravis are discussed.

INTRODUCTION

The human neuromuscular disorder, myasthenia gravis, is now well characterized as an autoimmune disease in which a major self-antigen is the nicotinic acetylcholine receptor. Antibodies to the receptor can be demonstrated in the serum of 90% of myasthenic patients. Experimental models of the disease can be readily induced in a range of species by immunization with nicotinic receptor purified from the electric organs of fish, such as Torpedo (see review by Barkas, 1979). One possible approach to therapy might be the production of anti-idiotype antisera as a means of specific suppression of autoantibody production. Preliminary work along these lines has been reported by Schwarz et al., (1978) who immunized mice with syngeneic lymphocytes previously educated in vitro with purified Torpedo receptor. Their anti-idiotype antisera showed considerable cross-reaction of anti-receptor antibodies raised in a number of species, including cross-reactions at the receptor-binding sites. For use as a possible means of therapy, it would obviously be advantageous if one anti-idiotype antiserum could be used for different patients and the cross-reactivity noted above is therefore encouraging. The techniques used to raise the anti-idiotype antisera, however, are not readily applicable to the human situation. A more practicable method is the purification of anti receptor antibodies which are then used to raise heterologous anti idotype antisera. We have adapted this approach using anti-Torpedo receptor antibodies, and have successfully produced anti-idiotype antisera. However, negligible cross-reaction at the receptor-binding site was found between anti-receptor antibodies raised in different animals. The relevance of these findings to possible immunotherapy of myasthenia gravis is discussed.
Materials and Methods

Preparation of nicotinic acetylcholine receptor and antisera. The nicotinic acetylcholine receptor was purified from the electric organs of Torpedo marmorata as described previously (Harvey et al., 1978). Antisera to the purified receptor were raised in rabbits, sheep and mice by two intramuscular injections of 100 µg (10 µg for mice) of receptor firstly in Freund's complete adjuvant then in incomplete adjuvant with an interval of 3 weeks. Animals were bled out when paralysis occurred.

Measurement of receptor levels and antibody titres. Alpha-bungarotoxin (Boehringer) was iodinated by the method of Lindstrom et al. (1976) to a specific activity of 100 Ci/mmol. Receptor activity was measured essentially as described by Schmidt & Raftery (1973).

Anti-receptor antibody levels were determined as described by Barkas et al. (1978) using a second antibody as precipitant. Sheep anti-rabbit IgG and anti mouse IgG and donkey anti sheep IgG were kindly provided by the Scottish Antibody Production Unit, Law Hospital.

Protein was estimated by the method of Dulley & Grieve (1975).

Preparation of antibody. Sheep antibodies to the receptor were purified by affinity chromatography. One hundred millilitres of crude Triton extract of Torpedo electric organ (100 g) were mixed with 20 ml of Sepharose-coupled α-cobratoxin (Barkas et al., 1978) for 2 hr at 20°C. After extensive washing with buffer containing 1 M sodium chloride, 50 ml of heat-inactivated sheep antisera to Torpedo receptor were applied for 1 hr at 20°C. After washing, the bound material was eluted with 25 ml 3 M potassium thiocyanate in phosphate-buffered saline, pH 7-2 (PBS). This treatment was shown to have no effect on antibody activity but irreversibly destroyed receptor antigenicity. After centrifugation, the beads were washed with 10 ml PBS and the combined supernatants dialysed against 2 x 11 PBS. Negligible toxin binding activity was detected in the dialysed sample (700 pmol compared with 33,100 bound). However, sheep IgG and antibody to Torpedo receptor could both be demonstrated by immunodiffusion. A precipitate formed on storage at 1°C was removed by centrifugation at 10,000 g for 20 min. The supernatant was then concentrated to 1 ml and applied to an 86 x 2 cm column of Ultragel ACA34. Elution was carried out in PBS and 1.8-ml fractions were collected. Two peaks of protein were eluted, one at the void volume and one at the position of elution of IgG. Antibody activity and IgG were demonstrated only in the second peak which was pooled and used for further work. Recovery of antibody activity was 137.8 nmol receptor binding sites from 2,630 nmol applied to the beads of which 444 nmol bound. This represents 11.0 mg antibody. Total protein recovered was 19.2 mg.

Rabbit antibody was purified in a similar fashion.

Preparation of normal sheep IgG and immobilization. A crude sheep IgG preparation was prepared by precipitation of normal sheep IgG with ammonium sulphate (40%) followed by dialysis against PBS. One hundred milligrams of this material were coupled to Sepharose CL-4B by the cyanogen bromide method (Parikh, March & Cuatrecasas, 1974). Absorption of anti-idiotype sera was performed by incubating equal volumes of immunoadsorbent and serum at 20°C for 90 min. Normal sheep IgG was prepared by the method of Ling, Bishop & Jeffers (1977).

Preparation of Fab'2. Purified sheep anti-receptor antibodies were concentrated to 10 mg/ml and Fab'2 prepared as described by Stanworth & Turner (1973).

Preparation and radiolabelling of mouse IgG and rabbit anti-mouse IgG. Mouse IgG was isolated by chromatography on protein A-Sepharose. The eluted material was further purified by gel filtration on Ultragel ACA34. IgG was radiolabelled to a specific activity of 500 µCi/mg by the method of McConahey & Dixon (1966). Rabbit anti-mouse IgG antiserum was raised by immunization at 3 weekly intervals with 100 µg amounts of mouse IgG in adjuvant.

Preparation of anti-idiotype antisera. Anti-idiotype antisera to the purified sheep antibodies were raised in rabbits by intramuscular injection in adjuvant at 3-weekly intervals, firstly in Freund's complete and secondly in Freund's incomplete adjuvant. For the first two injections, 35 µg of sheep antibody were used. This was increased to 100 µg for two further injections. Blood samples were collected at weekly intervals.

Assay of anti-idiotype antisera. (1) Fluid-phase assay. The antisera were tested for their ability to inhibit binding of radiolabelled receptor to anti-Torpedo receptor antibodies. All assays were in quadruplicate. One hundred microlitres (100 ng) of purified sheep antibody in 100-fold-diluted...
normal sheep serum or diluted normal serum alone were incubated with 20 µl buffer (10 mM phosphate, 0.1% Triton X100, pH 7.4), normal rabbit serum or test serum for 1 hr at 20°C. One hundred and fifty microlitres of toxin-labelled Torpedo receptor (1.6 pmol) were added and incubated at 4°C for 16 hr. Sufficient donkey anti-sheep IgG to precipitate the sheep IgG was added and incubated at 4°C for 4 hr, followed by centrifugation at 1,500 g for 10 min and washing with 10 mM phosphate buffer containing 0.1% Triton X100.

For cross-reaction experiments, suitably diluted anti-Torpedo antisera were substituted for the sheep antibody and precipitation was performed with the appropriate anti-immunoglobulin antiserum.

(ii) Solid-phase assay. Purified sheep antibody or normal sheep IgG was coated onto LP3 tubes (Luckham) by incubating 1-ml aliquots (10 µg/ml in PBS) at 4°C for 4-6 days. Excess protein was washed out with three 1-ml washes of PBS. One millilitre of PBS containing 0.01% gelatin was added and incubated at 20°C for 2 hr. The tubes were then washed or stored at -70°C. For the assay, aliquots of test sera or IgG fractions were added to the tubes followed by sufficient PBS containing 0.05% Tween 20 to make the volume 1 ml total. After 1 hr at 20°C, 15 µl of toxin-labelled receptor (1.6 pmol) were added and incubated at 4°C for 16 hr. All tubes were then washed three times with 1 ml of PBS and counted.

Measurement of anti-sheep IgG and anti-idiotypic antibodies in the anti-idiotypic antiserum. Purified sheep anti-receptor antibodies were iodinated by the method of McConahey & Dixon (1966) to a specific activity of 138 Ci/mmmol.

Antibodies to sheep IgG were quantitated by incubating 100 µl PBS-0.5% BSA buffer with 50 µl heat-inactivated rabbit antisera or NRS at 10-fold dilutions for 30 min at 20°C. Fifty microlitres of labelled sheep antibody (0.38 pmol) were then added and incubated for 60 min at 20°C. One hundred microlitres of 10% *Staphylococcus aureus* in PBS-0.5% BSA were added and incubated for 30 min at 20°C. Five hundred microlitres of PBS-BSA were added and the tubes spun at 1,500 g for 10 min. The supernatants were discarded and the pellets washed with a further 500 µl buffer, then counted.

Anti-idiotypic antibodies were measured by the same method except that in the first incubation, heat-inactivated autologous sheep serum from preimmunization bleeds diluted 10-fold in PBS-BSA was used in place of buffer. Experiments using undiluted sheep serum showed no difference compared with 10-fold-diluted serum.

Immobilization of purified sheep anti-receptor antibody and absorption of anti-idiotypic serum. The method used was based on that of Avrameas & Ternynck (1969). One millilitre (1 mg) of sheep antibody or PBS was added to 1 ml of heat-inactivated autologous preimmunization serum and dialysed against 0.9% saline, pH 6.6, at 4°C overnight. Potassium phosphate buffer, 1 m pH 7.0 (0.2 ml), was added and followed by 0.3 ml 25% aqueous glutaraldehyde. The samples were incubated at room temperature for 5 hr, then at 4°C for 24 hr. The gels formed were gently homogenized and washed extensively with PBS.

One millilitre of heat-inactivated antiserum from rabbit 2 was then added to each gel and incubated at 4°C for 2 hr. After centrifugation at 1,500 g for 5 min, the sera were tested for anti-idiotypic activity in the fluid-phase assay.

**RESULTS**

Preparation of anti-idiotypic antisera

After two injections of purified sheep antibody, antibodies capable of inhibiting the fluid-phase binding of receptor to the purified antibody were elicited (Table 1). As shown in Fig. 1, the effect was clearly detectable at dilutions greater than 100-fold, while normal rabbit serum inhibited the reaction only slightly. Identical results were obtained with heat-inactivated sera. After each boosting dose, the inhibitory activity increased, and remained fairly constant for a period of 3 weeks between injections (Table 1).
Fig. 1. Inhibition of the binding of radiolabelled receptor to purified sheep antibodies by anti-idiotype serum A. The serum used was from rabbit A 1 week after the third injection. Results are the mean and standard deviation of three experiments. (*) Serum from rabbit A, (•) serum from normal rabbit.

Definition as anti-idiotype
Inhibition of the precipitation assay could be explained by factors other than anti-idiotype antibodies. The anti-idiotype antisera were tested for anti-receptor antibody activity but none was found. The results were shown not to be due to non-specific effects on the subsequent precipitation by anti-sheep IgG antibodies. Absorption of the anti-idiotype antiserum with immobilized normal sheep IgG had no effect on the result. Moreover, no decrease in radiolabelled receptor precipitated was found if the anti-idiotype antiserum was added after the labelled receptor rather than before it. This was confirmed by the observation that the anti-idiotype antiserum did not affect the binding of

Table 1. Inhibition of binding of radiolabelled receptor to purified sheep antibody by anti-idiotype antisera

<table>
<thead>
<tr>
<th>No. of injections of antibody</th>
<th>Time after injection (weeks)</th>
<th>Percentage inhibition of binding of receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rabbit A*</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>65-6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>62-0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>78-1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>76-3</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>74-5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>83-0</td>
</tr>
</tbody>
</table>

Inhibition was calculated using the equation:

\[
\frac{(Ab + B) - (Ab + T)}{(Ab + B) - (N + B)}
\]

where (Ab + B) is the c.p.m. with sheep antibody plus buffer, (Ab + T) is the c.p.m. with sheep antibody plus test serum, (N + B) is the c.p.m. with normal sheep serum plus buffer. All other details are given in the Materials and Methods section.

* Source of serum.
labelled mouse IgG to sheep anti-mouse IgG as assessed by immune precipitation with anti-sheep IgG (Table 2). The antiserum was shown to be effective using F(ab')2 fragments of pure sheep IgG (Table 2). Direct confirmation that the inhibition observed was not due to altered precipitation came from experiments using solid-phase purified sheep antibodies (Table 3) where a similar blocking effect was observed.

However, the results might still be explained by anti-allotype antibodies if the allotype site was present in the F(ab')2 region in such a position that bound antibody could cause steric hindrance of the binding of antigen. That this was not so was demonstrated by the fact that exactly the same inhibition of receptor binding was found in the presence of a 100-fold excess of normal IgG obtained from preimmunization bleeds from the same sheep (sheep 1) used to prepare the purified antibody (77.0 ± 3.7%, mean and standard deviation of two experiments) and of IgG from a serum pool from other sheep (75.2 ± 8.0%). Identical results were obtained with a 100-fold or 200-fold excess of normal autologous IgG (74.9 ± 1.84%, with 100-fold excess, 78.5 ± 1.41% with 200-fold excess). This last experiment clearly defines the antisera as anti-idiotype. Moreover, the inhibitory activity of the antisera was absorbed by purified sheep anti-Torpedo antibody immobilized in the presence of autologous preimmunization serum, but not by the serum alone.

The final titres of anti-sheep IgG and anti-idiotype antibodies were respectively 682 and 85 nm for rabbit A and 866 and 108 nm for rabbit B.

<table>
<thead>
<tr>
<th>Table 2. Effect of anti-idiotype antiserum on precipitation of labelled mouse IgG by sheep anti-mouse IgG and on the precipitation of receptor by F(ab')2 fragments of pure sheep anti-Torpedo receptor antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum added</td>
</tr>
<tr>
<td>Normal rabbit</td>
</tr>
<tr>
<td>Anti-idiotype A</td>
</tr>
<tr>
<td>Anti-idiotype A after absorption with normal sheep IgG</td>
</tr>
</tbody>
</table>

Total mouse IgG added was 53,800 c.p.m. Total labelled receptor added was 49,940 c.p.m. The antiserum used was from rabbit A 1 week after the fourth injection. Results of blanks using normal sheep IgG have been subtracted.

<table>
<thead>
<tr>
<th>Table 3. Inhibition of binding of radiolabelled receptor to immobilized purified sheep antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample added</td>
</tr>
<tr>
<td>Buffer alone</td>
</tr>
<tr>
<td>IgG from anti-idiotype A</td>
</tr>
<tr>
<td>IgG from anti-idiotype A</td>
</tr>
<tr>
<td>Serum from anti-idiotype A</td>
</tr>
<tr>
<td>Normal rabbit serum</td>
</tr>
</tbody>
</table>

Total receptor added was 48,000 c.p.m. The concentration of the IgG from rabbit A was 1 mg/ml. Background counts have been subtracted. The antiserum used was from rabbit A 1 week after the fourth injection.
Table 4. Binding of radiolabelled receptor to anti-Torpedo receptor antibodies from a range of animals in the presence of anti-idiotype antisera raised to antibody from sheep 1

<table>
<thead>
<tr>
<th>Source of antibodies</th>
<th>Normal rabbit serum</th>
<th>Anti-idiotype A</th>
<th>Anti-idiotype B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified antibody from sheep 1</td>
<td>6,378±255</td>
<td>—</td>
<td>1,633±178</td>
</tr>
<tr>
<td>in normal sheep serum</td>
<td>7,465±590</td>
<td>2,573±129</td>
<td>—</td>
</tr>
<tr>
<td>Serum from sheep 1</td>
<td>5,646±91</td>
<td>—</td>
<td>2,772±209</td>
</tr>
<tr>
<td></td>
<td>6,332</td>
<td>4,017±83</td>
<td>—</td>
</tr>
<tr>
<td>Serum from sheep 2</td>
<td>12,003±267</td>
<td>—</td>
<td>11,370±9</td>
</tr>
<tr>
<td></td>
<td>14,827±2,584</td>
<td>15,073±2,415</td>
<td>—</td>
</tr>
<tr>
<td>Serum from sheep 3</td>
<td>10,047±9</td>
<td>—</td>
<td>9,380±2</td>
</tr>
<tr>
<td></td>
<td>12,746±1,312</td>
<td>12,630±3,174</td>
<td>—</td>
</tr>
<tr>
<td>Serum from rabbit 1</td>
<td>10,635±381</td>
<td>8,439±622</td>
<td>10,582±211</td>
</tr>
<tr>
<td>Serum from rabbit 2</td>
<td>3,706±310</td>
<td>4,677±490</td>
<td>4,250±389</td>
</tr>
<tr>
<td>Serum from rabbit 3</td>
<td>5,733±370</td>
<td>5,887±180</td>
<td>6,033±415</td>
</tr>
<tr>
<td>Serum from rabbit 4</td>
<td>11,091±705</td>
<td>11,866±630</td>
<td>11,673±152</td>
</tr>
<tr>
<td>Purified rabbit antibody</td>
<td>14,875</td>
<td>15,240±601</td>
<td>15,064±139</td>
</tr>
<tr>
<td>Serum from mouse</td>
<td>14,896±1,122</td>
<td>16,298±757</td>
<td>15,159±1,228</td>
</tr>
</tbody>
</table>

Total receptor added was 40,000 c.p.m. Anti-Torpedo antisera were diluted to give approximately equivalent amounts of antibody. The results are the mean and standard deviation of two experiments. The antisera were those taken 1 week after the fourth injection. Results of blanks using normal sheep serum with the appropriate rabbit serum have been subtracted.

Effect of anti-idiotype sera on antibodies from other animals
The antisera were tested for their ability to prevent the binding of receptor to antisera from three sheep (Tables 4 & 5). Inhibition was only observed with serum from the sheep (sheep 1) from which the antibodies were prepared. Inhibition was less than with purified antibody.

Similarly, with a number of antisera from rabbits and mice, little inhibition was observed (Tables 4 & 5).

Effect of anti-idiotype antisera on experimental myasthenia
Experimental autoimmune myasthenia gravis (EAMG) is readily and reproducibly induced in rabbits by two intramuscular injections of 100 μg purified Torpedo receptor in adjuvant. Animals producing the anti-idiotype antisera were tested for susceptibility to EAMG. Onset and severity of disease were identical to normal EAMG, showing no protection.

DISCUSSION

In the present work, we have demonstrated the feasibility of preparing heterologous anti-idiotype antisera to anti-acetylcholine receptor antibodies by immunization with relatively small quantities of purified antibody. The antisera produced are capable of blocking the binding of radiolabelled receptor to the inducing antibodies by a factor of up to 75% as compared with normal rabbit serum. The antisera have been clearly defined as being directed against the idiotypes of the antibodies. Firstly, inhibition of binding of antigen is often itself taken as evidence that antisera are
cross-reaction was envisaged, authors tested can therefore be
Secondly, the possible that binding of subsequent study present would be have to production or immunization animals. This is anti-idiotypic. The anti-idiotypic. We have also shown from the sheep used for the preparation of the idiotypes did not influence the result. The antisera can therefore be defined as anti-idiotypic.

The blocking effect of the antisera was shown to be restricted almost totally to the original inducing antibodies (Tables 4 & 5) and had no effect on anti-Torpedo antibodies from other animals. This is identical to the situation with anti-tetanus antibodies in man (Geha & Weinberg, 1978) but contrasts with the work of Schwarz et al. (1978) who used antisera raised in mice by immunization with syngeneic lymphocytes educated in vitro with purified Torpedo receptor. These authors tested their antisera by two methods; firstly, by direct binding to immobilized anti receptor antibody and secondly by inhibition of the binding of radiolabelled receptor to fluid-phase anti-receptor antibody. Possibly the difference might be explained by the different methodology for production or assay of the antisera. For example, it is not clear from the previous work whether pooled lymphocytes or antisera were used. However, if therapeutic use of antisera in humans was envisaged, the approach used would have to be that taken in the present work. If similar lack of cross-reaction was found with human antibodies, this would imply that individual antisera would have to be prepared for each patient to be treated. In those cases with low levels of antibody, this would be extremely impractical. However, the outlook might not be so bleak as this. Firstly, the present study relates only to those idiotypic sites where bound antibody can interfere with subsequent binding of receptor. Idiotype sites outside this area have not yet been studied but it is possible that they might show more cross-reaction of the type observed by Schwarz et al. (1978). Secondly, the antibody response in humans might be much more restricted than in experimental.
animals as shown for antibody production to thyroglobulin (Nye, Pontes de Carvalho & Roitt, 1980).

Another point of possible relevance is the observation that less inhibition was observed using whole serum from sheep 1 compared with purified antibodies from sheep 1 added back to normal sheep serum, even though the amount of antibody used present in the whole serum sample was less (Table 4). This suggests the possibility that only a subfraction of antibody has been purified by the present method. This could result from lack of binding of low-affinity antibodies to the affinity resin or lack of release of high-affinity antibodies from the resin. If this were the case, the method would require altering so as to obtain a full spectrum of idiotypes.

No protection against EAMG in rabbits was affected by prior immunization with purified sheep antibodies. Similar results were obtained using purified rabbit antibodies (results not shown). This would not be surprising if there were no cross-reaction between the idiotypes. However, rabbits immunized with receptor–rabbit antibody complexes were protected from EAMG (Barkas & Simpson, submitted). The difference between these two systems is being investigated.

This work was supported by the Muscular Dystrophy Group of Great Britain. The technical assistance of Mrs J. Gairns and Mr I. MacDonald are gratefully acknowledged, as is the secretarial assistance of Mrs M. McColl.

REFERENCES


BARKAS, T. (1979) Myasthenia gravis, the acetylcholine receptor and the immune response. *Int. J. Immunopharmacol.* 1, 263.


\[\alpha\]-Bungarotoxin binding to the nicotinic acetylcholine receptor is inhibited by two distinct subpopulations of anti-receptor antibodies

Affinity-purified antibodies to the nicotinic acetylcholine receptor of *Torpedo marmorata* were fractionated into two populations using a covalently cross-linked receptor-toxin immunosorbent lacking free toxin-binding sites.

The population of antibodies which bound to and were subsequently eluted from this resin, and which cannot possibly contain antibodies directed to the toxin-binding site itself, was effective in inhibiting the binding of toxin to receptor in solution. This unequivocally demonstrates that inhibition of toxin binding can be mediated by antibodies which are not directed against the toxin-binding site. A second minor population of antibodies which did not bind to the affinity resin but which did inhibit the binding of toxin to receptor in solution was detected.

Two subpopulations of toxin-binding inhibitory antibodies can therefore be distinguished. A clear differentiation should be made in future work describing “anti-toxin site” antibodies between antibodies directly binding to the toxin-binding site and the pseudo-anti-toxin-binding site antibodies described in this report.

1 Introduction

Circulating antibodies directed against the nicotinic acetylcholine receptor are found in 90% of patients with myasthenia gravis [1]. Anti-receptor antibodies can also be readily induced in experimental animals by immunization with receptors purified from a range of species [2, 3]. In general, these antibodies are estimated using a receptor prelabeled with iodinated \(\alpha\)-bungarotoxin, a specific nicotinic ligand. However, it has been suggested that antibodies which might also be present which are directed against the toxin-binding (TB) site itself, and that these antibodies might be especially relevant to the disease state [4–7]. Similar “anti-toxin site” antibodies have been described with hybridoma antibodies [8–10]. The evidence for such antibodies is that prior incubation of the receptor with antiestera inhibits the subsequent TB. However, inhibition of TB by a large molecule such as an antibody could be due to not only antibodies directed specifically to this site but also to steric hinderance or induced conformational changes. A monoclonal antibody which inhibits the TB to the receptor and which itself is inhibited from binding by the small mol. wt. toxin has been described [10], showing that antibodies to the TB site itself do exist. However, no detailed work has been performed to see if antibodies not directed to the TB site can also interfere with TB. To this end, we have isolated such antibodies by affinity chromatography and here present evidence that these antibodies not only can inhibit TB but can, in fact, account for the major part of the so-called anti-TB site antibodies of a polyvalent antiserum.

2 Materials and methods

2.1 Preparation of the nicotinic acetylcholine receptor

Nicotinic acetylcholine receptor was prepared from the electric organs of *Torpedo marmorata*. Freshly thawed electric organ (200 g) was chopped and homogenized for two periods of 1 min at 4°C in 200 ml of 10 mM phosphate buffer containing 10 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 0.01% sodium azide, pH 7.4 (buffer A). The homogenate was centrifuged at 20,000 x g for 1 h, and the pellet extracted with 200 ml of buffer A containing 1% (v/v) Triton X-100 at 4°C for 16 h. The extract was centrifuged at 100,000 x g for 1 h and the supernatant retained. Extract (100 ml) was applied in a batch to 50 ml of affinity resin, prepared by immobilizing 25 mg \(\alpha\)-cobratoxin (Miami Serpentarium, Miami, FL) on Sepharose CL-4B with cyanogen bromide [11]. After 2 h at 20°C, the beads were washed on a sintered glass funnel with three alternate 50-ml aliquots each of buffer A containing 0.1% Triton X-100 (buffer B) and 1 M NaCl or buffer B alone. The beads were then eluted with 20 ml of 1 M carbachol (Sigma, St. Louis, MO) in buffer B for 5 h at 20°C. After centrifugation, the supernatant was dialyzed overnight at 4°C against 2 l of buffer B without EDTA (buffer C). It was then applied to a 2-ml column of DEAE-cellulose (Whatman DE-52, Maidstone, Kent, GB) previously equilibrated with buffer C. The column was washed with 10 ml of buffer C, and receptor was eluted using buffer C containing 0.5 M NaCl. One-ml fractions were collected. Typical recoveries were 4–6 mg of protein and specific activities of 3500–6000 pmol TB/mg protein.

2.2 Labeling of \(\alpha\)-bungarotoxin and assay of receptor

\(\alpha\)-Bungarotoxin (Boehringer, Mannheim, FRG) was iodinated using chloramine-T to a specific radioactivity of 1600 Ci/mmol (= 59.2 TB/mmol) (Barkas and Simpson, submitted). Receptor was assayed by the method of Schmidt and Raftery [12].
2.3 Preparation of covalently-linked RT-Sepharose

α-Cobratoxin-Sepharose was prepared as above, using cyanogen bromide [11]. Dimethylsuberimidate dihydrochloride was prepared by the method of Davies and Stark [13]. One hundred ml of a crude extract of Torpedo electric organ was applied to 20 ml of toxin-Sepharose, at 4°C for 16 h. The beads were centrifuged at 1500 × g for 5 min and washed three times alternatively with 20 ml of 10 mM phosphate buffer, 10 mM EDTA, 0.1% Triton X-100, pH 7.4 (buffer D) and with buffer D containing 1 M NaCl. Then the beads were washed with 20 ml 100 mM triethanolamine-HCl buffer containing 0.1% Triton X-100, pH 8.1 (buffer E). Twenty-four ml of freshly prepared 10 mM dimethylsuberimidate dihydrochloride solution [14] in buffer E was added to the beads which were gently mixed for 2 h at 20°C. The beads were then washed with 20 ml of buffer E. Unbound receptor was eluted with 20 ml 1 M carbachol in buffer D for 4 h at 20°C. After centrifugation and washing of the beads, the eluted material was dialyzed and assayed for receptor and protein.

In a second stage, free TB sites on the affinity resin were blocked using α-bungarotoxin. Two mg of α-bungarotoxin in 20 ml phosphate-buffered saline (PBS), 0.1% Triton X-100, pH 7.1 were mixed with the RT beads at 4°C for 16 h. The beads were centrifuged and washed twice with 20 ml PBS, 0.1% Triton X-100 and once with buffer E. Twenty-one ml of freshly prepared 10 mM dimethylsuberimidate dihydrochloride in buffer E was added and the beads were mixed gently at 20°C for 2 h. The beads were then washed with buffer E (20 ml) followed by PBS, 0.1% Triton X-100 (20 ml).

2.4 Purification of anti-Torpedo receptor antibodies

2.4.1 By absorption to noncovalently linked RT beads

The method was as described by Barkas and Simpson [15]. Essentially, a crude extract of electric organ was mixed with 20 ml toxin-beads and the beads then washed as described above for receptor preparation. Heat-inactivated sheep anti-Torpedo receptor antiserum (50 ml) [15] was added at 20°C for 30 min and then the beads were washed three times each using buffer B and buffer B containing 1 M NaCl before eluting for 30 min at 20°C with 20 ml of 3 M potassium thiocyanate in buffer B. The eluate was dialyzed, concentrated to 1 ml by ultrafiltration, centrifuged at 15000 × g for 10 min, then applied to a column of Ultrogel AcA 34 (85 × 2 cm). The column was eluted with PBS and the IgG peak collected.

2.4.2 By two-stage absorption (total antibody)

The method was essentially the same as the previous one. However, after addition of the antiserum and washing, a further cycle of treatment with electric organ extract followed by antiserum was performed to isolate any antibodies which might be directed against the TB site. The first batch of receptor (a large excess over toxin) binds via the TB site. The volume of antiserum used was chosen to provide a large excess of antibody so as to saturate the receptor and leave free antigen-binding sites available. These then bind more receptor via sites other than the TB site, leaving TB sites exposed to the second batch of antiserum. The bound material was eluted, dialyzed, concentrated and gel filtered as described above.

2.4.3 Absorption to covalently linked RT beads

One volume of antibodies (640 µg/ml) purified by the method Sect. 2.4.2 was applied to an equal packed volume of covalently linked RT beads at 20°C for 30 min. The beads were centrifuged and washed with 1 vol. of PBS, 0.1% Triton X-100, pH 7.1 and the unbound material retained (fraction I). The bound antibody was eluted with 1 vol. of 3 M potassium thiocyanate in PBS at 20°C for 30 min, and the beads centrifuged and washed with 1 vol. of PBS. The eluted material was pooled (fraction II). Both fractions were filtered through 0.45-µm Millipore filters and dialyzed against two changes of PBS (100 vol.). After testing, fraction I was reabsorbed more extensively by incubation with an equal volume of RT beads at 4°C for 48 h. The unbound material was filtered and dialyzed without further dilution (fraction IA).

2.5 Assay using RT beads

Antibodies purified by the method 2.4.1 were labeled to a specific radioactivity of 138 Ci/mmol [15] by the method of McConahey and Dixon [16].

Replicate 100-µl samples of a 10% suspension of toxin- or RT-beads in PBS, 0.1% Triton X-100 were mixed with or without 10 µl curare (10⁻² m) for 30 min at 20°C. One hundred µl of labeled toxin (1.8 pmol) or labeled antibody (0.38 pmol) to Torpedo receptor were added and mixed for 45 min at 20°C. The beads were then washed twice with PBS, 0.1% Triton X-100 and counted.

2.6 Assay of anti-Torpedo receptor antibodies

One hundred µl of Torpedo receptor (30 fmol) was incubated with 50 µl of labeled α-bungarotoxin (125 fmol) at 20°C for 90 min. Sheep antibodies (100 µl) in heat-inactivated normal sheep serum diluted one hundred-fold were added and incubated at 4°C for 16 h. The equivalence amount (16 µl) of heat-inactivated donkey anti-sheep IgG (Scottish Antibody Production Unit, Law Hospital, Lanmark, Scotland) was added. The tubes were incubated for 4 h at 4°C, centrifuged, washed, and counted. All dilutions were in 10 mM phosphate buffer, 0.1% Triton X-100, pH 7.4 (buffer F).

2.7 Assay for inhibition of TB

Two sets of 100 µl of Torpedo receptor (30 fmol) or buffer F were incubated with 50 µl of labeled toxin (125 fmol) (set 1) or buffer (set 2) for 90 min at 20°C. Buffer (50 µl) was then added to set 1, and toxin to set 2, and incubation continued for 90 min at 20°C. For test samples, dilutions of the antibody in buffer were added to the receptor in place of buffer before and after toxin. After the second incubation the contents of the tubes were either filtered through two DEAE-cellulose discs (Whatman) or mixed with 1 ml of a 10% (v/v) suspension of DEAE-cellulose in buffer F for 30 min at 20°C, then centrifuged and washed twice with the same buffer. Inhibition of binding by antibody before or after toxin, was assessed and the difference calculated.
2.8 Protein determination

Protein in solution was determined by the method of Dulley and Grieve [17]. Protein bound to beads was determined by incubating with mixing 200–μl aliquots of a 10% (v/v) suspension of beads with 1 ml of the first reagent for 2 h at 20°C, followed by 100 μl of the second reagent for 30 min at 20°C. The tubes were then centrifuged at 1500 × g for 5 min at 20°C. Standards were treated identically.

3 Results

3.1 Preparation of covalently linked RT beads

The amount of receptor absorbed to the beads before coupling was estimated by TB to be approximately 120 nmol, which corresponds to 29 mg of protein. Material eluted from the beads by curare after the coupling stage was estimated by protein determination to be 3 mg. Protein determinations on toxin beads and covalently linked RT beads were 0.49 and 1.94 mg/ml, respectively, showing that 31.5 mg of material had been bound. Covalent attachment of this protein was demonstrated by treating the beads with 3 M potassium thiocyanate, followed by protein determination on the remaining bound material. More than 96% of the RT remained bound under these conditions.

The RT beads were tested for their ability to bind labeled toxin. Labeled α-bungarotoxin (630 pmol) could be bound, but only 262 pmol were blockable with 10–5 M curare. No binding was observed using toxin beads alone. After covalent attachment of unlabeled α-bungarotoxin, all curare-inhibitable TB was abolished. Binding of anti-receptor antibodies was demonstrated using radiolabeled purified antibodies. This was not affected by the second cross-linking stage (14 226 cpm per 100 μl of 10% suspension before, 14 561 cpm per 100 μl of 10% suspension after).

Table 1. Recovery of total protein and antibody activity in isolated fractions of anti-Torpedo receptor antibodies

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Preparation 1</th>
<th>Preparation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (μg/ml)</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Antibody activity (pmol TB/ml)</td>
<td>34.7</td>
<td>94.3</td>
</tr>
</tbody>
</table>

Table 2. Percentage inhibition of TB by isolated fractions I and II

<table>
<thead>
<tr>
<th>Dilution of fraction</th>
<th>Percentage inhibition of TB by fractions</th>
<th>Preparation 1</th>
<th>Preparation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>I</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>1:100</td>
<td>77.4 ± 9.8</td>
<td>96.8 ± 6.3</td>
<td>63.9 ± 7.6</td>
</tr>
<tr>
<td>1:200</td>
<td>68.7 ± 3.1</td>
<td>86.5 ± 14.6</td>
<td>55.2 ± 8.9</td>
</tr>
</tbody>
</table>

3.2 Fractionation of sheep anti-Torpedo receptor antibodies

Sheep anti-Torpedo receptor antibodies were prepared by the two-stage method in which excess receptor was applied to toxin beads, followed by an excess of antibody, then more receptor and more antibody. The material eluted by potassium thiocyanate (which destroys receptor activity and antigenicity) was gel-filtered and the IgG peak used for further work.

These purified antibodies were applied to RT beads to obtain two pools, fraction I which was not bound and fraction II which was bound and eluted with thiocyanate. Preliminary experiments used receptor noncovalently bound to toxin beads but this method was found to yield a fraction I preparation containing receptor activity. In all further work, covalently bound RT beads were used.

Recovery of antibody activity in the combined fractions I and II in two preparations was estimated as 63–75%, whereas recovery of protein was 91–95%.

3.3 Characterization of the antibody fractions

3.3.1 Protein content and antibody activity

The protein and antibody content of fractions I and II are shown in Table 1. A second more extensive absorption of fraction I yielded fraction IA. Essentially 86–90% of the total protein and 96–98% of the total detectable antibody was removed by the two absorption stages.

3.3.2 Inhibitory effect on TB

The specificity of this assay for inhibition of TB was tested using heat-inactivated sheep anti-Torpedo receptor antiserum or normal sheep serum at a dilution of one in ten. Toxin bound to receptor in the presence of normal serum was 38 745 ± 950 cpm when the serum was added after the RT incubation, 37 420 ± 1646 cpm when mixed with the receptor before the toxin was added. In contrast, the results using the anti-receptor antisem were 40 890 ± 1364 cpm and 16 440 ± 3720 cpm, respectively (inhibitions of 3.4% and 59.8%).

The ability of the original antibody pool, on preincubation with receptor, to block subsequent binding of toxin is shown in Fig. 1.

The effect of the isolated fractions I and II is shown in Table 2. Both fractions were found to be effective. In particu-
lar, fraction II (that bound to and eluted from immobilized RT) was more effective than fraction I.

Reabsorption of fraction I demonstrated that whereas most of the antibody detectable using receptor prelabeled with toxin was removed, a high proportion of the inhibitory effect was not absorbed (Table 3).

![Figure 1. Percentage inhibition of TB to receptor by purified sheep anti-Torpedo receptor antibody. Affinity-purified sheep anti-Torpedo receptor antibodies (640 μg/ml) were prepared by the two-stage method. Dilutions of antibody were incubated with Torpedo receptor before or after the addition of radiolabeled toxin as described in Sect. 2.6, and the degree of inhibition in each case determined by adsorption to DEAE-cellulose. Percentage inhibition of binding is derived from the difference between the inhibition of binding when antibody was added before toxin and that when added after toxin. Results are the mean and standard deviation of 6 determinations.](image)

### 4 Discussion

Antibodies to the nicotinic acetylcholine receptor of *Torpedo marmorata* have been fractionated by affinity chromatography into two populations. The starting material for this work was purified antibodies isolated in such a way as to contain both those antibodies directed to sites other than the TB site and those, if present, which bind to the toxin site itself. Fractionation of the antibody pool was achieved using Torpedo receptor linked to α-bungaro-toxin-Sepharose beads. Preliminary experiments using receptor noncovalently linked to the beads indicated that both unbound and bound antibody fractions (I and II) could inhibit the binding of labeled toxin to receptor, suggesting that much of the inhibitory effect was mediated by antibodies not directly specific for the TB site. However, this approach had two drawbacks. Firstly, fraction I was found to contain active receptor, suggesting that receptor was eluting or being displaced from the toxin beads. The antibody contained in this fraction therefore could also contain or consist of antibody capable of binding to RT. Secondly, it has also been reported that much of the receptor prepared from electric organs exists as the dimer rather than the monomer [18-20]. In this case, fraction II could also contain antibodies specifically directed at the toxin site if only one TB site per dimer was used in the binding to the toxin beads. To overcome these difficulties, two steps were taken. Firstly, receptor was covalently bound to toxin-Sepharose using the cross-linking reagent dimethylsuberimidate. This overcame the leakage or displacement phenomenon. Second, free TB sites were irreversibly blocked by saturation with soluble α-bungarotoxin followed by a second treatment with the cross-linking reagent. This resulted in an affinity resin without detectable free TB sites in which all protein was covalently-bound to the Sepharose as demonstrated by the stability to potassium thiocyanate. Fractionation of the antibody pool yielded two fractions designated I (not bound) and II (bound, and subsequently eluted). Reabsorption of fraction I yielded a third fraction designated IA. Protein content, antibody activity and TB inhibitory activity were measured in all fractions. Protein estimates indicated that 45-50% of the recovered material was contained in fraction I, 50-55% in fraction II and 10-14% in fraction IA. Antibody assays showed that, of the antibody recovered, 29.9-28.1% was in fraction I, 71.9-73.1% in fraction II and 1.6-4.1% in fraction IA. This clearly demonstrates that the covalently linked receptor is still highly antigenic and that virtually all of the antibody activity can be removed by two absorption stages. Also, since most of the protein was also absorbed, one can conclude that at least 85% of the purified antibody pool was active antibody. However there is some discrepancy between the 1.6-4.1% of antibody and 10-14% of protein left in fraction IA. This will be discussed below.

Comparison of the TB inhibitory activity is more difficult. Calculation of total recovery in fractions I and II compared to the starting pool is not possible as the slopes of the inhibition curves differ. The curve obtained with the unfractionated material is less steep than that for either fraction. This results in the anomaly that, at higher concentrations, both fraction I and II appear more effective than the original pool by a factor of two, whereas this effect is lost at lower concentrations. No simple explanation can be offered, unless competition between different subpopulations of antibodies in the original pool might be responsible. However, comparison of the activities contained in the isolated fractions is possible as the inhibition curves are similar in shape. The most obvious fact is that fraction II, which consists of material purified on the RT complex, is highly effective at TB inhibition. This clearly dem-

### Table 3. Percentage inhibition of TB by isolated fractions I and IA

<table>
<thead>
<tr>
<th>Dilution of fraction</th>
<th>Percentage inhibition of TB by fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preparation 1</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1: 50</td>
<td>68.9 ± 7.9</td>
</tr>
<tr>
<td>1: 100</td>
<td>63.0 ± 3.4</td>
</tr>
<tr>
<td>1: 200</td>
<td>48.2 ± 8.8</td>
</tr>
</tbody>
</table>

a) The percentage inhibition of TB was determined as described in the legend to Fig. 1. The results are the mean and standard deviation of 2 determinations.
onstrates that much of the TB inhibition observed in the original antibody pool can be attributed to antibodies directed against sites other than the TB site itself. In other words, the antibody in fraction II can bind to the RT complex whereas toxin cannot bind to the receptor-antibody complex. This absorbable material represents 80–85% of the total recovered TB inhibitory material and is presumably acting either by binding near to the TB site and sterically hindering access for toxin or by binding at a distance and causing a conformational change which prevents TB. A second observation is the selective enhancement of TB inhibitory activity in fraction IA when compared with fraction I. Although IA contains only 6–13% of the anti-receptor antibody contained in fraction I, nevertheless it still retains 50% or more of the TB inhibitory material. This suggests that a second population of antibodies is present. These do not bind to RT absorbents but do inhibit the TB and presumably are directed either to the TB site itself or very close to it. This population represents 15–20% of the total TB inhibitory material recovered. Further evidence for the presence of this subpopulation is the discrepancy between the 1.6–4.1% of antibody remaining in fraction IA (compared to fraction I) and the 10–14% protein. Some or all of this unbound protein might represent anti-toxin site antibodies, while some might be inactive antibody or other contaminants.

It appears therefore that two distinct subpopulations of antibodies to the nicotinic acetylcholine receptor are capable of inhibiting the binding of α-toxin. One of these (80–85%) is directed at sites outside the TB site itself and can bind to RT complexes. The second (15–20%) does not bind to such complexes and could represent a true antibody to the TB site. A careful distinction should be made between these two types of 'anti-toxin site' antibodies in future work.

The secretarial assistance of Mrs. M. McColl is gratefully acknowledged.
**α-BUNGAROTOXIN DISPLACING ANTIBODY IN MYASTHENIA GRAVIS**

T. Barkas* and J. A. Simpson  
Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF, UK  
(Received 21 December 1981)

**SUMMARY**  The majority of sera from myasthenic patients cannot precipitate all available receptor-toxin complex even in conditions of a 4,000-fold excess of antibody. This effect was traced to displacement of toxin from the receptor-toxin complex. Pure IgG prepared from the serum of a myasthenic patient was as effective as the original serum in displacing ability. The possible effect of such antibodies on the correlation of antibody titre and disease state is discussed.

**INTRODUCTION**

Myasthenia gravis is now well established as a classical autoimmune disease in which autoantibodies to the nicotinic acetylcholine receptor play a major role (1, 2). Circulating antibodies are normally detected by immunoprecipitation of receptor specifically prelabelled with iodinated α-bungarotoxin (9). This method detects antibodies directed against sites other than the toxin-binding site. Antibodies capable of inhibiting the subsequent binding of toxin have been described by other workers (4-6). We here report the identification of a further antibody function in myasthenia gravis, namely the ability to displace bound toxin from the receptor.

**MATERIALS AND METHODS**

**Patients**

Blood samples were collected from patients with myasthenia gravis attending the Department of Neurology, Southern General Hospital, Glasgow. All samples used in the study were positive for the presence of antibodies to the nicotinic acetylcholine receptor (control level 0-1.2 10^-10M). Severity of disease at the time of sampling was assessed by three criteria:

(i) Using the Osserman classification (7).

(ii) By dosage of pyridostigmine.

(iii) By response to pyridostigmine treatment (when last required, in cases no longer on anticholinesterase medication).

**Treatment of Sera and Preparation of IgG**

Sera were separated from clotted blood and stored at -70°C. Heat-inactivation was performed at 56°C for 30 min when required. IgG was prepared by precipitation of immunoglobulin at 40%, saturation with ammonium sulphate, followed by dialysis against 10 mM potassium phosphate buffer, pH 8.0 and chromatography on DEAE-cellulose equilibrated with the same buffer. The non-bound peak was collected and shown to be pure IgG by immunodiffusion. A second fraction eluted by 1 M NaCl contained Igm and some IgG. Both fractions were concentrated to the volume of the original serum using a YM10 membrane on Diaflo apparatus.

IgG and Igm levels were determined by radial immunodiffusion (8).

**Indication of Toxin and Fractionation of the Products**

α-bungarotoxin (Boehringer) was iodinated by two methods. All reagents were in 50 mM potassium phosphate buffer, pH 7.5.

(i) Low Specific Activity (8-100 Ci/mmol)

The method used was essentially that of Lindstrom (9). 10 μl of toxin (5 mg/ml) was mixed with 10 μl of buffer and 10 μl (1 mCi) of Na[I-125] (New England Nuclear). 2 μl of freshly prepared chloramine T (260 μg/ml) was added and stirred at 4°C for 10 min. 2 μl of sodium metabisulphite (260 μg/ml) was added followed by 500 μl of buffer. The mixture was fractionated on a 20x1 cm column of Sephadex G25 equilibrated with buffer containing 1% (w/v) bovine serum albumin (BSA). 1 ml fractions were collected and the toxin peak pooled.

(ii) High Specific Activity (1600-2500 Ci/mmol)

5-10 μl α-bungarotoxin (0.5 mg/ml) was mixed with 10 μl (1 mCi) Na[I-125], then 10 μl freshly prepared chloramine T (5 mg/ml) was added. After stirring at 20°C for 60 sec. 750 μl of sodium metabisulphite (160 μg/ml) and 200 μl sodium iodide (10 mg/ml) were added. The mixture was then fractionated as above.

Separation of mono- and di-iodinated toxins was performed by the method of Vogel (10).

**Preparation and Assay of Human Muscle Extracts**

Human muscle was obtained from limbs freshly amputated from patients with peripheral vascular disorders or from post-mortem material. All subsequent operations were at 4°C. The muscle was minced then homogenised using an MSE Omnimix for two periods of 1 min in two volumes of phosphate-buffered saline (PBS) pH 7.4 containing 10 mM EDTA, 0.2 mM PMSF and 0.01% sodium azide. The homogenate was centrifuged at 20,000 g for 1 hr, and the pellet washed with a further two volumes of buffer. The pellet was then extracted overnight with one volume of 10 mM potassium phosphate buffer pH 7.4 containing 1% (w/v) Triton X 100, 10 mM EDTA, 0.2 mM PMSF and 0.01% azide. The suspension was centrifuged at 100,000g for 1 hr, and the supernatant filtered through glass wool.

Extracts were assayed by a modification of the method of Lindstrom.
(i) Ammonium Sulphate Precipitation
107 μl saturated ammonium sulphate was added and mixed. After incubation at 4°C for 16 hr, the samples were filtered using glass fibre papers (Whatman GF/C). Tubes were rinsed and filters washed with three 1 ml aliquots of 40% ammonium sulphate. The precipitated radioactivity was then measured.

(ii) Antibody Precipitation
5 μl of myasthenic serum diluted to 20 μl with 10 mM phosphate buffer pH 7.4 containing 0.1% Triton × 100 was added and incubated at 4°C overnight. A volume of heat-inactivated goat anti human IgG antiserum (Scottish Antibody Production Unit, Law Hospital) sufficient to precipitate all the human IgG was added, and the tubes incubated at 4°C for 4 hr. 500 μl of 10 mM phosphate buffer + 0.1% Triton × 100 was added and the tubes centrifuged at 1500 g for 10 min. The pellets were washed with a further 500 μl of buffer and counted.

(iii) Binding to DEAE-Cellulose
This more rapid method was used to follow the time-course of binding of toxin to receptor. 100 μl aliquots of extract were treated with curare and toxin as described above. At timed intervals after the addition of toxin, 1 ml of a 10% (v/v) suspension of DEAE-cellulose in 10 mM phosphate buffer pH 7.4 containing 0.1% Triton × 100 was added. After mixing at 20°C for 15 min, the tubes were centrifuged at 1500 g for 5 min and the pellets washed twice before counting.

In most experiments, extracts containing 0.2–0.4 nM receptor were used. An extract from a motor neurone disease patient contained 2 nM receptor.

Antibody Assay
Antireceptor antibodies were measured by a micro-method based on a published method (3). Quadruplicate 100 μl samples of extract (0.01–0.1 pmole receptor) were incubated at 20°C for 90 min with 10 μl curare (10−4 M) or distilled water. 50 μl (0.2 pmole) high specific activity toxin was added and incubated for a further 90 min at 20°C. 5 μl of the test serum, or tenfold dilutions of the serum in pooled normal human serum, diluted to 20 μl with 10 mM phosphate buffer pH 7.4 containing 0.1% Triton × 100 was added and incubated overnight at 4°C. The appropriate volume of heat-inactivated goat anti-human IgG antiserum was added, incubated at 4°C for 4 hr then centrifuged and washed as described above. Positive high titre samples and blanks of normal human serum (NHS) were included.

Gel-filtration of Extracts
100 μl of extract was labelled with 50 μl toxin (0.2 or 3.5 pmole) after curare or water pretreatment as described above. 5 μl of heat-inactivated test serum (myasthenic or control) diluted to 20 μl with

Table 1 Maximal precipitation of labelled receptor-toxin complex by myasthenic sera. Percentage precipitation is the percentage of receptor toxin precipitated by the sera (5 μl undiluted, 2–4,000-fold excess of antibody) of that available as measured by ammonium sulphate precipitation. Antibody titres were measured as described in the text. High specific activity toxin was used throughout. Extract A contained 0.2–2 pmole/ml receptor, extract B 0.1 pmole/ml. Severity of disease was assessed by three methods. In the third method, A denotes a full response, B a good response, C a slight response and D no response to pyridostigmine.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Osserman group</th>
<th>Pyridostigmine dosage (mg/day)</th>
<th>Response to pyridostigmine</th>
<th>Antibody titre (10^10 M)</th>
<th>Maximum receptor-toxin precipitated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extract A</td>
</tr>
<tr>
<td>1</td>
<td>IIB</td>
<td>16</td>
<td>B-C</td>
<td>1.234</td>
<td>101</td>
</tr>
<tr>
<td>2</td>
<td>IIA</td>
<td>4.5</td>
<td>A</td>
<td>41</td>
<td>54:5</td>
</tr>
<tr>
<td>3</td>
<td>IIA</td>
<td>0</td>
<td>A</td>
<td>34</td>
<td>50:0</td>
</tr>
<tr>
<td>4</td>
<td>IIA</td>
<td>6</td>
<td>B</td>
<td>54</td>
<td>50:0</td>
</tr>
<tr>
<td>5</td>
<td>IIA</td>
<td>4</td>
<td>A</td>
<td>261</td>
<td>59:1</td>
</tr>
<tr>
<td>6</td>
<td>IIA</td>
<td>4</td>
<td>A</td>
<td>122</td>
<td>63:6</td>
</tr>
<tr>
<td>7</td>
<td>IIA</td>
<td>9</td>
<td>B-C</td>
<td>228</td>
<td>63:6</td>
</tr>
<tr>
<td>8</td>
<td>IIA</td>
<td>2</td>
<td>A</td>
<td>2.032</td>
<td>63:6</td>
</tr>
<tr>
<td>9</td>
<td>III</td>
<td>16</td>
<td>B</td>
<td>460</td>
<td>73:2</td>
</tr>
<tr>
<td>10</td>
<td>III</td>
<td>11</td>
<td>B</td>
<td>56</td>
<td>72:4</td>
</tr>
<tr>
<td>11</td>
<td>IIB</td>
<td>5</td>
<td>A</td>
<td>65</td>
<td>74:4</td>
</tr>
<tr>
<td>12</td>
<td>IIA</td>
<td>4</td>
<td>A</td>
<td>103</td>
<td>53:8</td>
</tr>
<tr>
<td>13</td>
<td>III</td>
<td>0</td>
<td>B</td>
<td>576</td>
<td>46:2</td>
</tr>
<tr>
<td>14</td>
<td>IIA</td>
<td>6</td>
<td>B-C</td>
<td>262</td>
<td>38:5</td>
</tr>
<tr>
<td>15</td>
<td>IIA</td>
<td>6</td>
<td>B</td>
<td>7,546</td>
<td>53:8</td>
</tr>
<tr>
<td>16</td>
<td>IIA</td>
<td>6</td>
<td>B</td>
<td>91</td>
<td>68:8</td>
</tr>
<tr>
<td>17</td>
<td>IIA</td>
<td>4</td>
<td>A</td>
<td>343</td>
<td>81:6</td>
</tr>
<tr>
<td>18</td>
<td>IIA</td>
<td>9</td>
<td>B</td>
<td>169</td>
<td>50:9</td>
</tr>
<tr>
<td>19</td>
<td>IIA</td>
<td>3</td>
<td>A</td>
<td>34</td>
<td>26:8</td>
</tr>
<tr>
<td>20</td>
<td>IIA</td>
<td>2</td>
<td>B</td>
<td>266</td>
<td>80:7</td>
</tr>
<tr>
<td>21</td>
<td>I</td>
<td>4</td>
<td>B</td>
<td>37</td>
<td>36:5</td>
</tr>
<tr>
<td>22</td>
<td>I</td>
<td>2</td>
<td>A</td>
<td>41</td>
<td>46:6</td>
</tr>
<tr>
<td>23</td>
<td>IIA</td>
<td>10</td>
<td>B</td>
<td>93</td>
<td>67:1</td>
</tr>
<tr>
<td>24</td>
<td>IIA</td>
<td>14</td>
<td>B</td>
<td>126</td>
<td>57:2</td>
</tr>
<tr>
<td>25</td>
<td>IIA</td>
<td>9</td>
<td>C</td>
<td>55</td>
<td>38:1</td>
</tr>
<tr>
<td>26</td>
<td>IIA</td>
<td>6</td>
<td>B</td>
<td>182</td>
<td>80:0</td>
</tr>
</tbody>
</table>

114 BARKAS AND SIMPSON
buffer was added and incubated at 4°C overnight. The sample was then fractionated on a 16 × 0.8 cm column of Ultragel ACA34 using PBS eluting buffer containing 0.1% Triton \times 100. Two 7 ml fractions were collected for each sample and the radioactivity measured. Preliminary experiments demonstrated that the first pool contained only receptor-bound toxin while the second pool contained only free toxin.

**RESULTS**

**Precipitation of Toxin-labelled Receptor by Myasthenic Sera**

During routine assay of myasthenic sera it became apparent that, even in large excess of antibody (2-fold to 4,000-fold excess), the majority of sera could not precipitate all of the offered receptor-toxin complex (Table 1). Values ranged from 27–82% (extract A) of that precipitated by serum from patient 1. This last sample was capable of precipitating all of the offered complex (101 ± 9.2%, mean and standard deviation of six determinations) as assessed in comparison with the ammonium sulphate method. Table 1 also shows results with a second extract (B) of lower receptor concentration. The values were similar but higher than those with extract A (regression coefficient 0.795, \( P < 0.001 \)). Assays using an extract of higher receptor concentration (2 nM, data not shown) gave values very similar to those using extract A. Sample 1 was tested for ability to bind toxin in the absence of added receptor with negative results.

The suboptimal precipitation of bound toxin observed in the presence of the majority of sera was not due to trivial causes. Heat-inactivation of all sera had no effect, showing that complement solubilisation of complexes was not occurring. Varying the amount of goat anti-human IgG used as precipitant showed that the results were not caused by inadequate precipitation of abnormal amounts of immunoglobulin in the sera. Increasing the amount of myasthenic sera produced no further precipitation of receptor-toxin. The effect could not be ascribed to incomplete labelling of the receptor before the addition of antibody as time course determinations of toxin binding clearly demonstrated that labelling of receptor was complete after 90 min (99.8 ± 10.8%, by binding to DEAE-cellulose, 94.5 ± 2.6% by precipitation using serum 1, mean and standard deviation of three experiments in comparison with a 24-hr incubation period). Incomplete precipitation by sample 8 was also observed when receptor and toxin were preincubated for 24 hr before the addition of antibody (47.0 ± 6.8%, mean and standard deviation of four experiments).

**Sequential Addition of Test Sera**

The possibility that two antigenically distinct acetylcholine receptors might be present in the extract was investigated. In the first series of experiments, serum samples from one patient were added to the labelled extract and were precipitated by the equivalent amount of heat-inactivated goat anti-human IgG antiserum. The supernatants from this experiment were then retested with other myasthenic sera. As shown in Table 2, sera (Nos. 14, 15) which only precipitate a portion of the available receptor-toxin completely prevent subsequent precipitation by sample 1.

In a second approach (Table 3) sequential addition of myasthenic sera was performed but without precipitating the IgG of the first sample before the addition of the second. The results again demonstrate the inhibitory activity of sample 15 on the subsequent ability of sample 1 to precipitate the expected amount of receptor-toxin. However, it can also be seen that if sample 1 was added before sample 15 much less inhibition occurred.

**Table 2** Addition of two consecutive myasthenic samples to labelled extract with precipitation of the first sample before addition of the second. Results are the mean and standard deviation of two experiments. High specific activity toxin and extract A were used.

<table>
<thead>
<tr>
<th>Sera added</th>
<th>Receptor-toxin precipitated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First stage</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3** Addition of two consecutive myasthenic samples to labelled extract without precipitation of the first sample before addition of the second. High specific activity toxin and extract A were used. Percentage inhibition is the inhibition of the precipitation of that receptor-toxin complex precipitated by sample 1 alone but not by sample 15 alone.

<table>
<thead>
<tr>
<th>Sera added</th>
<th>Receptor-toxin precipitated (%)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First stage</td>
<td>Experiment 1</td>
</tr>
<tr>
<td>NHS</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>NHS</td>
<td>7.8</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>8.7</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>9.8</td>
</tr>
<tr>
<td>NHS</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>NHS</td>
<td>15.9</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>14.4</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>12.5</td>
</tr>
</tbody>
</table>

\[
\text{Percentage inhibition} = \left( \frac{\text{Cpm (sample 1 alone)} - \text{Cpm (sample 15 alone)}}{\text{Cpm (mixture)}} \right) \times 100
\]

NHS indicates normal human serum.
Table 4 Recovery of receptor-toxin on gel filtration of labelled extracts in the presence of sera. Results are the mean and standard deviation of three determinations. High specific activity toxin and extract A were used.

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>Receptor-toxin complex (fmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human serum</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Sample 1</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Sample 15</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Sample 8</td>
<td>9.6 ± 1.5</td>
</tr>
</tbody>
</table>

Toxin Displacement by Myasthenic Sera

Gel filtration of toxin-labelled receptor extracts demonstrated that sera incapable of precipitating the expected amount of receptor-toxin complex also gave a low recovery of radiolabel in the macromolecular region of the Ultragel elution profile (table 4), whereas sera which do not contain the displacing activity give the expected yield of receptor-toxin complex.

Results Using Different Preparations of Iodinated Toxin

The toxin used in our routine assays is approximately tenfold higher in specific activity than that used by most other workers. However, further experiments also demonstrated that serum 8 also failed to precipitate the expected amount of toxin labelled with low specific activity toxin (43 ± 4-3% with high specific activity toxin, 70-4 ± 6-0% with low specific activity toxin, mean and standard deviation of three experiments).

To investigate whether the displacement could be explained by preferential removal of di-iodinated toxin, the two radioactive toxin preparations used above were fractionated by ion-exchange chromatography (10). Mono-iodinated toxin in the high specific activity preparation was 15% of the total whereas it represented 84% of the low specific activity preparation. As mono-iodinated toxin binds more rapidly to receptor preparations (11, 12) binding of low specific activity toxin would be expected to be at least 84%, if serum 8 was displacing only di-iodinated toxin.

Characterisation of the Agent Responsible

IgG was isolated from a serum sample of patient 8 and tested in the gel-filtration assay. Pure IgG was found to be as effective as the original serum at the equivalent concentration of IgG. However, a second fraction containing a third as much IgG as the first fraction and high levels of IgM was also effective.

Discussion

We here report the detection of a new type of anti-receptor antibody in myasthenia gravis. In contrast to previously described “antibodies to the toxin-binding site”, the factor described displaces toxin from the receptor-toxin complex. That these two activities are different is apparent from the present work. The previously described factor acts by preventing the subsequent binding of toxin. In the present work, the receptor is prelabelled with toxin. Evidence that this is so stems from a number of experiments. Previous experience with extremely dilute solutions of Torpedo receptor and high specific activity toxin had shown binding of toxin to be complete in 90 min at 20°C (Barkas, unpublished results). Similar time course experiments with human extracts confirmed this finding. Binding of receptor-toxin complex to DEAE-cellulose after 1-5 or 22-hr preincubation of receptor and toxin gave similar results for toxin binding. Similarly, precipitation of receptor-toxin complex by serum 1 also gave identical results after either 1-5 or 24-hr preincubation of receptor and toxin. Finally, a clear distinction can be made between these two “anti-toxin-binding” activities as sample 1 which does not displace toxin can block subsequent toxin binding if added to the receptor immediately before the toxin (62-8 ± 1-4%, of expected label precipitated, mean and standard deviation of two experiments).

Serum-mediated proteolysis is unlikely to explain the effect. Firstly, the order of addition of the sera is important (table 3). This could suggest that the length of time of incubation of the effective sera with the receptor was important. However, similar results were obtained when single sera samples were incubated for either 20 or 44 hr with the receptor, suggesting that this is not the case (table 3). Secondly, no effect is observed with normal sera or serum 1. Finally, the effect can be mediated by pure IgG.

The actual mechanism is unclear. Previously reported “anti-toxin-binding site” antibodies could act by direct binding to the toxin site, by binding at an adjacent site and causing steric hindrance, or by binding at a distance and causing a conformational change. From work using antisera from animals with experimental myasthenia, it is apparent that much of this activity can be mediated by antibodies not specifically directed against the toxin-binding site itself (in preparation). In the present study, both the first two possibilities seem unlikely as the toxin-binding site is already occupied.

The results provide further evidence for subpopulations of antibodies in myasthenia gravis (6, 13, 14). Twenty-five out of twenty-six samples contained this factor. Lack of effect was a constant finding for patient 1, six samples taken over a period of four years all being negative. Some evidence for close proximity of antigenic sites might be inferred from the observation that prior binding of antibodies from sample 1 inhibited the subsequent displacing activity of other samples (table 3).

The lack of ability of many myasthenic sera to precipitate the expected amount of receptor-toxin complex has been observed by others (Harrison and Lunt, personal communication). However, these results
conflict somewhat with those of other workers (6), who have found that of sera from 152 patients with myasthenia gravis, only 15% contained antibodies to the toxin-binding site and that none of these could displace bound toxin. Our results obtained with high specific activity toxin could possibly be explained by the increased sensitivity. However, qualitatively similar results were observed with low specific activity toxin. Preferential displacement of di-iodinated toxin cannot be involved as the low specific activity toxin (30% block) contains only 16% di-iodinated toxin, and mono-iodinated toxin is known to bind more rapidly to the receptor (11,12). Preferential displacement from either junctional or extrajunctional receptor is unlikely as similar results were obtained using relatively normal muscle and motor neurone disease muscle as a source of antigen, the latter of which would be expected to be enriched in denervated receptor. One possible explanation of the discrepancy between results might be that the previous report utilized denervated rat muscle as a source of antigen while we have used human. However, it has been reported (3) that, using human extracts similar results are obtained for receptor titres by gel-filtration in the absence of serum or precipitation by myasthenic antibodies.

No obvious correlation of the presence of the factor with either overall antibody titre or severity of disease as assessed by three methods can be found. We are currently measuring the levels of the factor in various sera by adding dilutions of the test sera before precipitation with serum 1 (as in table 3). Preliminary data shows that serum 8 is effective at a dilution of one in a hundred (24% inhibition). However, the presence of an antibody capable of displacing toxin and the possibility of different relative proportions of this factor and other antibodies might explain some of the lack of correlation of antibody titre with disease severity within a group of patients (15, 16). For example, sera from patients with severe disease and without antibodies detectable by the normal precipitation assay might give this result because of the presence of toxin-displacing antibodies. As patient-specific subpopulations of antibodies have been reported as relatively constant with time (14), it might be expected that the presence of such factors might not influence the comparison of titres and severity within samples from a single patient, as in fact has been observed by several groups (e.g. 17, 18).

ACKNOWLEDGEMENTS
Serum samples were kindly provided by other members of the medical staff of the Department of Neurology, in particular Dr. I. D. Melville. The technical assistance of Mrs. J. Cairns and Mr. H. Kerr and the secretarial expertise of Mrs. M. McColl are gratefully acknowledged. Statistical expertise was provided by Dr. S. Hansen. This work was supported by the Muscular Dystrophy Group of Great Britain.

REFERENCES

The last 25 years have changed myasthenia gravis from a rare disease confidently described in textbooks as a biochemical disorder resembling curare poisoning to a prototype of autoimmune disease exhibiting a newly recognised category of immunoreceptor disorders. The observation that the neuromuscular defect is commonly associated with disorders of other organs(1) now agreed to be immunological is widely confirmed. Physicians, like other observers, tend to notice only phenomena which fit into the contemporary mould. Recognition of an animal model for a myasthenic disease induced by inoculating immunodeficient animals with various species of nicotinic acetylcholine receptors (AChR)(2) and the use of the specific ligand α-bungarotoxin to detect the presence in serum of immunoglobulins with special affinity for some part of the receptors (3) in about 90% of patients (4) have convinced most people that myasthenia gravis is an autoimmune disease. A consequence of this acceptance is a reduction in the number of papers introducing the subject with the statement that myasthenia gravis is probably a mixture of different disorders of neuromuscular transmission.
The argument of contemporary interest concerns the possibility that there are different types of myasthenia gravis with different genetic and aetiological background.

Before examining this proposition it is necessary to define the terms used. English-speaking physicians should be reminded that the French "myasthénie" embraces a wider field of muscle weakness. The English term "myasthenia" is restricted to that type of weakness, often described as fatiguable, in which over a period of about one minute a maintained or repeated series of muscular contractions progressively decrements in power (and in the associated electromyographic activity), and this failure is rapidly but temporarily restored by a short rest or, for a more prolonged period by administration of an anticholinesterase drug. Everybody is familiar with the myasthenic syndromes of Lambert and Eaton (though only conforming to the above definition with slow rates of supramaximal motor nerve stimulation) and of motor neuronopathies including amyotrophic lateral sclerosis. It is not disputed that these myasthenic disorders are different from myasthenia gravis, though an immunological basis for the Lambert-Eaton syndrome has recently been indicated by therapeutic improvement after plasma exchange, and passive transfer of the disease from man to mouse (5).

There is more controversy about the status of
Congenital Myasthenia. An increasing number of patients with congenital myasthenia are being reported without elevated serum titres of anti-AChR antibody (6,7). I have two patients (sisters) in this category and have agreed with Walsh and Hoyt (8) that the striking symmetry of myasthenic weakness, generalised hypotonia, common history of diminished fetal movements, absence of remissions and tendency to improve in later life, place these cases closer to the benign congenital hypotonias of infancy than to true myasthenia gravis. Contemporary opinion is against an immunological basis for congenital myasthenia. Nevertheless a puzzling phenomenon is that low serum IgA is more common in congenital myasthenia than in acquired MG (9,10). A recent series of studies from the Mayo Clinic makes it clear that a number of congenital neuromuscular disorders may present in this way due to developmental abnormalities of the transmission mechanism. Three types have been identified (11), one prejunctional, attributed to a defect of resynthesis or mobilization of acetylcholine (ACh), and two postjunctional. In one of the latter the authors have identified a significant deficiency of ACh esterase. In the other, with similar myasthenic transmission defect associated with repetitive endplate responses, the esterase was present in normal amounts and it is postulated that there was an abnormality of the ACh induced ion channel. Five cases investigated by Vincent et al (12) had morphological and
physiological evidence of postjunctional abnormality. In two cases examined, the conductance of the single postjunctional ion channel was normal. One case had a normal number of \( \Delta \)-bungarotoxin binding sites despite a very small amplitude of spontaneous miniature endplate potentials. The authors suggested a number of possible mechanisms including reduced number of ACh molecules in each quantum, but a molecular abnormality of the AChR ion channel is not excluded. Clearly a wide variety of genetic mutations of the receptor macromolecule, ACh esterase, or the prejunctional apparatus may produce similar functional disorders and in principle the same comment could be made about acquired myasthenia gravis. (The adjective "gravis" or symbol MG will now be used to indicate the acquired disease).

**Antibody specificity in myasthenia gravis**

I reviewed the clinical and pathological clues pointing to an autoimmune mechanism for MG at the Milan Congress in 1969 (13). All the essential points of the original hypothesis (1) and its morphological basis (14) have been validated (15) since the identification of experimental autoimmune MG and of anti-AChR antibodies (4). It is generally agreed that fluctuations of disease severity in the individual patient are paralleled by changes in antibody titres and by the proportion of damaged receptors (16), but that in a population of myasthenics there is poor correlation
between antibody titre and clinical severity. In addition to absence of anti-AChR antibodies (detectable by current techniques) in about 10% of patients with MG, it is possible to have significantly raised titres without apparent clinical disease in babies born to myasthenic mothers, siblings or parents, or in patients with thymoma (17). I have recently reviewed the reports published up to 1980 (18).

It is becoming increasingly apparent from the lack of correlation between antibody titres recognised by AChR-toxin from different species that there is not complete cross-reactivity and that some but not all of the antigenic sites of the receptors are species specific. Most assays detect globulin which blocks access of bungarotoxin to the receptor sites by binding in close proximity to the latter, but cannot detect antibody directed against the ionophore protein. In view of the possibility of preparing an antibody against the pathogenic antibody, a major attack has been made on the investigation of subclasses of anti-AChR antibodies. It is a reasonable task since there are probably a limited number (about 10) of antibody combining sites on each AChR macromolecule (19). That a particular subpopulation of antibody may be significant is indicated by the surprising constancy in individual patients of the ratio between anti-human AChR antibodies and those cross-reacting with other species and the consistency for each patient of the percentage
precipitation of AChR by excess of antiserum (20).

Inter-patient differences in disease severity and course may depend on the patterns of subpopulations of anti-AChR antibody.

There are a number of important consequences from the observation of heterogeneous anti-AChR antibodies in a MG population with individual consistency. In the first place the site of immunological attack may vary from patient to patient and this could depend on different risk factors or be quite arbitrary. Secondly, a heterologous anti-idiotype antiserum would have little therapeutic value. We have found that heterologous anti-idiotype serum to anti-AChR antibodies has a blocking effect restricted almost totally to the original inducing antibodies (21). If similar lack of cross-reaction was found with human antibodies it would be necessary to prepare individual antisera for each patient to be treated. In those cases with low antibody this would be extremely impracticable. The antibody-mediated autoimmune chronic stage of the experimental animal disease is preceded by cell-mediated immunopathology in an acute stage if pertussis is used as an adjuvant in addition to complete Freund’s adjuvant, but phagocytic invasion of endplates is not a characteristic of human MG (22).

It is becoming clear that antibodies need not be directed against the ACh binding site, but the position
of attachment to antigenic determinants on the extracellular surface of the AChR molecule should allosterically reduce the binding affinity for ACh or interfere with the regulation or function of the ionophore. Cross-linking of closely packed receptors by a molecule of antibody into large aggregates may be necessary for abnormal conformational changes, antigenic modulation, lysis and internalisation of receptors for destruction by lysosomes (23). Antibody dependent membrane lysis is probably complement mediated (24). One of the earliest indications for immunopathology of MG was the observation by Nastuk et al (1956) that serum complement may be reduced during a myasthenic relapse (25). Complement-mediated lysis of the postsynaptic membrane is the likely reason for destruction of the architecture of the postjunctional membrane. The primary cleft is widened, secondary clefts are shallow or abolished, and the receptors at the crests of the folds are destroyed (22,24,26). These important changes limit the response to anticholinesterase drugs and determine the end stage status. Fortunately there is active regeneration not only of endplates as previously suggested (14) but of receptors synthesised and inserted into the membrane. The latent period for recovery after plasma exchange in human MG (2-3 days) has been interpreted as an indication of the recovery time (27), but this assumes that the previous loss of function was due to loss of receptors. It is then
necessary to ask why removal of antibodies is beneficial to only a small group of MG patients.

Three mechanisms for antibody induced failure of receptor function have been identified. The most severe is lysis of AChR and destruction of the postjunctional membrane. This lesion can unquestionably reduce or abolish the safety factor for transmission (28,29). Recovery is only possible by formation of new receptors. Loss of AChR also occurs from antigenic modulation, independent of complement, causing degradation of receptors. Conformational change would be equally damaging for function but could respond more rapidly to removal of antibody. The third mechanism is antibody blockade of otherwise intact receptor sites, either by ACh recognition site binding or steric hindrance from neighbourhood binding. There is now considerable evidence that this mechanism exists but that it is unlikely to be functionally significant (16,30). It is certainly rare for plasmapheresis to cause immediate return of muscle power. Nevertheless it is difficult to account for the results reported from thoracic duct drainage of lymph (rapid improvement followed by rapid deterioration on reinjection of the humoral component of lymph)(31) except by "immunopharmacological blockade" as relapse appears to have occurred during the retransfusion of cell-free lymph.
It is necessary to sort out whether all three mechanisms occur as different grades of severity of one disease process (since this will affect the prognosis and choice of treatment at any particular time) or whether, in analogy with congenital myasthenia, they represent different sites for immunological attack. I do not believe in the lumped-splitter dichotomy. In the early stage of analysis of the disease I am convinced of the advantages of unification for extraction of general principles and I would find it tedious to subdivide MG according to the exact site and extent of immunological attack on the receptor sites. Nevertheless it may be important to split the case material now to see whether the different aspects of receptor damage correlate with different risk factors or with the clinical stage. Once these points have been established it is my feeling that we can leave the further fate of the receptor to the molecular biologists since the lysed AChR structure is beyond therapeutic intervention. Treatment must, as in the past, concentrate on removing or limiting the immunological attack on the endplates, promoting resynthesis and placement of new receptors, and maximising the use of surviving receptors.

Immunogenesis of myasthenia gravis

At the 1969 Milan Congress it was still necessary for me to advocate an autoimmune hypothesis (13). By the time of the 3rd Congress in Newcastle (1974) the suggested mechanism was widely accepted and the 1978
Montreal Congress devoted a whole section to the concept. My remit at Montreal was to review the general principles of treatment (32). It was not possible to examine the modifications required for different stages and types of the disease, including the wider question of single entity versus clinical syndrome. In the eight years since the Newcastle meeting the proliferation of papers on immunological aspects of MG has become too great for me to cite more than the key references. In this review I will simply summarise the present situation and refer readers to the comprehensive book edited by Eldefrawi and Albuquerque (33). It has not changed significantly since Audrey Penn reviewed the immunological features of MG at Montreal (34). There is still no evidence for a contribution of cell-mediated immunity to the initiation of autoimmunity in the human disease and the antibody-mediated damage of the endplate involving several of the surface antigenic determinants of the AChR is polyclonal, involving subspecies of anti-receptor antibodies but also autoantibodies directed against other tissue, as in the original hypothesis. This points to a breakdown of immunological tolerance rather than response to antigens from a single tissue such as the widely advocated myoid cell of the thymus (18,25,36).

Loss of tolerance to self antigens may be due to
production of T-helper cells or suppression of T-suppressor cells regulating B-cell response to homologous antigens and many centres are concentrating on the analysis of T subsets to identify the abnormality in MG. There is evidence for both AChR-specific T-helper cells (37) and for defective T-suppressor cell function (38,39). It is possible that these are both caused by an immunoregulatory antibody (39-41). Some investigators report that a defect in suppressor cell activity is restricted to a subgroup of myasthenic patients associated with HLA-B8 antigen (42,43). As with the different but related endplate lesions, we are faced with the question - do these differences reflect different stages of a single disease or are they evidence for a group of related immunological disorders with a common endpoint but different genesis? I have always been impressed with the smoothness of the age distribution curve for non-thymoma cases of MG which does not suggest that we are dealing with a miscellany of diseases (1) and still believe that the various immunological disturbances have a common cause in a disorder of the controlling function of the thymus, probably exerted by genetically influenced thymic hormones such as one of the thymosins (36). Dalakas and colleagues have recently demonstrated thymosin-β1 in the epithelial cells, even of "involuted" MG thymuses (44). The discoverer of thymosin, A.L. Goldstein, suggests that excess thymosin-β1 secretion may
exacerbate MG by facilitating overproduction of helper T-lymphocytes sensitized against nicotinic AChR which stimulate B-lymphocytes to produce the specific antireceptor antibodies (45) but we are still left with the questions why specific antigens are selected for attack and why the control should be abnormal in MG.

I am sceptical about models based on the presence of AChR antigen within normal thymus for a number of reasons, particularly the polyclonal response involving a number of tissue antigens (35,36) unless the primitive stem cells which can differentiate to myoid cells are pluripotent (46). A more plausible model would be that immunosurveillance is defective because of some interference with whatever mechanism is normally responsible for differentiating self from non-self, a distinction which is only gradually improved by removal of the thymus. The NZB mouse is an indication that this function is under genetic control. Myasthenia precipitated by penicillamine or betablockers indicates the possibility of exogenous tolerance breakers, including viruses, but the only well defined risk factors are genetic and the presence of a thymoma. Geographical studies do not suggest an environmental factor (47).

Genetic factors

The proposal for a genetic factor (1) was not supported (48) until account was taken of my suggestion
of alternative expressions of the gene disorder based on recognition of familial linkage with other autoimmune diseases (1,49). Support was obtained from extensive family studies (50,51). All genetic studies indicate that there is polygenic or multifactorial inheritance, or that an additional environmental factor is necessary. It is now agreed that one of the human leucocyte antigens HL-A1,B8 is significantly more frequent in Caucasian and Indian myasthenics, especially in women with onset of myasthenia below the age of 30 years (52) but that it is not obligatory (53). There is a less strong linkage with a lymphocyte defined gene HLA-Dw3. In Japanese patients (54) the linkage is with HLA-B12 and with Gm haplotypes which regulate immunoglobulin allotypes determinants (55).

Linkage disequilibrium of the types mentioned point to a role for the major histocompatibility complex of chromosome 6 but it is not obligatory and probably concerns increased susceptibility to autoimmune disorders in general. The B8 linkage is less strong in male patients and is absent for late onset MG associated with thymoma in Caucasians (56) but, on the contrary, in Japanese it is linked more strongly with thymoma than with thymic "hyperplasia" (54). At present it seems probable that the genetic factor influences age of onset and possibly susceptibility to unknown tolerance-breaking factors but not sex of patient or the titre of anti-AChR antibody (57). For recent views see
Investigations of susceptibility of inbred and congenic mouse strains to EAMG suggest that the tendency to recognise AChR and to produce anti-AChR antibody may be controlled by the H-2 complex towards the K end of the complex (60,61). Clinical expression might depend on the safety factor for neuromuscular transmission which may also be strain dependent. Tubocurarine sensitivity tests indicate that this factor cannot account for the strain differences seen in the mouse but that factors additional to the H2 haplotype determine susceptibility to EAMG, namely alleles of loci which are linked to or identical with those which regulate immune responsiveness in the IgCH allotype locus (62). Comparable studies on Gm allotypes in human MG are awaited with interest as they would throw light on the discrepancy between clinical severity and antibody titres in human MG. This may be due simply to variation of specificity or affinity of anti-AChR antibodies or to toxin-displacing antibodies (63) but it raises the question whether some of these species may be protective to the endplate. We also have some evidence that receptor-antireceptor complexes suppress the synthesis of antibody and protect against EAMG (64). An inhibitory affect of alpha-fetoprotein in amniotic fluid on the binding of myasthenic antibodies to AChR has been proposed to account for the favourable effect of
pregnancy on MG and the in utero protection of the fetus from placentally transferred antibody (65).

Whatever the mechanism turns out to be, the facts that the level of antibody does not correlate with clinical severity and that there is a possibility of blocking the immunoreactivity or its effects on the receptor by blocking antibodies and protective serum proteins have important implications for the suppression and arrest of myasthenia gravis since they may not have the patient-specific limitations which threaten to restrict the use of anti-idiotypic antibodies (21). Meanwhile the roles of the various therapies described at the last Congress (32) continue to be assessed. We have had very encouraging short term improvement with plasma exchange either alone or with immunosuppression (66) but the initial enthusiasm for plasma exchange is subsiding. It still has a useful place in the treatment of myasthenic crisis or in the preparation of a patient for thymectomy, but the effect is transient and accompanied by increased susceptibility to infection. Azathioprine (67) and steroids (68) are being used almost routinely in many countries. I consider that the hazards of these drugs are not negligible and it is necessary to stress that death or major long term disability are now very rare with early thymectomy supported by judicious anticholinesterase treatment. The former are dangerous forms of treatment and should be confined to desperate cases.
Nevertheless, steroids occasionally induce remarkable remissions. At the last Congress I hinted that steroids may have a direct action on the safety factor for transmission, including possibly endplate remodelling (32). We now have definite evidence that the early steroid weakness is due to a prejunctional factor which is followed 10 days later by improved postjunctional response (69). We still do not know whether this is due to increased receptor density or other morphological change. Phosphodiesterase inhibitors or hormones which increase cyclic nucleotide levels are reported to increase the turnover of ACh receptors in skeletal myotube cultures (70), but it is encouraging to consider that the next advances in the management of MG may include measures to protect the receptors against immunological attack and to encourage regeneration.
References


47. KUROIWA Y. Epidemiological aspects of myasthenia gravis in Japan. In Japan Medical Research Foundation (eds) Myasthenia Gravis: Pathogenesis and Treatment, 1981, 9-17, Tokyo, University of Tokyo Press.


57. SMITH C.I.E., HAMMARSTRON L., MOLLER E., LEFTVERT A-K., MATELL G. No significant correlation of HLA-B8 and amount of antibodies directed to acetylcholine receptor protein in patients with myasthenia gravis. Tissue Antigens 1978, 12, 387-395.


63. BARKAS T., SIMPSON J.A. Toxin-displacing antibody in myasthenia gravis. (Submitted for publication, 1982).


70. BLOSSER J.C., APPEL S.H. Reciprocal regulation of acetylcholine receptor and myosin in chick myotubes by a phosphodiesterase inhibitor and cyclic nucleotide analogues. Society for Neuroscience, Abstracts 1979, 9, 753.
THE MYASTHENIC (EATON-LAMBERT) SYNDROME ASSOCIATED WITH CARCINOMA. ENZYME INDUCTION AS A POSSIBLE MECHANISM OF PARANEOPlastic SYNDROMES

J. A. Simpson

Glasgow University Department of Neurology, Institute of Neurological Sciences
Southern General Hospital, Glasgow

Summary. A myasthenic syndrome associated with small-cell tumours of the bronchus and with autoimmune diseases (Eaton-Lambert syndrome) has been attributed to diminished probability that a nerve action potential will release acetylcholine (ACh) from terminals of cholinergic nerves (somatic motor and autonomic). This model derives from evidence for reduced quantal content of the transmitter released by a nerve impulse. The test procedure implies certain constancies of postsynaptic response: Abnormal responses to ACh-agonists indicate that receptor response is not normal. It is suggested that all previously described neuromuscular responses are compatible with a new model: the subsynaptic apparatus produces excess acetylcholinesterase (AChE) which limits the endplate conductance changes produced by normal output of ACh. This model is supported by earlier ultramicroscopic studies which cannot be accounted for by the contemporary model.

It is proposed that enzyme induction by peptide or immunoglobulin may also be responsible for other paraneoplastic syndromes.

Amyasthenic syndrome associated with oat-cell tumour of the bronchus in man reported by Eaton and Lambert (1) and in non-tumour cases by Simpson and Lenman (2) is given its eponymous title after the first describers as it appears to be identical in both types. Clinical correlations indicate that the second type is associated with autoimmune disease. The syndrome is characterised by a defect of neuromuscular transmission causing slow initiation of voluntary contraction of muscle, depressed tendon jerks, certain autonomic disturbances (decreased salivation, blurred vision, impotence) and muscular pains. A supramaximal stimulus to a motor nerve evokes a subnormal muscle response which must be a transmission defect as the antidromic nerve action potential is normal and full muscular response is obtained by direct stimulation of the muscle. Repeating the supramaximal stimulation at slow rates (<10/sec) evokes decrementing responses. At faster rates the muscle response rapidly increments to the normal level (Fig. 1). Microelectrode studies show that these events parallel changes in amplitude of the evoked endplate potentials (e.p.ps). Pharmacological analogies have led to the current model which interprets the defect as a failure of release of acetylcholine (ACh) at motor nerve terminals.

The most detailed analysis of neuromuscular transmission was made by Elmqvist and Lambert (3,4) on biopsied intercostal muscle from patients with myasthenic syndrome. Their findings were the same whether or not the patient had a bronchogenic carcinoma. Their deductions, that a very low number of acetylcholine quanta were released from the nerve ending by a nerve impulse and that the defect is like that produced by magnesium ions and botulinum toxin, have been accepted as conclusive in all subsequent reports and textbook descriptions.

The facts described were as follows (3). Needle electrode electromyography (in vivo) showed no abnormal injury potentials of muscle and no spontaneous fibrillation potentials. Voluntarily recruited motor unit potentials had a mean duration just below the normal range but varied in amplitude from moment to moment. The proportion of polyphasic motor unit potentials was normal. These findings indicate a reduction of the
mean muscle fibre content of the motor units with a variability suggesting a defect of neuromuscular transmission rather than degeneration of muscle fibres. This interpretation is supported by single fibre electromyography (5).

A single maximal stimulus to the motor nerve evoked a low amplitude compound action potential of the muscle it supplied, with a twitch response of subnormal tension but when the muscle was stimulated directly there was a normal twitch tension. This also indicates a defect of neuromuscular transmission. Conduction velocities of motor and sensory nerves were normal and nerve action potentials (examined in a sensory nerve) were normal. The conclusion that the abnormality responsible for the small muscle twitch is at the neuromuscular junction is entirely reasonable.

The unusual feature of the syndrome is the effect of repetitive stimulation of the motor nerve at different frequencies. At a slow rate (2/sec) the initially small muscle action potential progressively decreased in amplitude during the first few responses. At higher rates of stimulation (10 to 50/sec) a progressive increase in amplitude of the muscle action potential occurred and at 40/sec the action potential reached a normal amplitude within about four seconds. These data are representative of most cases of myasthenic syndrome, whether accompanied by bronchogenic carcinoma or not.

When they excised a specimen of muscle from their patient, Elmqvist and Lambert (3) noted that only very few muscle fibres twitched when the nerves were cut, unlike the usual experience. In a nerve-muscle bath the normal response to direct stimulation of muscle and abnormal response to indirect (nerve) stimulation was confirmed. (Their Figure 4 shows that in the case examined the relaxation phase of the directly stimulated twitch contraction was shorter than in muscle from a normal subject or one with myasthenia gravis). The tension of an indirectly stimulated twitch was markedly increased by raising the Ca⁺⁺ concentration in the bath fluid.

The calcium effect, however, was anomalous when studied with a microelectrode placed intracellularly at the endplate of the biopsied muscle. In response to low-frequency nerve stimulation the endplate potential (e.p.p.) was smaller than normal, but when occasional larger e.p.ps occurred they elicited an action potential of the muscle fibre at a threshold for excitation within the normal range for mammalian muscle. This was an important observation suggesting to the authors that the postsynaptic mechanism must be normal. It was supported by their finding that the mean amplitude of spontaneous miniature endplate potentials (m.e.p.ps) was also normal, in contrast to myasthenia gravis. They therefore concluded that the small e.p.ps from indirect stimulation necessarily indicated a reduced quantum of acetylcholine released by each nerve stimulus until facilitated by repetitive stimulation at a
sufficient rate. This is the logic that I challenge in the next section, but before doing so it is necessary to analyse the further studies which likened the defect to a magnesium block and to intoxication with botulinum toxin or neomycin which also cause variable amplitude e.p.ps and tetanic ‘facilitation’.

Since this paradigm is the accepted model for the myasthenic syndrome it is important to underline a number of differences recognised by Lambert and Elmqvist. In their first case (3) the m.e.p.p. frequency was twice normal. In their larger experience (4) the average frequency of 12 cases was slightly above normal but not statistically significant. In contrast, in magnesium-blocked normal muscle the frequency is normal or slightly reduced (6). If the motor nerve ending is partially depolarised with excess K+ the frequency of m.e.p.ps in the magnesium-blocked preparation is increased by addition of Ca++ to the bath. In the myasthenic syndrome, on the contrary, Ca++ reduced m.e.p.p. frequency. If the potassium level is kept normal, addition of Ca++ ions to the magnesium-blocked preparation normalises the m.e.p.p. frequency if it is reduced and increases the e.p.p. amplitude. In the myasthenic syndrome the e.p.p. was also increased but m.e.p.ps were reduced both in frequency and in amplitude (Fig. 7 of ref. 3).

Lambert and Elmqvist drew attention to these differences but were encouraged to interpret the block in the myasthenic syndrome as being essentially similar because the e.p.p. amplitude and the evoked muscle action potential and twitch tension could be improved strikingly by administration of guanidine to the patient or addition of calcium in the in vitro preparation. Both of these procedures encourage the release of acetylcholine from motor nerve terminals in response to nerve impulses. In terms of their model, this would point to normalisation by the drugs of a defective active release of ACh from terminals which nevertheless have normal or even increased exocytosis of the ACh vesicles presumed to cause spontaneous miniature endplate potentials. The biophysical nature of the postulated release failure is not apparent.

Clearly the essential point is to account for small e.p.ps in the presence of normal m.e.p.ps. I submit that the Elmqvist and Lambert model has the same fallacy as the Elmqvist model for the transmission defect in myasthenia gravis (7). In that disorder abnormally small m.e.p.ps were attributed to a presynaptic lesion producing pathologically small quanta of ACh and the small e.p.ps were therefore interpreted as evoked by adequate number but small quantum packages of ACh. In two theoretical papers (8,9) I showed that this conclusion depended on the model adopted by the Swedish workers which assumed that the morphology of the endplate structure was normal, whereas the observations (and certain anomalous responses to quaternary ammonium drugs applied to the endplate) could be equally well accounted for by a morphological change such as wider separation of receptor sites (Fig. 1 of Ref. 9), possibly caused by immunological damage previously postulated (10). This mechanism and its immunological causation were confirmed by Engel and colleagues (11). The observations of other workers were correct but they were wrongly interpreted because the model was wrong. This applied particularly to the interpretation of drug effects on the myasthenic neuromuscular junction.

The current model of the Eaton-Lambert syndrome

Elmqvist and Lambert postulated that the abnormality in the Eaton-Lambert syndrome was a decrease in the number of quanta of acetylcholine released by a nerve impulse such as appears to occur in magnesium-block. As formerly with myasthenia gravis, this is a model to account for the small e.p.ps in the presence of normal m.e.p.ps. The evaluation of the quantal release of the neurotransmitter is not improved by using three methods of calculation (12) since they all involve the assumption that no alteration of postsynaptic sensitivity or responsiveness occurs during the period of observation and that m.e.p.p. amplitudes have a normal distribution (13). The problem is to assess the amount of transmitter released by measuring its postsynaptic effect. A simple analogy will
clarify the difficulty. Imagine that the power output of a pulsed DC electric generator is to be assessed but the only measuring instrument available is the variation of luminance of a batch of light bulbs, known to be non-linear in their responses. But the observer is unaware that this batch of light bulbs is faulty because of a non-linear resistance in series. The reduced luminosity is attributed to a defective generator.

The model appeared to be supported by some pharmacological studies. Twitch response is increased to normal by guanidine (14), 4-aminopyridine (15), or calcium ions (3) in that order of potency. As these substances increase the amount of ACh released from motor nerve endings by a nerve impulse, the therapeutic response has been accepted as confirmation of the presynaptic model although it was a similar analogy that allowed a presynaptic model to dominate thinking about myasthenia gravis for so long. As in the latter condition, the anomalous effects of drugs acting postsynaptically were ignored. Drugs binding to the ACh receptors (ligands) are of two types i) competitive blockers, e.g. D-tubocurarine ii) ACh-agonists e.g. 'depolarising' blockers.

Elmqvist and Lambert (3) were unable to detect any abnormality of sensitivity of the muscle end-plate receptors to acetylcholine added to the bathing solution in an organ bath. Intra-arterial injection of adrenaline hydrochloride caused a decreased response (16) whereas this procedure prevents the intermittent failure of impulses to traverse all branches of the terminal nerve arborization of normal muscle during stimulation at rates exceeding 50/second (17).

With blocking drugs, the sensitivity to D-tubocurarine is increased (18). The depolarising drugs decamethonium and succinylcholine have a biphasic effect. In small doses they may cause transient improvement of neuromuscular transmission (19), but with larger doses they block transmission (20). Wise and McDermott (21) found that decamethonium could be more effective than the naturally released transmitter. In the normal human subject, the decamethonium block is prolonged by anticholinesterases. In the myasthenic syndrome transmission blocked by decamethonium is promptly though incompletely improved by edrophonium (21,22). Anticholinesterases (edrophonium, neostigmine, or pyridostigmine) (14) given alone cause some improvement in the weakness caused by the myasthenic syndrome though less than in myasthenia gravis (confirmed by personal experience). The therapeutic effect is inferior to that of guanidine (14). This is considered to favour the presynaptic model but nobody has attempted to account for the anomalous effects of drugs acting mainly or exclusively postsynaptically (acetylcholine agonists).

In summary, the main deficiencies of the current model are (i) inconsistencies with all known disorders of formation or release of ACh from nerve terminals (ii) failure to account for unusual effect of drugs acting postsynaptically, (iii) interpretation of a low amplitude ratio of e.p.p. to m.e.p.p. by postulating an unique disorder, lowered quantal response to a nerve impulse.

On the hitherto firm grounds that the amplitude of endplate potentials depends on postsynaptic response, it is necessary to consider whether any disorder there could account for the phenomena described. It is a question of non-specificity of the lowered 'safety factor' for transmission as determined by postsynaptic events (8,9). Given only that there are unoccupied postsynaptic receptors, it is not legitimate to regard a beneficial response to guanidine, 4-aminopyridine or calcium as necessarily indicating a presynaptic defect. With alpha-hungarotoxin labelling it has been shown that the muscle endplates of patients with the Eaton-Lambert myasthenic syndrome have a normal content of ACh receptors, unlike those with myasthenia gravis and they do not have antibodies bound to their receptors (23).

Proposed alternative model
I propose a novel model, excess cholinesterase activity of the subsynaptic part of the endplate (Fig. 2).

The frequency of the miniature endplate potentials would not be lowered, their amplitudes would not be measurably decreased. Their duration might be
decreased and this should be looked for. The evoked endplate potentials would show statistical lowering of amplitude (as described) and the time course of the recovery phase might be shortened. The kinetics of the depolarisation caused by decamethonium could be shifted towards the constants of acetylcholine at the normal endplate. Curare would certainly be potentiated. The depressant effect of intra-arterial adrenaline described above is unexpected with either model since it reduces the activity of cholinesterases (24).

I am unable to trace any literature on the effects of excess of cholinesterase at the motor endplate. However, the model does have some morphological support. The only consistent abnormality of the endplate seen with the electronmicroscope was first described by Engel and Santa (25). The typical abnormality was an over-development of the post-synaptic region (Fig. 3). The secondary clefts and folds were highly complex and the sarcoplasmic folds contained numerous pinocytotic vesicles. Histometric analysis shows a significant increase in the mean area of clefts and folds per nerve terminal and in the mean post-synaptic to pre-synaptic membrane length ratio.

No significant changes were found in the number and dimensions of presynaptic vesicles and the mean nerve terminal area was normal. Its mitochondrial content was slightly reduced.

The function of the post-synaptic folding has recently been re-assessed. Formerly thought to provide a structure for carrying the greatest possible number of receptor sites (AChR) near the release sites for ACh, it is now clear that the AChR are found only at the crests of the folds. The receptor-poor depths of the secondary clefts are rich in acetycholinesterase (AChE). Among other possible functions, Porter and Barnard (26) discuss the possibility that the secondary...
clefts may serve as hydrolysis traps for ACh. In addition to limiting the number of collisions of ACh with AChR and the duration of the quantal conductance change, AChE limits the lateral spread of ACh molecules to adjacent receptors (27) and so limits the endplate current with respect to duration of its falling phase (28) and amplitude. Morphological studies suggest that AChE is synthesized within the muscle fibre and transported to the post-junctional membrane where it is released by exocytosis into the folds of the synaptic cleft (29), where it is bound in part to the intersynaptic matrix (26). Synthesis depends on innervation of the muscle fibre and on its activity. It is rapidly decreased by tetrodotoxin block of the motor nerve (30), by botulinum toxin and denervation (31), and by disease of the lower motor neurone in amyotrophic lateral sclerosis (32). On the contrary, there is no reduction of AChE at the subsynaptic site in myasthenic syndrome endplates as judged histochemically (25). Indeed Engel and his colleagues (33) have specifically stated that endplate AChE is preserved or is even more abundant than normal.

My hypothesis is that the myasthenic syndrome associated with oat-cell carcinoma of the bronchus is due to excessive production of a cholinesterase in the subsynaptic apparatus of the muscle. It could be an isoenzyme, one of the low molecular weight forms of AChE found in embryogenesis, or a 'pseudo'-cholinesterase. This should be easy to establish if the proposed excessive activity is confirmed. The limitation of the excess hydrolysis effect to the initially released transmitter might point to a 'false enzyme' but an alternative mechanical explanation worth considering is that excess enzyme accumulating in the synaptic folds during rest is pumped into the
primary cleft by the first few contractions of muscle fibre.

The other signs of the disease are entirely compatible with this model, dryness of the mouth, impotence and blurring of vision, since muscarinic as well as nicotinic synapses would be affected and, obviously, reduced or absent tendon reflexes. Pain in the limbs occurs in other neuromuscular disorders, notably polymyelitis and amyotrophic lateral sclerosis and its nature remains obscure.

How could such a disorder occur as a remote, non-metastatic effect of cancer? Subsynaptic cholinesterase is reduced by 60% following denervation. Neural tissue is claimed to have an active factor which maintains cholinesterase at neuromuscular junctions.

It is postulated to be, or to contain a peptide (34). Alternatively, ACh-receptor binding may release a second messenger, Ca+ or a nucleotide cyclase, which promotes exocytosis of membrane bound granules. Activation of intracellular and membrane bound enzymes following appropriate ligand binding is well established in immunology (35). In the Eaton-Lambert syndrome, activation could be caused by a peptide released from cancer cells or by an immunoglobin acting directly on the postsynaptic membrane or as an immune complex with hormonal activity (36). Passive transfer from human to mouse has recently been reported, but apparently required daily injection of immunoglobin for 1 to 2 months (37). The synapse-specific 16S species of acetylcholinesterase is formed in the synaptic basal lamina components of aneural muscle cells in culture but possibly only where the cell line is derived from previously innervated muscle (38), and ligand-receptor binding may be necessary for its activation. In the two clinical forms of the myasthenic syndrome, ACHE activation may be promoted by either a tumour-released peptide or an immunoglobin (or both). Tumour activation of an enzyme (acid phosphatase) is already recognised in prostatic carcinoma, though not 'at a distance', and in the hormone secreting tumours.

Significance of the model
The proposed model offers a more complete explanation of the pharmacology and histopathology than the present presynaptic model. The novel form of pathogenesis suggests a new insight into the baffling field of paraneoplastic diseases known as 'remote non-metastatic effects of cancer'. For instance, it may not be insignificant that the two sites of attack in the central nervous system, the cerebellar cortex and hippocampus, are particularly rich in acetylcholinesterase. Although in these sites the function of this widely occurring enzyme may be different, and not concerned with cholinergic transmission (39), some modification of brain function might be expected from surplus enzyme. Selective fibre-type degeneration is probable as the gliosis of the granular layer of cortex is of the isomorphous type. There are no quantitative enzyme studies at present and these could be rewarding, but clearly induced synthesis of enzymes need not be restricted to ACHE. The relevance to 'neurotransmitter deficiency' syndromes in dementia will occur to the reader.

Enzyme deficiency states are familiar, causing arrest of an appropriate metabolic reaction with deficiency of an endproduct or accumulation of a precursor. In most organs of the body, overproduction of a natural enzyme would be unlikely to cause detectable clinical symptoms. The nervous system may be uniquely favourable for its discovery owing to the time scale of enzymatic destruction of neurotransmitters (less than 5 msecs) and the amplifying and threshold properties of synaptic function.

REFERENCES


Hubbard JJ. The effect of calcium and magnesium on the spontaneous release of transmitter from mammalian motor nerve endings. J Physiol (Lond) 1961; 159:507-517


Cull-Candy SG, Miledi R, Trautmann A, Uchitel OD. On the release of the transmitter at normal, myasthenia gravis and myasthenic syndrome affected human endplates. J Physiol (Lond) 1980; 299:621-638


Wise RP. A myasthenic syndrome complicating bronchial carcinoma. Anaesthesia 1962; 17:488-504


Lindstrom JM, Lambert EH. Content of acetylcholine receptor and antibodies bound to receptor in myasthenia gravis, experimental autoimmune myasthenia gravis and Eaton-Lambert syndrome. Neurology (Minneap) 1978; 28:130-138

Benson WM, Meck WJ. Hydrolysis of choline esters in the presence of adrenaline. Amer J Physiol 1949; 158:327-331

Engel AG, Santa T. Histometric analysis of the ultrastructure of the neuromuscular junction in myasthenia gravis and in the myasthenic syndrome. Ann NY Acad Sci 1971; 183:46-63


Hartell HC, Kuffer SW, Yoshikami D. Postsynaptic potentiation: interaction between quanta of acetylcholine at the skeletal neuromuscular synapse. J Physiol (Lond) 1975; 251:427-463

Kordas M. On the role of junctional cholinoesterase in determining the time course of the end-plate current. J Physiol (Lond) 1977; 270:133-150


Butler JH, Drachman DB, Goldberg AM. The effect of disease on cholinergic enzymes. J Physiol (Lond) 1978; 274:593-600
31 Drachman DB. Neutrotrophic regulation of muscle cholinesterase: effects of botulinum toxin and denervation. J Physiol (Lond) 1972;226:619-627


38 Silberstein L, Inestrosa NC, Hall ZW. Aneural muscle cell cultures make synaptic basal lamina components. Nature (Lond) 1982;295:143-145

39 Crawford JM, Curtis DR, Voorhoeve PE, Wilson VJ. Acetylcholine sensitivity of cerebellar neurones in the cat. J Physiol (Lond) 1966;186:139-165
Current Concepts and History of the Autoimmune Nature of Myasthenia Gravis

John A. Simpson

Contents
1.1 Historical 3
1.2 Serum antibodies in MG 13
1.3 Production of immunoglobulins 25
1.4 Immune deficiency and the thymus 26
1.5 Genetic factors 29
1.6 Summary 30

Abbreviations
ACh acetylcholine
AChR acetylcholine receptor
ACTH adrenocorticotropic
ANF antinuclear factor
BGT bungarotoxin
EAMG experimental autoimmune
MG myasthenia gravis
HLA human leukocyte antigens
Ir immunoreactive
LD lymphocyte defined
LE lupus erythematosus
MEPP miniature endplate
potential
MG myasthenia gravis
NMJ neuromuscular
junction
RF rheumatoid factor
SLE systemic lupus
erythematosus

1.1 HISTORICAL
1.1.1 Clinical recognition of the disease
Myasthenia gravis (MG) was not recognized as a clinical entity until late in the nineteenth century but it is now accepted, since Guthrie (1903) drew attention to it, that this was probably the disorder in a patient described in detail by the English physician Thomas Willis in 1672.

The next recorded observation was also in England by the London physician Samuel Wilks (1877) and he was the first to notice the striking absence of gross pathology in the nervous system. The disease then became better known in the German Schools with the magnificent contributions of Erb (1879) and Goldflam (1893). The main contribution made by Erb was the establishment of the clinical picture. Though he was an early exponent of electrodiagnosis he...
apparently failed to notice the typical myasthenic decrementing muscular response to faradic current applied to a motor nerve. Goldflam (1893) particularly emphasized the rapid exhaustion of affected muscles and the short-term variability, which is so striking, as well as longer term remissions and exacerbations. He also drew attention to the normal or lively reflexes but noted that the knee jerk could be exhausted by repeated stimulation. The clinical picture was now so completely described as to more than justify the eponym 'Erb–Goldflam' disease still used in European literature.

The name 'myasthenia gravis pseudoparalytica' was first given by Jolly (1895). He noted that when the muscles were stimulated repeatedly by faradism (alternating current) a reaction of asthenia was promptly demonstrated, yet when the muscle no longer responded to this type of stimulus it would respond immediately to galvanism (direct current). This fundamental observation is still the basis of all electrodiagnostic tests. Jolly termed it 'the myasthenic reaction'. He correctly interpreted it as evidence for a defect of neuromuscular transmission, though the synaptic nature of this was not yet known. He also suggested the use of physostigmine as a form of treatment but there is no record that he ever used it.

Although it seems certain that cases had been observed by Byrom Bramwell of Edinburgh (1892; cited by Simpson, 1960) and correctly diagnosed in the last 5 years of the century in Europe and in the USA (reviewed by Viets, 1953), it was the comprehensive review of Campbell and Bramwell (1900) that made 'myasthenia gravis' a well-recognized diagnostic entity to English-speaking neurologists.

1.1.2 Concepts of mechanism of neuromuscular block

(a) Myasthenic toxin

After reviewing the clinical data and the essentially negative pathological findings, Campbell and Bramwell (1900) reached a conclusion which is worth quoting:

The view we ourselves put forward is that the disease is due to a poison probably of microbial origin acting upon the lower motor neurons and interfering with their functional activity without necessarily producing discoverable change in structure. We suggest that the poison acts upon the motor fibre (axon) or end-plates; as to whether it also acts upon the cell-body [in bulbar nuclei or anterior horns], we can form no opinion... The poison may conceivably act upon the motor nervous system, or on the muscles themselves.

The concept of a 'myasthenic toxin' remained in the literature from that date. In 1901 Laquer and Weigert reported a case of MG associated with a thymic tumor. Weigert (1901) reported the presence of round-cell infiltration in the muscles and thought that they were metastases from a thymic tumor but...
Buzzard (1905) soon disproved this and termed them 'lymphorrhages' because they appeared to leak from blood vessels. He also found them in the adrenal glands and other organs, including a posterior root ganglion, and concluded that 'it is necessary to assume that the toxic agent is capable of exerting an influence on the function of other tissues besides muscle in order to account for the sensory, mental, vasomotor and secretory disorders occasionally met with in the disease'. Buzzard advocated directing more attention to the condition of the blood, the marrow, the lymphatic system and the fatty and connective tissues as the best hope of solving the pathogenesis of MG.

Before the introduction of anticholinesterase treatment Keschner and Strauss (1927) gave an admirable review of the disease. In their opinion 'the symptoms of the disease are best explained by assuming the presence of some toxic, possibly autotoxic, agent which has a special influence on the protoplasmic constituent of voluntary muscle and a less specialized influence on the function of other tissues'. 'It would seem', they comment later, 'that in all probability the thymus is of significance on the physiologic and pathologic processes merely by virtue of its lymphoid character', and 'the peripheral site of origin of the myasthenic reaction is most likely the periterminal network'.

A major landmark in the history of MG was Mary Walker's demonstration of the dramatic relief afforded by physostigmine, and later of its analogue neostigmine (Walker 1934, 1935). In fact earlier workers, including Oppenheim (1887) and Jolly (1895), recognized a resemblance between MG and curare poisoning and suggested using physostigmine, the antidote to curare, in the treatment of MG. Improvement of muscle 'fatigue' in MG was in fact reported with physostigmine by Murri in 1895, and with neostigmine by remen in 1932, but abandoned because of the muscarinic symptoms.

(b) Biochemical models

Walker's report, and a demonstration to a major medical society, was more dramatic and it came at the right time. The possibility that neuromuscular transmission was mediated chemically was being investigated by a number of physiologists and indeed in the same year Dale and Feldberg (1934) confirmed the role of acetylcholine (ACH) in neuromuscular transmission. In the next 25 years the rapid advance of neurophysiology and of pharmacology suggested several mechanisms for such a block, leading to many arguments. Thus an important series of experiments by Grob and his colleagues in Baltimore in the 1950s showed that the myasthenic reaction was due to a reduction of the safety factor for transmission at the neuromuscular junction (NMJ), with characteristics indicating a 'competitive block'. They suggested that the time course and other properties of the 'late' depressant effect of ACH applied by close intraarterial injection in myasthenic patients indicated that it may be produced by choline release after the hydrolysis of ACH by cholinesterase (Grob, Johns and Harvey, 1956).

Review papers of that period invariably prefaced a discussion of MG with a
brief discussion of possible mechanisms, including some of the following possibilities:

1. Deficient formation or release of ACh from the motor-nerve endings.
2. Excessively rapid removal of ACh after its release.
3. Competition for receptors between ACh and a sterically related molecule ('myasthenic toxin', 'curare-like substance').
4. Abnormal prolongation of the action of ACh.
5. False transmitter (e.g. choline).
6. Abnormality of structure or function of the ACh receptors (AChRs).
7. Failure of activation-contraction coupling in the muscle fiber.

It is unnecessary to survey the evidence (the interested reader is referred to Simpson, 1969) but by the end of the decade it was widely accepted that the defect was due to a biochemical lesion and the argument concentrated on its site. Those impressed by the evidence of Churchill-Davidson and Richardson (1953) and others for aberrant endplate responses to quaternary onium drugs favored a postsynaptic lesion. Others interpreted the Baltimore studies and those of Desmedt (1957) (comparing post-stimulation events with those in hemicholinium poisoning) as indicating a presynaptic lesion. The presynaptic hypothesis was supported by observations of Elmqvist and his colleagues that in biopsied intercostal muscle of myasthenic patients spontaneous miniature endplate potentials (spontaneous MEPPs) were markedly reduced in amplitude. For various reasons Elmqvist et al. (1964) chose to interpret this finding as evidence of reduction of the quantal content of ACh released spontaneously at the nerve terminals. The physiological and pharmacological data on which these opinions were based are critically reviewed by Simpson (1969, 1971). Accepting all the reported phenomena (and there is no reason to doubt them) it was shown that all of them were compatible with a reduced safety factor of transmission associated with altered structure of the endplate with wide separation of receptors, with or without receptor blockade. Clearly the opinions of the presynaptic and postsynaptic proponents, based on the pharmacology of normal mammalian NMJ, would be invalid if the endplate geometry was abnormal.

1.1.3 Histopathology

Until the use of intravital staining with methylene blue it was standard teaching that there was no histological abnormality of the NMJ in MG. This method was used by Mott and Barada (1923). In the one case reported by these authors, no abnormality was detected. Either they were unlucky or, as suggested by contemporary critics, the diagnosis of MG was erroneous. It was by a similar method, supplemented by staining for cholinesterase in the subneural apparatus, that Coërs and Woolf (1954) and Coërs and Desmedt (1959) showed florid morphological changes of the intramuscular nerve
endings. Terminal knobs were reduced in number and arranged serially on a long endplate region. There was also aberrant ultraterminal sprouting. In the 1960s various authors described abnormalities of the ultrastructure of the NMJ and attention began to be paid to the importance of endplate regeneration (Simpson, 1969, 1971, 1978). The definitive studies of endplate structure by Engel and his collaborators showed gross disorganization of the postsynaptic membrane with reduced number of receptor sites to a degree which correlates linearly with the decreased MEPP recorded from that synapse. Presynaptic vesicles are normal in numbers and size (Santa, Engel and Lambert, 1972; Engel, Lambert and Howard, 1977) (see Chapter 4). There can be no doubt that the major lesion is postsynaptic and includes destruction of AChRs (Albuquerque et al., 1976). The postulate that endplate structure regeneration may be important in accounting for the variability of clinical symptoms (Simpson, 1969) is supported by recent findings. An unexpected result of the new techniques for receptor analysis has been the discovery that AChRs regenerate very rapidly, probably within 2 days in man. There is presently no reason to believe that receptor synthesis is abnormal in MG but the unusual ultraterminal sprouting type of regeneration is certainly inefficient and represents an aspect of the pathology which may point to some additional presynaptic disorder and which may be amenable to treatment.

1.1.4 Pathogenesis models

This historical review has so far been arranged to present the development of concepts regarding the mechanism of the neuromuscular defect in MG. Each concept may be regarded as a model or schema, for indeed that is the way the human mind conceptualizes, using a model until later observations render it untenable. Popper and Eccles (1977) have emphasized the necessity of such models for adaptive procedures of learning new things. Equally, an inappropriate model can hold back further observations. Unfortunately the acceptability of a model depends on the climate of informed opinion.

The seventeenth-century model (Willis, 1672) was based on the theory of Animal Spirits which was at that time appropriate for the analysis of a neuromuscular disorder. In the nineteenth century an appropriate model was curare-type poisoning and this was the model adopted by Goldflam and Jolly. It was a powerful model, since it could incorporate the next observational advances. The neurotoxin model of Campbell and Bramwell (1900) is essentially the same, and the extract quoted above could stand unchanged for botulism and other disorders. It was strikingly prescient. The fact that microbes can cause pathology by immunological as well as toxic actions was unknown at that time but by the time of Keschner and Strauss (1927) the autotoxin model was in favor and obviously a rudimentary immunological theory. But medicine was changing. Under the impact of the new sciences of biochemistry and experimental pharmacology, the fashionable models in
Myasthenia Gravis were biochemical. From 1930 to 1960 few doubted that MG was a disorder of the recently demonstrated chemical transmission at the NMJ.

Towards the end of that period I wrote (1958):

‘Few diseases have been more satisfying to the teacher of medicine than myasthenia gravis for no better meeting ground exists for clinician, physiologist and pharmacologist. Ten years ago the standard teaching was that the myasthenic response must indicate one of three possible “chemical lesions” at the neuromuscular junction: (i) insufficiency of acetylcholine, (ii) excess of cholinesterase, or (iii) a “curariform” block of transmission, presumably due to a substance carried in the blood. More recently the logical fourth possibility of an abnormality of the motor endplates of muscle has been postulated. It seemed only a matter of time and of refinement of physiological and pharmacological techniques before the true lesion would be demonstrated. In that same period knowledge of the physiology of the neuromuscular junction has made its greatest advances, yet the solution of the problem of myasthenia evades us despite renewed and world-wide interest.’

(a) Relationship with thymus pathology
The paper (Simpson, 1958) went on to stress the necessity to include the thymus in any model of MG. A possible biochemical linkage might have been through the thyroid gland. In the first half of the twentieth century a literature had grown up on the association between MG and thyrotoxicosis. The present review does not call for a detailed study (see Simpson, 1968). The theory that hyperthyroidism in some way ‘causes’ MG was disproved by the careful metabolic experiments of Engel (1961). Nevertheless, the development of the ductless-gland model made it reasonable to postulate that the thymus was also an endocrine gland. In the absence of any apparent function after birth, it was suggested that it might secrete a curare-like substance to suppress fetal movements and as this was no longer required after birth the gland would normally involute. However, on occasion, as in thymic tumors, it might be supposed to secrete enough of this substance to act as a ‘myasthenic toxin’. After Weigert’s description of a case of MG associated with a thymic tumour, many cases were reported but in addition Norris (1937) drew attention to a high incidence of thymic lymphoid ‘hyperplasia’ in myasthenics and Sloan (1943) stressed the importance of ‘germinal centres’ in the medullary portion of the gland.

In the 1940s there were conflicting reports of ‘curare-like’ action of saline extracts of thymus from human and other species. Wilson, Obrist and Wilson (1953), using different solvents, reported that extracts of human thymus glands removed surgically from myasthenics had definite neuromuscular blocking activity which correlated with the beneficial therapeutic response to thymectomy. The further studies of the Liverpool group on human and whale fetal
thymus are of only historical interest. The quaternary nitrogen bases extracted from these glands can no longer be considered important in the pathogenesis of MG. Meanwhile the nature of lymphorrhages remained so uncertain (and their incidence so irregular) that most workers agreed with Russell (1953) that they were non-specific muscular changes of little or no importance.

The validity of the thymic version of the 'myasthenic toxin' models depended on the evidence that removal of the thymus would cause remission of MG: otherwise the thymic abnormalities must, like the lymphorrhages, be considered as epiphenomena. The first report on thymectomy performed by Sauerbruch for MG describes marked improvement of the MG but no effect on an associated exophthalmic goiter (Schumacher and Roth, 1913). This trial was not followed up (and it is interesting to note that they observed that lymphocytosis present before operation returned to normal after thymectomy) until Blalock, in Baltimore, started during the Second World War. His important paper (Blalock et al., 1941) reawakened interest in thymectomy but with continuing experience the results were considered discouraging (Grob, 1953) and American surgeons in general were abandoning the operation by the mid-1950s.

On the other hand, Keynes in London in a series of reports (e.g., Keynes, 1955) claimed good results. His belief that the difference was due to selection of non-tumor cases rather than thymoma and early rather than late cases was confirmed by an independent review (Simpson, 1958) which established the criteria for operation that are now generally accepted. It was now clear that thymectomy would induce remission of MG after a considerable latent period (1–2 years) but less certainly if the disease had been present for more than 7 years or if the thymus contained a lymphoepithelial thymoma. On the other hand, myasthenia could relapse or even occur for the first time years after thymectomy (Koch, Regli and Reine, 1970). An acceptable model for MG must find a regulating but not obligatory role for the thymus.

(b) Autoimmune models
When I had the opportunity to examine a large number of myasthenic patients in London in 1953–5 to assess the value of thymectomy (Simpson, 1958) it was decided to forget all that the textbooks had to say about biochemical lesions and to listen to the patients. A proforma was prepared and every ascertainable fact noted about the previous history of these patients (some 400, of whom about 250 were questioned and examined), including every illness whether it seemed relevant or not. Certain features were noted to recur in the histories. On returning to Glasgow in 1955 I conceived the idea that instead of rejecting as 'irrelevant' any data which were 'not statistically significant', it would be worth considering whether there was some correlation between them – e.g. that the muscular disorder might be part of a multisystem disease. For instance, it was found that MG was correlated not only with thyrotoxicosis but with all other non-tumor types of thyroid disease (Simpson 1960, 1968). It was also
noted, for the first time, that there was an apparent linkage between MG and 'rheumatoid' arthritis (including ankylosing spondylitis), pernicious anemia, sarcoidosis, reticuloendothelial disorders and diabetes mellitus. It was also observed that these related diseases occurred with more than expected incidence in the relatives of myasthenic patients. This was the first suggestion of a genetic factor with alternative forms of expression (Simpson, 1960).

It was then noted that lymphorrhages had been dismissed by Russell (1953) as 'non-specific' because they occurred in thyrotoxicosis, Addison's disease, rheumatoid arthritis and other diseases. It seemed more profitable to consider what factor was common to these diseases and an immunological reaction suggested itself. In the way as the pathology was being dismissed as 'non-specific' in the 1950s the opinion was often mooted that myasthenia was a type of muscular reaction and not a disease entity. Indeed a myasthenic reaction is not uncommon in a number of connective tissue diseases (to use the terminology of that time), including systemic lupus erythematosus (SLE).

In 1954 when Harvey et al. published their classic paper on SLE, I immediately recognized the similarity in the age and sex incidence and the fluctuating natural history. I had also had the opportunity in 1952–3 of giving adrenocorticotrophin (ACTH) and cortisone to myasthenic patients and to observe the early deterioration and 'rebound' improvement described by Torda and Wolff (1951) and later authors. These considerations led me to consider that MG might be an autoimmune disease with occasional multi-organ involvement and a natural history resembling SLE. This was a new pathogenesis model but could it include all known clinical and pathological phenomena and account for the neuromuscular defect? It is necessary to recall that at that time (1955–60) pernicious anemia was regarded as a deficiency disease due to malabsorption and thyrotoxicosis and diabetes as endocrine disorders. For that reason a possible hypothalamopituitary factor was discussed. I was unaware that Schwartz (1960) had evidence for an immunological mechanism in pernicious anemia. Autoimmunity was accepted in the pathogenesis of Hashimoto's disease but not yet in thyrotoxicosis (Anderson et al., 1964; Volpe et al., 1972) or diabetes mellitus (Bottazzo, Florin-Christensen and Doniach, 1974). In 1960 the thymus was considered to be an endocrine gland with possible roles in growth or maturation in the fetus, and the neuromuscular defect of MG was regarded as biochemical, probably due to a curare-like substance. A major difficulty with that theory was that myasthenia had never been transferred passively to another human subject and experiments purporting to show a blocking substance in laboratory animal nerve–muscle preparations did not stand up to rigorous examination (Nastuk, Strauss and Osserman, 1959). On the other hand a myasthenic woman could sometimes give birth to a baby with neonatal myasthenia (about one in seven live births in my series) and this disorder would persist for up to 12 weeks and then recover completely. The duration of the neonatal disorder was too long to be accounted for by persistence of a curare-sized molecule. The new model had
to accept these facts. It occurred to me that an antibody against AChR protein would account for:

1. Reversible competitive-type block in the affected patient.
2. Transplacental passage in the fetus with persistence for the required time.
3. Apparent specificity to genetically related subjects.

An antibody against AChR substance would be a perfect competitor for ACh, entirely compatible with the available facts about neuromuscular transmission. The logic of the argument led me to conclude that the thymus—with germinal centres reminiscent of lymph nodes rather than of a ductless gland—might be responsible for cellular and humoral immunity as a surviving residue of a wider fetal role as a regulator of cell differentiation. The concept was naive but, of course, was formulated before Miller (1961) had demonstrated the immunological role of the thymus.

From 1955 onwards, attempts were made to produce an experimental autoimmune myasthenia gravis (EAMG) in mice. The methods were crude and the observation of MEPP amplitude as a marker had not yet been made: the mouse has too high a safety factor for neuromuscular transmission to detect any myasthenic effect we may have produced. Nevertheless, the idea was pursued with renewed vigour when Smithers (1959) suggested that the myasthenic thymus may be immunologically damaged. He made no suggestions about the endplate disorder. In the same year, the experiments by Nastuk, Strauss and Osserman (1959) in New York disproving the curare-substance hypotheses showed that serum from some myasthenic patients (and a few control subjects in lesser degree) caused lysis of frog muscle cells. I still had no direct evidence of antibody when I gave the Honvman-Gillespie Lecture in Edinburgh on 28 April 1960. In the month after its journal publication the New York group, following up the cytolysis observation, announced the discovery of a complement-fixing antibody against the myosin of skeletal muscle in the blood of many myasthenic patients (Strauss et al., 1960). Since the antimyosin globulin was inconstant (and later shown to be in higher titer in the presence of a thymoma), and since physiological evidence required an endplate-acting substance, these authors came to regard it as an epiphenomenon and speculated that an antibody developed against skeletal muscle found a similar antigen in the thymus, which became damaged and released a neuromuscular blocking substance (Strauss et al., 1966) — an unrecognized return to 'curare-like substance', although it was their final refutation of this concept that had started their immunological studies.

In the 1960s there was not the present acceptance of autoimmune pathogenesis. Contemporary techniques could not detect anti-AChR antibody and presynaptic models such as the 'small quantum' theory were dominant. The 'myasthenic toxin' model was given a new run as a secretion from the thymus, which was now suggested as a target organ for immunological damage
Myasthenia Gravis

(Smithers, 1959; Marshall and White, 1961; Strauss et al., 1966; Goldstein and Whittingham, 1966). The rediscovered myoid cells of thymus were suggested as targets for an antimyosin antibody (Strauss et al., 1966; and many later authors).

Meanwhile autoimmune pathogenesis was being accepted for an increasing number of diseases and many of these were being reported in association with MG, for example myxedema (Simpson, 1960), Hashimoto's disease, sarcoidosis, vitiligo, hemolytic anemia, nephritis and hepatitis (Simpson, 1960; 1964), ulcerative colitis (Alarcon-Segovia et al., 1963), Sjögren's disease (Downes, Greenwood and Wray, 1966; Simpson, 1966a) and pemphigus vulgaris (Wolf et al., 1966). The association of MG and SLE has been recorded many times since the first report by Harvey et al. (1954). Since it was the recognition of these relationships which had originally led to the autoimmune hypothesis for MG, I have consistently argued for a model including defective immunological tolerance rather than a thymitis theory. Unfortunately most contemporary critics failed to notice the different heuristic values of the different autoimmunity models which I have contrasted in a review (Simpson, 1978).

(c) Neurotrophic theory

The obvious deficiencies of the thymitis model and general failure to reproduce the reported animal model of Goldstein and Whittingham (1966) influenced a rejection of all immunological models and a swing towards the newest fashionable model, deficiency of trophic factor (Engel and Warmolts, 1971).

1.1.5 Experimental autoimmune myasthenia gravis (EAMG)

A chance finding restored the attraction of the immunological model by demonstrating that the proposed mechanism could actually happen. The factor which made further advances possible was the demonstration by Lee, Tseng and Chiu (1967) that snake neurotoxins bind specifically and with high affinity to the ACh-binding site of muscle. Labeled toxin permitted identification of AChR substance extracted from the membrane of muscles (Miledi, Molinoff and Potter, 1971) and to purify AChR on affinity columns. To confirm that a toxin-binding protein obtained from electric organs of eels was, in fact, AChR, Patrick and Lindstrom (1973) prepared rabbit antibodies to the purified protein. When rabbit's serum was applied to the electrophleque it blocked the action of ACh. The new discovery which validated the immunological model of MG was that, a few weeks after injection, the immunized rabbits developed muscular weakness with the characteristics of MG. Other workers soon showed that this animal model of MG could also be produced in monkeys, guinea-pigs and rats and that the antigen could be AChR from motor endplates of a number of mammalian species including the
human. For further details of the development of the animal model (EAMG) and its immunology, see Chapters 11 and 13.

1.2 SERUM ANTIBODIES IN MG

1.2.1 Antibody against nicotinic AChRs

The successful production of EAMG depends on there being a certain amount of interspecies cross-reactivity of AChR. Animals immunized with Torpedo electroplaque first develop antibodies which recognize the electric organ in much higher concentration than antibodies recognizing syngeneic AChR. At a later stage of the immune response the absolute amount of antibody against the recipient animal’s AChR is high (Lindstrom et al., 1976b), suggesting true autoimmunity. The theory that the proximate mechanism of EAMG is autoimmune is supported by experiments demonstrating passive transfer of EAMG by lymph node cells in guinea-pig (Tarrab-Hazdai et al., 1975) and rat (Lennon, Lindstrom and Seybold, 1975) and γ-globulin in rat (Lindstrom et al., 1976a). In human MG Matell et al. (1976) have reported that patients go into remission after drainage of lymph from the lymph duct and relapse promptly if their cell-free lymph is reinjected. Passive transfer of MG from man to mouse by human myasthenic serum has been described by Toyka et al. (1977). On the other hand, the presence of circulating anti-AChR globulin is not necessarily associated with detectable neuromuscular transmission failure (Simpson, 1980), this could be because the antibodies measured by contemporary techniques are not pathogenic (see below), or it may be necessary for the safety factor for neuromuscular transmission to be reduced by associated disease, such as cell-mediated immunity (Chapter 11), before failure occurs. However there is little evidence for involvement of cell-mediated immunity at the endplate itself.

(a) Assay methods

The ability to identify AChR sites also made it possible to prepare extracts of muscle with a high concentration of binding sites and to use these as antigens to detect the presence of anti-AChR substances in the serum of myasthenic patients. It also became possible to detect an immunoglobulin capable of blocking access of neurotoxin to nicotinic AChRs because of high affinity of the immunoglobulin for AChR or closely adjacent material. Early assays based on measuring the inhibition of binding of γ-bungarotoxin (γ-BGT) to extra-junctional AChRs of denervated rat muscle (Almon, Andrew and Appel, 1974) or to junctional AChRs of human muscle (Bender, Ringel and Engel, 1976) were of low sensitivity. (Nevertheless it should be remembered that the more ‘sensitive’ assays do not take account of antibodies directed at the toxin binding site of the receptor.) Using complement fixation, Aharonov et al. (1975a) provided indirect evidence of an antibody in human myasthenic serum which
cross-reacts with AChR from electric tissue of \textit{Torpedo californica}. More sensitive assays are based on the binding of antibody to solubilized AChR linked to isotope-labeled \textit{z}-BGT. An anti-immunoglobulin or \textit{Staphylococcus aureus} protein A (Barkas, Farrar and Watson, 1979a) precipitates the antibody-AChR-neurotoxin complexes along with carrier immunoglobulin. The radioactivity of the resulting pellet is, after washing, a measure of the AChR bound to antibody (Appel, Almon and Levy, 1975; Lindstrom, 1977). It is, however, becoming increasingly clear that there is a multiplicity of immunoglobulins that may be produced against the AChR in myasthenia, one of which may block access of toxin to the AChR sites. Antibodies may also be directed against sites on the ionic channel of the AChR which block neuromuscular transmission but would probably pass undetected in the Lindstrom assay. On the other hand, antibodies directed against antigenic determinants on the intracellular surface of the AChR would be detected by the assay which uses detergent-solubilized \textit{[125I]}\textit{z}-BGT-labeled AChR as antigen, while it is pathologically irrelevant since they could not bind \textit{in vivo}. This should be borne in mind when considering the following studies on myasthenic patients. Additionally, different antibodies, or fractions of antibodies may be recognized by AChR-toxin from different species as there is not complete cross-reactivity. For instance, Lindstrom \textit{et al.} (1976c) could detect anti-AChR antibody in at least 87\% of patients otherwise thought to have MG, but only if the test AChR was from human muscle, and the titer did not correlate closely with disease severity in the different patients. AChRs contain a number of antigenic sites and not all of them are species specific or immunologically equivalent.

\textbf{(b) Correlation between antibody titer and neuromuscular block} Patently, assays from different laboratories are not comparable with respect to titers though standardization should soon be achieved. Although Lindstrom \textit{et al.} (1976c) found correlation between anti-AChR titer and clinical severity, most workers find that the correlation is rather poor (Lefvert \textit{et al.}, 1978; Newsom-Davis \textit{et al.}, 1978; Barkas \textit{et al.}, 1979b). On the other hand, the changing titers of anti-AChR antibody are inversely associated with changing clinical state in patients responding to thymectomy, plasmapheresis, lymph duct drainage, steroid therapy and immunosuppression (Dau \textit{et al.}, 1977; Lefvert \textit{et al.}, 1978; Newsom-Davis \textit{et al.}, 1978; Limburg, Oosterhuis and The, 1981; Carter \textit{et al.}, 1980; Savage-Marengo \textit{et al.}, 1980). With steroid therapy the relationship between antibody level and clinical status is less striking than the association of either with the level of steroid dosage (Seybold and Lindstrom, 1979). It is possible to have anti-AChR antibody without apparent clinical disease. This has been reported in the serum of apparently normal babies born to myasthenic mothers (Keesey \textit{et al.}, 1977; Lefvert \textit{et al.}, 1978) and in siblings or a parent of a myasthenic child (personal observation). Recently Guenouch \textit{et al.} (1980) have reported detection of antibodies to AChR in three of 11 patients with a thymoma removed by operation but without MG. Follow-up
of these patients will be of great importance with regard to the understanding of those cases of MG developing years after removal of a thymoma.

(c) **Subclasses of antibody**

Since, as shown above, there is some parallel between antibody titer and degree of neuromuscular block in the individual (which may not necessarily indicate disease severity) but rather poor group correlation, it is considered that there may be heterogeneous antibodies and that any one detected by a particular immunoassay may not be pathogenic (though it could still be a useful marker if idiotypes are linked, as seems likely from the studies of Savage-Marengo et al. (1980)). The ratio between anti-rat AChR and anti-human AChR antibodies remains surprisingly constant in individual patients, indicating that a particular subpopulation may be significant (Savage-Marengo et al., 1980). Indeed the antibody(s) assayed, although undoubtedly responding to receptor antigen, may not include the important one for pathogenesis. Furthermore, the changes in antibody titer described in (b) seem to precede the clinical improvement, whether the AChR used in the assay is human (Newsom Davis et al., 1978) electric eel (Barkas et al., 1979b) or monkey (Limburg, Oosterhuis and The, 1981). Many laboratories are studying the specificity of anti-AChR subclasses. Nevertheless, the fact that they are heterogeneous but not unlimited points to there being a limited number (about 10) of antibody combining sites on each AChR molecule (Vincent and Newsom-Davis, 1979a).

In animals with EAMG, immune sera do not protect against α-BGT block of ACh recognition sites and electrophysiological studies indicate that antibody does not block ionophore sites (Takamori, Ide and Tsujihata, 1981). The possible sites of action of antibody are discussed in Chapters 3 and 12. Are these antibodies a cause of receptor failure/blockade as in the Simpson model, or could they be a response to receptor damage and hence protective? The effect of plasmapheresis makes the latter possibility unlikely.

### 1.2.2 Antimyosin antibodies

It may well be that a more directly pathogenic antibody remains to be discovered but there are other factors which indicate that the known antibodies are sufficient. A critical observation goes back to the early days of the immunological models. It was shown above that the Strauss model was developed from the observation, during a search for a 'curare-like' substance, that serum from myasthenic patients caused lysis of frog muscle (Nastuk, Strauss and Osserman, 1959). Although some control sera showed similar activity (and, surprisingly, the basic observation has not been repeated) the occurrence of cytolysis suggested that serum complement might be used because of its known role in immune hemolysis (Nastuk, Plescia and Osserman, 1960). Nastuk and co-workers found that serum complement (determined on
the basis of degree of lysis produced by the test serum in a standard suspension of sensitized sheep erythrocytes) was distributed over a wide range in myasthenic patients but tended to fall during clinical relapse and rise during remission of MG (sometimes to 'supernormal' levels). One of the authors (O. J. Plescia) suggested that the fall in serum complement activity may be the result of its uptake by an antigen–antibody complex. Simultaneously the New York group reported the finding of a complement-fixing globulin in sera from some myasthenic patients which would bind to striated muscle, where it could be shown by the indirect immunofluorescence technique (Strauss et al., 1960). I had been searching for anti-muscle or anti-endplate antibodies since 1955 without success, though techniques were similar. It soon became clear that the new antibody, which bound to cross-striations of muscle, was inconstant but appeared in highest titer in the sera of those patients who had a thymoma. Whereas I had tested individual sera, Strauss and his colleagues had used pooled sera and at least six of their first ten patients had a thymoma whereas a further 16 individual sera were negative.

Antibodies to skeletal muscle proteins have been shown by different serological methods: immunofluorescence (Strauss et al., 1960), antiglobulin consumption (Van der Geld and Oosterhuis, 1963), indirect hemagglutination (Djianian, Beutner and Witelsky, 1964) and precipitation (Shulman et al., 1966). Immunofluorescence studies have used direct and indirect methods of showing globulin-binding to muscle: it is generally agreed that the direct method originally used by Strauss et al. (1960) is unreliable. Unfortunately, the criteria for interpretation of indirect immunofluorescence studies have not been standardized. In the first report, Strauss et al. (1960) localized the antibody–antigen reaction in the anisotropic or A-bands of skeletal muscle, which contain actomyosin. Veters (1965) found that in many myasthenic patients and normal control subjects the immunofluorescent staining affected the isotropic or I-bands of muscle which contain actin. Indeed he came to the conclusion that A-band binding of globulin was confined to sera from patients with a thymoma (Veters, 1967) and he regards 1-band binding as non-specific and of no diagnostic importance. Because of the differences of criteria for this and other immunological tests which will be described, no attempt will be made to list all reports with a view to identifying the incidence of different types of antibodies in sera from myasthenic subjects. Only early papers and large series will be cited and a general indication of incidence reported.

Namba, Sato and Grob (1967) studied in vivo binding of myasthenic sera to rat skeletal muscle and reported greater binding to muscle fibers of smaller diameter, described as having a higher activity of ATPase and succinic dehydrogenase (fiber type not identified). They also reported that serum from patients with MG reacted with ribonucleoprotein isolated from human skeletal muscle in 30 (48%) of 63 cases. This reaction was occasionally found in patients with other diseases of muscle (Namba, Hime and Grob, 1967). These reactions, which are not specific for MG, have not been reported by other
workers and cannot be assessed without comparative studies with anti-nuclear and anti-sarcolemmal reactions. It is not even certain that they are immunological.

Using the antiglobulin consumption test, Aarli (1970) showed that normal human γ-globulin combines with muscle tissue by the Fe fragments only (presumably non-antibody binding) but sera from myasthenic patients have γ-globulins which attach to muscle and thymic tissue by the F(ab)_2 fragments (possibly but not necessarily an antibody binding). This Fab binding induces structural changes in the Fe part of the molecule which can be 'recognized' by rheumatoid factor (RF). Reduction of the RF titer of test sera when reacted with myasthenic sera was demonstrated by Aarli (1971). The non-specific 1-band binding described by Vettes may be mediated by Fe fragments of immunoglobulins (Aarli and Closs, 1972). If the A-band binding is immunological, what is the antigen used? If it is indeed confined to the A-band it is likely to be related to actomyosin (Strauss et al., 1960). With the method of precipitation in Ouchterlony gel, Ricken (1969) reported that activity was confined to the myosin-containing fraction of muscle. Rule, Bartlett and Osserman (1973) showed that absorption with either actomyosin or myosin removed all activity shown by the indirect immunofluorescent assay. They also found that quantitative complement fixation showed that active fractions of purified human muscle proteins included actomyosin and myosin, the highest activity being in the myosin fraction. Actin preparations had no antigenic activity. Some anti-striational antibodies also react with heart muscle (Beutner et al., 1962; Van der Geld and Oosterhuis, 1963) and with myoid cells of the thymus (Feltkamp-Vroom, 1966; Van der Geld and Strauss, 1966) but not with other substrates, including smooth muscle.

The reader may feel that too much space has been devoted to 'an epiphenomenon' (Strauss et al., 1966) which is now only of historical interest since the discovery of anti-AChR antibodies. The reasons for giving it this emphasis are: (1) the demonstration of multiple antibodies has important implications with respect to the pathogenesis of autoimmunity; and (2) the diagnostic significance of the antistriational antibody. Vettes carried out his studies on material from the writer's patients and the detection of genuine A-band fluorescence (using the criteria of Vettes, 1965) has made it possible to detect the presence of a thymoma in the absence of radiological evidence. The converse is not true: antistriational antibody may be undetectable at one time and found later in patients with thymoma (Weiner and Osserman, 1966). Although it is not certain that other workers have made the distinction between I-band and A-band fluorescence, Feltkamp et al. (1974), using indirect immunofluorescence, detected antibodies to skeletal muscle in 12 out of 13 myasthenic patients with a thymoma and in only two out of 43 patients in whom the presence of a thymoma could be excluded by thymectomy. Their earlier report, that 47 (42%) of 111 cases showed globulin binding to muscle, included sarcolemmal and nuclear fluorescence as well as I and A band. They
showed that sarcolemmal fluorescence also occurred with control sera (Feltkamp, Van der Geld and Oosterhuis, 1963). Different criteria may also account for the experience of Weiner and Osserman (1966) that high titers of antistriational antibody occurred in patients who had no detectable thymus at autopsy. The A-band binding globulin has also been detected in sera from patients with a lymphoepithelial thymic tumor who do not have clinical MG. Many of these sera also showed antinuclear reactions (Strauss et al., 1966). There is some evidence to suggest that expression of this antibody is linked to the HLA-A2 antigen (Feltkamp et al., 1974).

1.2.3 Antinuclear factors

Positive reactions for the presence of antinuclear factor (ANF) were reported in the sera of six of 16 myasthenic patients by White and Marshall (1962). The usual variation of morphological pattern of the nuclear fluorescence was found. Both of their patients with thymoma had a negative reaction. Two patients with systemic or cutaneous LE showed positive reactions. Patients who also had rheumatoid arthritis or thyrotoxicosis appeared in both groups. In larger series, Van der Geld et al. (1963) found 11 (10%) of 111 cases positive and Simpson (1964) eight (20%) of 40 cases, while Downes, Greenwood and Wray (1966) found 22 (30%) positive in a series of 74 myasthenics and Feltkamp et al. (1974) found 44 of 100 patients to be positive, using one or more substrates (controls 4% positive). The incidence clearly depends on the associated diseases as well as on the techniques used. Strauss et al. (1966) suggested that presence of ANF was related to a thymoma. Oosterhuis, van der Geld and Feltkamp (1967) reported that patients with a thymoma have an increased production of ANF but no other relation with the severity of the myasthenia or with age and sex could be found. They noted 21 (19%) positive in 110 cases without thymoma, but eight (53%) positive in 15 cases with thymoma. Of the non-thymoma positive cases, 25% had an associated autoimmune disease. Reviewing six early reports, Rule and Kornfeld (1971) found incidences of positive ANF varying from 0 to 89%. Their figure of 21% is fairly representative for uncomplicated MG; when patients with myasthenia and one other autoimmune disease were reviewed, ANF was present in 90–100% and the titer was much higher.

1.2.4 Rheumatoid factor

In 1960 I drew attention to an apparent linkage between MG and ‘rheumatoid’ arthritis (Simpson, 1960). The qualification was used to indicate the possibility that the joint disorders might not be conventional rheumatoid arthritis because they could be transitory and were often seronegative. Some patients had ankylosing spondylitis (Simpson, 1964). These findings were
confirmed by White and Marshall (1962), Van der Geld et al. (1963) and others, especially in women (Oosterhuis and De Haas, 1968). Aarli, Milde and Thunold (1975) have confirmed the different clinical types of arthritis. They consider that both definite rheumatoid arthritis and juvenile rheumatoid arthritis may develop during myasthenia but that there is also a form of arthropathy which is probably not of rheumatoid nature. Some cases in each of the series quoted were probably associated with SLE and occasional cases of arthritis have had Sjögren’s syndrome (Downes, Greenwood and Wray, 1966; Simpson, 1966a).

That a surprising proportion of these arthropathies are seronegative was confirmed by White and Marshall (1962); Van der Geld et al. (1963); Szobor, Bosóky and Gáspari (1969); Aarli, Milde and Thunold (1975) and others, using sensitized sheep cell or latex fixation tests for RF. Aarli has suggested that there is autoreactivity between skeletal muscle antibodies and RF so that reduction of titer of RF might be caused by in vivo binding of RF to skeletal muscle tissue (Aarli, 1971; Aarli, Milde and Thunold, 1975). An interesting difference from the autoimmune reactions already reviewed is that I have not traced any record of arthritis or positive test for RF in a myasthenic patient who had a thymic tumor without evidence of SLE. The frequency of positive RF tests reported are 0–6% of myasthenics, (Van der Geld et al., 1963; Simpson, 1964; Adner et al., 1964; Downes, Greenwood and Wray, 1966; Oosterhuis, Van der Geld and Feltkamp, 1967) and 0–28% of myasthenics with arthropathy (Simpson, 1964; Downes, Greenwood and Wray, 1966; Aarli, Milde and Thunold, 1975). In a population of 'normal' rheumatoid arthritis, RF factor is present in significant titer in about 65% of cases. It is not, however, unique to rheumatoid arthritis, being found in patients with sarcoidosis and other hyperglobulinemic states. Rheumatoid factor is considered to be an auto-antibody to altered human γ-globulin (Aho and Simons, 1963). Aarli (1971) has demonstrated that RF is absorbed from serum by muscle treated with myasthenic serum and then washed, interpreted as combination of RF with muscle-bound γ-globulin. Clearly, RF is an indicator of altered γ-globulin and is in no sense an antibody against joint tissues. The significance of the negative correlation with thymoma is not apparent.

1.2.5 Antithyroid antibodies

A relationship between MG and thyrotoxicosis has been recognized for a long time although, in fact, the incidence of MG in thyrotoxic patients is about 1 in 3000 (Simpson, 1958) whereas 21% of female and 9% of male myasthenic patients have a thyroid disorder at some time, but this includes all types of thyroid disease; about 5% of myasthenics have thyrotoxicosis though not contemporaneously (Simpson, 1968). The first reported case associated with Hashimoto’s disease (Simpson, 1964) is of some importance as the thyroiditis appears to have manifested shortly after thymectomy. Immunological studies
showed an even higher incidence of thyroid disease than did thyroid function tests: of myasthenics without clinical evidence of present or past thyroid disease, 21% of female and 15% of male myasthenics had complement-fixing antibodies against thyroglobulin or thyroid microsomes (Simpson, 1964, 1968). Clinical thyroid disease occurred in another 24% of female and 15% of male patients.

Downes, Greenwood and Wray (1966) demonstrated thyroid antibodies in 42% of myasthenic patients compared with 22% of matched controls. An unexplained difference was that in their series thyroid antibodies were more common in male (59%) than in female (35%) myasthenics. (Their figures included clinical as well as subclinical cases of thyroid disease.) These authors tested for cytoplasmic antibody, colloid antibody on fixed tissue sections, and tanned erythrocyte hemagglutination. With the hemagglutination technique for thyroglobulin antibodies Adncr et al. (1964) found 17 (35%) positive in 48 cases: their test gave negative results in all control sera. Oosterhuis et al. (1967) measured antibodies to thyroid cytoplasm and colloid. One or both tests were positive in 26% of non-thymoma and 40% of thymoma patients with MG. The sex ratio was 35% positive in females and 15% in males. The difference between sexes was statistically significant but the higher incidence associated with thymoma was not.

Unfortunately other published series do not differentiate thymoma cases, but personal and reported experience (Van der Geld et al., 1963; Aarli, 1971) confirms that thyroid antibodies occur in more than one third of myasthenic sera and not significantly more commonly in association with thymoma. Aarli (1971) has confirmed that thyroglobulin antibodies do not cross-react with muscle.

1.2.6 Gastric parietal-cell antibody

A linkage between MG and Addisonian pernicious anemia was recognized by Simpson (1960, 1964, 1966a) and further studied by Howard, Silverstein and Mulder (1965) but the incidence is certainly low, of the order of 0.5–2%. At about the same time evidence was accumulating that antibodies against gastric intrinsic factor were present in the sera of patients with pernicious anemia (Schwartz, 1960; Irvine et al., 1962; Jeffries, Hoskins and Sleisinger, 1962). Antibodies to gastric parietal cells were found in 6–18% of myasthenic patients (Simpson, 1964; Downes, Greenwood and Wray, 1966; Wright and Kerr, 1967; Oosterhuis, Van der Geld and Feltkamp, 1967). This is a significantly greater incidence than in control sera tested with the same criteria (Feltkamp et al., 1974). Antibody against intrinsic factor was not found by Downes, Greenwood and Wray (1966) in their cases with positive gastric parietal-cell antibody. Cases with parietal-cell antibody and no anemia have been followed up for a number of years without later development of pernicious anemia. Feltkamp et al. (1974) found a relationship between HLA-W10 antigen and
gastric parietal-cell antibodies in myasthenic patients. No reported case with pernicious anemia had a thymoma though parietal-cell antibody has been detected (Oosterhuis, Van der Geld and Feltkamp, 1967).

(a) *Erythrocyte aplasia and hemolytic anemia*
Contrary to pernicious anemia, the very small number of patients with erythrocyte aplasia and MG have all had a thymoma. No antibody has been identified but Holborow *et al.* (1963) found a positive ANF in one case. The aplasia (which occurs also with non-myasthenic thymoma) is not always confined to the erythrocytes but may be pancytopenic, as in the first case reported in association with MG (Wintrobe, 1946) and with hypogammaglobulinemia. The literature is reviewed by Simpson (1976) along with occasional reports of acute hemolytic anemia, also rare but not so clearly associated with thymic neoplasia.

### 1.2.7 Adrenal gland antibodies
Thymic germinal centres have been described in Addison’s disease and occasional thymoma (Sloan, 1943). It has long been known that primary myxedema is a common accompaniment of idiopathic Addison’s disease (Schmidt’s syndrome). Anderson *et al.* (1957) described the occurrence of a complement-fixing antibody against adrenal cortex in the serum of two of eight patients with idiopathic Addison’s disease, one of whom also had Hashimoto’s disease. Idiopathic Addison’s disease is often accompanied by chronic gastritis and by the presence of gastric parietal-cell antibody (Irvine, 1963). It would accordingly be expected that an occasional overlap of autoimmune disorders would cause coincidence of MG and Addison’s disease. In fact, this has rarely been reported and the autoimmune status of the adrenal disease is uncertain in some of the reported cases. In more than 600 cases of MG I have recognized adrenocortical failure in only one and that was shown, at autopsy, to be due to tuberculous adrenalitis (Simpson, 1966a). The cases described by Kane and Weed (1950), Boulet *et al.* (1959) and Thiodet *et al.* (1961) are of uncertain status.

On the other hand, I have a patient with MG and thyrotoxicosis (no thymoma at thymectomy) who has a son with idiopathic Addison’s disease who has adrenal cortex antibodies. Oosterhuis *et al.* (1967) were unable to demonstrate antibody reacting with adrenal cortex in their myasthenic patients but in a later series reported it in 3% (Feltkamp *et al.*, 1974). None had clinical hypoadrenalism and the number of cases investigated is not stated. Simpson (1966b) reported subnormal excretion of 17-hydroxy-corticosteroids and 17-ketosteroids in some cases of MG, but adrenocortical function has not been investigated by modern methods of steroid assay. Clearly an overlap with clinical autoimmune Addison’s disease is rare and familial linkage not confirmed but it seems reasonable to suggest that it is one of a cluster of
Myasthenia Gravis

Genetically linked autoimmune diseases. Bosch, Reith and Granner (1978) recently reported Schmidt's syndrome associated with MG.

1.2.8 Diabetes mellitus

Although there is an increased incidence of diabetes mellitus in a population of myasthenic patients (Simpson, 1960, 1964) and an immunological basis for insulin-dependent diabetes was later established, no studies have been reported on islet-cell or insulin antibodies.

1.2.9 Gut and liver

I have seen two patients in whom MG was associated with adult gluten-sensitive enteropathy, one also having dermatitis herpetiformis. Gluten-sensitive enteropathy (celiac disease) is associated with low serum IgA levels in some cases and high levels in others and there is probably defective synthesis of IgM. Anti-reticulin antibody is commonly detected (Fry and Seah, 1974). These disorders are also highly correlated with HLA-B8 antigen.

Ulcerative colitis has also been associated with MG and SLE (Alarcón-Segovia et al., 1963). It is currently considered to be an immunopathy with anti-colonic antibodies and immune complexes in the serum of some patients and a depressed lymphocyte phytohemagglutinin response (Zeromski et al., 1971).

Acute and chronic hepatitis has been reported in a small number of myasthenic patients but no information about the causation has been published.

1.2.10 Skin

A myasthenic syndrome is not uncommon transiently in dermatomyositis, usually early but occasionally later in the course (Vasilescu et al., 1978). Though it differs quantitatively from MG (Simpson, 1966c) it is perhaps surprising that dermatomyositis has not been reported in continuing MG.

There may be hyper- or agammaglobulinemia, RF and antinuclear antibodies, but tissue specific antibodies have not yet been identified. The LE factor is rarely found unless dermatomyositis coexists or overlaps with SLE (Jablonska and Chorzelski, 1974).

Four diseases of skin now recognized as autoimmune have been associated with MG. The first was vitiligo (Simpson, 1964; Durance, 1971) but the immunology has not been studied in detail. Pemphigus and pemphigoid have been associated on a number of occasions and antiepipithelial antibody detected in the serum (Noguchi and Nishitani, 1976). There is also an increased incidence of RF and ANF in pemphigus. In pemphigoid the antibody is directed against the basement membrane of the dermoepidermal junction as
 distinct from the epithelial intercellular cement substance in pemphigus (Beutner et al., 1965). The role of these antibodies in pathogenesis is still uncertain (Sams, 1974) but there is little doubt that the diseases use immunological mechanisms. Complement binding to the basement membrane has been demonstrated in the skin of patients with pemphigoid (Chorzelski and Cormane, 1968). One myasthenic patient with sclerodema is mentioned by Pirskanen (1977). Since our recognition of immunodeficiency with low serum IgA level in MG (Simpson, Behan and Dick, 1976) we have been searching for a linkage with gluten-sensitive enteropathy. As mentioned above, we now have two cases and one of these has had dermatitis herpetiformis (Simpson, 1980). This skin disease is associated with immunological defects (Fry and Seah, 1974). The skin lesions are related to gluten ingestion.

Despite the linkage with celiac disease, patients with dermatitis herpetiformis tend to have a higher serum IgA level and lower IgM than a control group. There is, however, no consistent pattern of abnormality of serum immunoglobulin levels. Antinuclear antibodies are detected in more than 30% of cases and an antireticulin antibody has been reported. Immunoglobulin and C3 component of complement are deposited in the skin. Patients with dermatitis herpetiformis have a high incidence of HLA-B8 haplotypes, and lymphocyte abnormalities have been described.

1.2.11 Plasma and cerebrospinal fluid globulins

Early reports on plasma proteins in patients with MG recorded decreased γ-globulin (Thévenard and Mende, 1955), raised γ-globulin (Lowenthal and van Sande, 1956) or no change (Osserman, 1958). Simpson (1960, 1966b) examined 55 cases and found hypergammaglobulinemia and raised erythrocyte sedimentation rate only in those cases where myasthenia was accompanied by a related autoimmune disease or thymoma. Excluding all cases with associated diseases, Kornfeld (1964) found no electrophoretic abnormality in 61 cases of MG. In all reports of hypergammaglobulinemia in myasthenics (e.g. Oosterhuis et al., 1964) there has been one of these associated disorders. The abnormality may be sufficient to give abnormal cephalin-cholesterol flocculation and thymol-turbidity tests.

In a number of cases, abnormal globulin levels have been present in the cerebrospinal fluid (Simpson, 1966b).

Further subdivision of γ-globulin into immunoglobulin fractions has shown that selective IgA deficiency is more common in myasthenic patients than in the normal population, especially in patients with juvenile onset congenital myasthenia (Bundey, Doniach and Southcll, 1972; Simpson et al., 1976; Bramis et al., 1976). (The contemporary use of ‘congenital’ is retained as the more recently delineated non-immunological category of ‘congenital myasthenia’ is not yet confirmed to include all congenital cases.) It is not influenced by
thymectomy and is probably an indication of immunodeficiency, or immune deviation. Lisak and Zweiman (1976) found no significant mean alteration of IgA levels in non-thymectomized myasthenics but one case had a low level (it was elevated in four). Their series contained no cases of 'congenital' myasthenia. Bramis et al. (1976) found lower IgA concentrations in patients with thymoma.

Lisak and Zweiman (1976) reported a slight but significant depression of the group mean IgM level and an elevated mean IgG level in serum compared with control subjects. The raised levels of all three classes of immunoglobulins were unrelated to the presence of thymoma or associated autoimmune diseases. Lefer et al. (1978) noted antireceptor antibody of IgM as well as IgG type. Sometimes the IgM type preceded the IgG antibody. On the other hand, a number of myasthenic patients with hypogammaglobulinemia have been described, usually in association with thymoma (Te Velde, Huber and van der Slikke, 1966). No doubt the immunoglobulin levels vary according to the activity of the disease. Monoclonal gammopathy has been described by Rowland et al. (1969) and Fauré et al. (1975). In both cases the abnormal IgM gammaglobulin did not contain anti-AChR antibodies.

1.2.12 Complement and immune complexes

Serum complement is a complex biological system of globulins which are activated serially along 'classical' and 'alternative' pathways ending in a common chain of reactions mediating a number of reactions including some immune responses, notably Type III (immune complex) reactions. Nastuk, Plescian and Osserman (1960) reported reduced serum complement levels during clinical relapse of MG, and reduced levels of C2, C4 and inhibitors of these components were observed by Plescia, Segovia and Strampe (1966). Simpson, Behan and Dick (1976) found no abnormality of Clq, C3, C4, C7 and C3 proactivator. They also measured C3 conversion products with C3 activator levels, and CH50 units. All were normal though examined in active and remittant cases. Their patients were re-examined by Behan and Behan (1979) when Engel, Lambert and Howard (1977) demonstrated localization of IgG and C3 at motor endplates, and they found that C4 component was depressed in 34% of cases while 29% had circulating immune complexes. The greatest immunological abnormalities were found in patients with mild disease. Almost certainly the hypocomplementemia is of consumption type, caused by complement binding to immune complexes. Circulating immune complexes, identified in 42% of myasthenic sera, have not yet been characterized (Barkas, Boyle and Behan, 1980) and complement is certainly fixed by some of the other antibody reactions described above, notably the anti-A band antibody associated with thymoma (Strauss et al., 1960).

A young woman with typical MG was heterozygous for hereditary deficiency of C2 component of complement (Riggs et al., 1980). Patients with deficiency of
various components of complement suffer an increased incidence of autoimmune disease. In the case reported, total complement and C3 and C4 levels were normal. The patient had an HLA haplotype A32, B18 which segregated with the gene for C2 deficiency. She also had HLA haplotype A1, B8 (presumably linked with her MG) but this was inherited from her mother and was not associated with hereditary complement deficiency. The role of C2 deficiency in this case is uncertain. Subjects with C2 deficiency are considered to activate the complement chain more readily than normal.

Engel, Lambert and Howard (1977) suggest that complement activation is necessary to induce receptor lysis when anti-ACh receptor antibody is attached to receptor sites. The abundance of IgG and C3 at sites of remaining AChR receptors in mild cases leads Engel to conclude that interference with receptor function by immune complexes is not of primary importance. The sites where destruction of receptors is most marked are characterized by deposits of C3, the terminal and lytic component of complement, and this is also present in debris in the synaptic space and between layers of basal lamina near remaining endplate regions. Engel considers that the junctional folds undergo complement-mediated lysis and that this induces relocation of the nerve terminal and hence increased spatial separation of endplate regions on the muscle fiber.

A role for the earlier components of the complement cascade is also suggested by the observation that myasthenic serum passively transferred to mice produces clinical signs in the recipients which are significantly less if the mice are depleted of C3, but recipient mice genetically deficient in the fifth component of complement are affected as severely as normal mice (Toyka et al., 1977). In the experimental model (EAMG) it appears that AChR antibodies are not pathogenic to rats in the absence of complement (Lennon et al., 1978). Clearly it will be important to determine the complement status of human myasthenics who have a high titer of anti-AChR antibody in their serum despite mild clinical disease, sometimes confined to the extraocular muscles. However, assay methods based on complement fixation (e.g. Aharanov et al., 1975b) do not appear to correlate any closer than anti-AChR with the severity of MG.

1.3 PRODUCTION OF IMMUNOGLOBULINS

The possible role of cell-mediated immunity in MG is discussed in Chapter 13 and will not be elaborated here, but it is necessary to give brief consideration to the source of antibody immunoglobulins. It is accepted that a given antibody molecule is synthesized by a given clone of lymphocytes. The above review of the many types of autoreactive antibodies commonly associated with anti-AChR, and of autoimmune disease of other organs often preceding MG in time, strongly suggests that the disorder is not monoclonal.

Immunoglobulin is synthesized in plasma cells and displayed on the surface
of B lymphocytes when these cells are stimulated by recognized antigens or by non-specific mitogens. Stimulating antigen may be in solution, but blast transformation is more effective when antigen is presented to the lymphocyte by macrophages. It seems probable that the latter mechanism is important in EAMG, stimulated by various adjuvants, but it has not been defined in the human disease. Macrophages require serum constituents to recognize foreign cells. The stimulated immunoglobulin secreting cell may switch from IgM production to an IgG molecule containing the same light chain and the same variable part, and hence the same antibody activity, coded for by C and V genes (two genes for each immunoglobulin polypeptide). Humoral antibodies of the type described in this chapter are formed by lymphocytes as suggested by Simpson (1960). Since Miller (1961) showed that neonatal thymectomy caused immune deficiency, particularly of cell-mediated immunity but also to a lesser extent of humoral immunity, it has become clear that immunoglobulin production is an important function of B cells but that this function requires the co-operation of T cells. The T cells are bone marrow-derived lymphocytes differentiated under the influence of the epithelial cells of the thymus, either by direct contact, or by the action of thymic hormones. Further maturation of T cells occurs outside the thymus but only those deriving from the medulla of the thymus are involved in B cell co-operation.

It is postulated that maximal response of T cells to conventional antigens only occurs when the latter are associated with antigens coded for the major histocompatibility complex, differing according to the type of T cell (cytotoxic, helper, etc.) - a 'self or non-self recognition' system. A third type of lymphocyte (K cells) attacks target cells coated with IgG. It will be apparent that in the fullest sense it is not possible to consider humoral and cellular aspects of autoimmunity in isolation unless the terms are restricted to the final effector mechanisms and to the possibility of passive transfer of immunity by cell or serum. Some understanding of the production and control of immunoglobulins is necessary for proper assessment of antibody reactions. By the criteria of passive transfer (Toyka et al., 1977) and effect of replacing cellular or humoral component of lymph (Matell et al., 1976) the major disorder in MG is humoral autoimmunity, a conclusion supported though not proved by the therapeutic benefit of plasmapheresis.

1.4 IMMUNE DEFICIENCY AND THE THYMUS

The immunology mechanism is part of the homeostatic control of the body's internal environment and cell population. The immunological system plays a normal scavenging role in the recognition and removal of effete or dead cells (and probably of cell mutations) including some components of the NMJ, but not, apparently, the AChRs which are removed by internalization into the muscle cell. It is further postulated that in normal development tissue antigens recognized as 'self' are 'tolerated' as compared with the prompt and amplified
response to 'non-self' antigens. For reasons discussed elsewhere (Simpson, 1978) I consider that MG and its associated 'autoimmune' diseases result from a loss of immunological tolerance.

The nature of 'tolerance' remains obscure. Mode and continuity of presentation of antigen to recognition sites are important, but so are protective mechanisms such as 'blocking' antibodies and unknown chemical substances. The relative improvement of tolerance in the remission of myasthenia in the second and third trimesters of pregnancy and for immunity from myasthenia in the majority of babies born to myasthenic mothers has been attributed to a blocking effect of placental and fetal proteins such as alpha-fetoprotein (Abramsky et al., 1979; Brenner and Abramsky, 1980).

Alternatively, fetal thymic hormone may cross the placenta to restore immunocompetence in the mother. Until the mechanisms of tolerance and recognition of self and non-self are better understood, it is impossible to account for breakdown of tolerance and development of autoimmune disease and it is profitless to review the numerous theories.

In our experience (Behan, Behan and Simpson, 1975; Simpson, Behan and Dick, 1976) there has been impairment of T-cell function in MG, relatively high incidence of decreased plasma IgA, an increased incidence of autoimmune diseases associated with anergy (Simpson, 1964), all pointing to an immunodeficiency state. (Preferable terms may be immune deviation or aberrant immunity (Hobbs, 1968) since antibody production is high and response to challenge with bacterial antigens is normal.) Dawkins et al. (1976) have also suggested that patients with MG may be deficient in humoral immune competence.

Despite an apparent paradox, it is increasingly recognized that autoimmunity is correlated with immunological deficiency states and anergy (Fudenberg, 1968). T cells may have two functions—a co-operative function with B cells increasing antibody formation against many antigens, and a feedback control function which limits the formation of certain antibodies (Allison, Denman and Barnes, 1971). Thus T-cell incompetence could be associated with high antibody titers. A feedback control system including IgA has been reported so that IgA deficiency may encourage autoimmune manifestations (Good and Rodey, 1970). A suggested schema is that T-cell cooperation with B cells is required for formation of antibodies against self-constituents but that T cells are normally made unresponsive. Secondly, Allison, Denman and Barnes (1971) suggest that T cells may also be able to exert an inhibitory function (using feedback control) and that this provides a surveillance mechanism against aberrant immune reactions. A consequence of this theory would be that deficiency of T-cell function could lead to hypogammaglobulinemia, but also to defective surveillance. The inhibiting function could be (though not necessarily) a property of a separate type of T cell (suppressor) and this model is favored at present. The model fits well with the evidence for increasing autoimmunity and decreasing surveillance of tumor
cells in states of immune incompetence such as ageing and lymphomatous diseases. No doubt thymic hormones play an important role (Goldstein et al., 1976).

Viruses or drugs (e.g. penicillamine) could act as tolerance breakers and induce autoimmune disease. The possibility of viral precipitance of autoimmunity has been considered for many years (Simpson, 1960) but no association with particular viral antigens has yet been found (Smith, Hammarstrom and Berg, 1978). Tindall et al. (1978) reported evidence of infection with cytomegalovirus but this is as likely to be a consequence of immunodeficiency as it is a cause.

Presumably some imbalance of T cells or of thymic hormones is responsible for immunodeficiency associated with thymic disease. In the present state of knowledge one cannot suggest how removal of the gland would correct the deficiency, in a time scale of months. Apparently the body is better off without a thymus than with a malfunctioning gland: the same is true in ulcerative colitis (Oda et al., 1975).

An attractive solution could be that a continuing source of antigenic stimulus exists in the myasthenic thymus. Marshall and White (1961) showed that injection of bacterial antigen into guinea-pig thymus provoked hyperplasia of the gland with formation of germinal centres. Their suggestion that the human thymus possesses a barrier to circulating antigen has influenced many later workers to look for an antigenic source within the thymus. There has been surprisingly little interest in factors which may lower the barrier. In addition to lymphocytes the thymus of many young animals including man contains myoid cells and these were proposed as a source of muscle antigen at a time when the only recognized anti-muscle antibody was the antimyosin (striational) antibody (Van de Velde and Friedman, 1970). With the recognition of anti-AChR antibodies it is important to ascertain whether these primitive cells also have nicotinic AChRs. At the time of writing, this is controversial.

Some authors claim to have identified AChR antigen in thymic extracts (Aharonov et al., 1975b; Lindstrom et al., 1976b) and to have demonstrated surface AChRs on cultured thymic myoid cells (Kao and Drachman, 1977); others have not been convinced that AChRs were present (reviewed by Vincent et al., 1979). The latter authors point out that most of the data could be explained if there were a class of receptors which do not bind z-BGT but are antigenically similar to skeletal muscle receptors. Demonstration of z-BGT binding in the thymus is not adequate evidence for muscle-type receptors since preliminary evidence suggests that there may be acetylcholine binding sites on membranes of mononuclear cells in the peripheral blood of untreated myasthenic patients and not in control subjects (Morrell, 1979).

Clearly the possibility of antigenic stimulation within the thymus is an attractive model for MG. One major objection, the problem of accounting for development of myasthenia many years after surgical removal of a thymoma, may be less important if the recent report is confirmed that anti-AChR...
antibody may be found in serum of patients operated on for a thymoma, without current MG symptoms (Cucnoud et al., 1980). A more fundamental difficulty is that ad hoc explanations would be necessary for the many other antibodies associated with MG unless the primitive stem cells which can differentiate to myoid cells are pluripotent, as suggested by Wekerle and Ketelsen (1977). There is, at present, no satisfactory evidence for production within the thymus of the wide range of antigens necessary to account for the antibodies found in the serum of myasthenic patients. A more plausible model would be that immunosurveillance is defective because of some interference with whatever mechanism is normally responsible for differentiating self from non-self (as in NZB mice), a distinction which is only gradually improved by removal of the thymus.

1.5 GENETIC FACTORS

For many years, the occurrence of familial cases of MG has suggested the possibility of a genetic factor (Rothbart, 1937; Simpson, 1960; Herrmann, 1966; Kott and Bornstein, 1969). Twin studies have reported one monozygotic (Simpson, 1965) or both of monozygotic and dizygotic twins affected (Hokkanen, 1969; Namba et al., 1971). There seems no doubt that familial occurrence of true MG has occurred sufficiently often to require acknowledgement. Since Levin (1949) it has been tentatively agreed that 'congenital myasthenia' is a different disorder and it is now clear that it is not associated with circulating anti-AChR antibody (Vincent and Newsom-Davis, 1979b).

This must lead to a reassessment of all familial cases. Jacob, Clack and Emery (1968) pointed out that the age distribution of familial cases is significantly different from that of non-familial cases, the familial form having an earlier age of onset. The study of Bundy (1972) suggested that familial cases presenting under the age of 2 years had a male excess and probable autosomal-recessive inheritance, whereas those with onset in later childhood and adolescence were more like sporadic myasthenia in sex distribution and relationship with other autoimmune diseases. The latter group had no certain genetic mechanism but there was some evidence to support the suggestion of Simpson (1960, 1968) that MG may be one of several alternative (autoimmune) expressions of a genetic predisposition. Alternative gene expression is also shown by the Finnish series of Pirskanen (1977). All genetic studies indicate that there is polygenic or multifactorial inheritance, or that an additional environmental factor is necessary. In 1972 Pirskanen, Titikainen and Hokkanen found that one of the human leukocyte antigens HL-A1, B8 (formerly HL-A8) was significantly more frequent in myasthenics, especially in women with onset of myasthenia below the age of 30 years. This finding has been confirmed by many others (see review by Pirskanen, 1976) but many patients in this group do not have the serologically defined HLA-B8 haplotype; in some familial cases HL-B8 may be present in some and not in other affected members (Dick et al., 1974; Pirskanen,
30 Myasthenia Gravis

1976). Homozygosity for B8 does not increase the chance of developing MG (Dick et al., 1974). Clearly the relationship with HL-B8 antigen is not a direct one.

Pirskanen and her colleagues have looked for some specific lymphocyte defined (LD) gene with a strong positive disequilibrium linkage with HL-B8, but one of these, LD-8a which may include LDm (now called HLA-Dw3) occurred only a little more often in myasthenics than in control subjects (Kaakinen, Pirskanen and Tiilikainen, 1973). The lymphocyte defined antigens occur at a gene locus in the human 6th chromosome corresponding to the Ir loci in experimental animals, which control immunological responsiveness. The HL-B8 and linked genes are associated with several other diseases, all of which seem to have immunological characteristics (Svejgaard et al., 1975).

In Japanese myasthenics there is an increased association of HLA-B12, and the linked HLA-A10, especially in young females with early-onset disease and thymic hyperplasia (Yoshida et al., 1977). In the Japanese study there was a high frequency of HLA-B5 in myasthenic patients with thymoma, an antigen associated with other autoimmune diseases in Japanese (Ono et al., 1975). The importance of HLA type in patients with thymoma is not well established. In Caucasian populations, increase of HLA-A2 or HLA-A3 has been reported in these cases (Feltkamp et al., 1974; Fritze et al., 1974) but not confirmed by Pirskanen (1976), who noted an 'almost significant increase' of W10 antigen.

In the animal model (EAMG) susceptibility to the disease correlates with the H-2 haplotype possessed by the strain of inbred mice (Fuchs et al., 1976) and by some congenic strains of mice (i.e. those differing genetically only at the H-2 locus) (Christadoss, Lennon and David, 1979) and rat (P. O. Behan and A. I. Weir, personal communication). The HLA associations with MG are reviewed by Behan (1980).

It is not appropriate in this chapter to review the possible mechanisms of HLA haplotype influence on disease. The HLA-B8 linked genes are probably 'markers' for a currently unidentified immunoreactive (Ir) gene, on the sixth chromosome in the human, its presence predisposing to autoimmune disease but not specifying the latter. The fact that autoimmunity is limited to a number of self-antigens and never generalized indicates that there is no failure of a generalized tolerance-inducing mechanism. I have elsewhere drawn attention to flare up of a number of autoimmune diseases after thymectomy for MG as indicating that there can be no loss of generalized suppressor function in that disease (Simpson, Behan and Dick, 1976). On the other hand, the overlap of autoimmune diseases argues against forbidden-clone or induction hypotheses. The possible role of immunoglobulin V genes requires evaluation.

1.6 SUMMARY

For the present it appears that some aspect of immunological tolerance is broken by whatever is the primary stimulus for MG, most easily in those with a
Current Concepts and History

hereditary predisposition, for which the HLA-B8 antigen and female sex are markers. Failing this, tolerance is broken when a thymoma develops, regardless of HLA status, or when certain substances such as penicillamine are taken. The thymus is apparently an essential intermediary during the active first stage of the disease but not later. The defect is manifested by immunodeficiency of lymphocyte function and sometimes of IgA. In these circumstances autoantibodies are produced which in many, but not all, cases damage cells containing their specific antigens. Anti-AChR antibodies may require complement activation to become pathogenic.

REFERENCES


Lennon, V. A., Seybold, M. E., Lindstrom, J. M. et al. (1978) Role of complement in the
Nastuk, W. L., Strauss, A. J. L. and Osserman, K. E. (1959) Search for a neuromuscular
Current Concepts and History

37


Schumacher and Roth (1913) Thymektomie bei einen Fall von Morbus Basedowii mit Myasthenia. Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 25, 746–63.


Myasthenia Gravis

EDITED BY

E. X. Albuquerque and A. T. Eldefrawi

Department of Pharmacology and Experimental Therapeutics,
University of Maryland School of Medicine,
Baltimore, Maryland, USA

London    New York
CHAPMAN AND HALL
NEONATAL MYASTHENIA GRAVIS

John A Simpson
Glasgow University Department of Neurology, Institute of Neurological Sciences.

Neonatal Myasthenia Gravis is a neuromuscular disorder occasionally and transiently present in a baby born to a woman with myasthenia gravis. It is to be distinguished from Familial Myasthenia Gravis (the rare association in siblings of adult-type myasthenia gravis inherited from either parent) and from Congenital Myasthenia. The latter is a group of disorders of neuromuscular transmission due to one of several congenital abnormalities of the presynaptic or postsynaptic mechanism, present in the fetus and remaining through life. The mother is never myasthenic.

Neonatal myasthenia affects the offspring of myasthenic women about once in seven live births, in my experience. (Spontaneous abortion is more common in pregnant myasthenics.) It was first recognised by Strickroot et al (1942) and confirmed by Wilson and Stoner (1944) who reported two cases. Further reports soon followed. In 1960 Greer and Schotland were able to summarise 20 cases in the literature and Millichap and Dodge (1960) added 10 further examples. By 1964 I had recorded obstetrical histories from 59 myasthenic women with 81 known pregnancies leading to 70 live births. Of these, 64 babies were normal (Simpson, 1964). There were three definitely myasthenic babies and another three in which the baby was weak at birth but recovered without specific treatment. An affected child or a normal child is not a marker for the result of the next pregnancy.

In the last twenty years I have recorded similar histories retrospectively but the weakness has always been so limited in duration that I have only twice had the opportunity to follow the progress of the baby and I have not found the diagnosis easy because of the difference from adult myasthenia. Further cases reported by Namba et al (1970) and Fenichel (1978) have consolidated the clinical picture.

Where the fact is mentioned, there appears to be general agreement that fetal movements are normal before birth but the neonate is very weak immediately or within a few hours and, if untreated, is likely to die from respiratory failure. Sucking is weak and the mouth tends to remain open. There is general hypotonia. Ptosis is common but, as Levin (1949) pointed out, there is rarely external ophthalmoplegia. This distribution is different from the average case of adult myasthenia gravis in which ophthalmoplegia is common and early and respiratory weakness a late manifestation.
The exact duration of the illness is a little uncertain as the baby has usually died within a few hours or has been maintained on neostigmine for several weeks, but from the vague accounts in the reported cases it appears to be more than one week but probably less than twelve weeks. Recovery then takes place and no recurrence has been reported except for the possible case described by Osserman (1958). His patient, the child of a myasthenic mother, had difficulty swallowing and suckling after birth. This persisted for "several weeks" and then disappeared without specific medication. Symptoms of muscular weakness reappeared at two years of age.

The diagnosis of neonatal myasthenia is straightforward if power is restored to normal by an anticholinesterase drug. Recommended test doses for intramuscular injection are 1mg of edrophonium or 0.1mg of neostigmine, and for treatment 4-10mg of pyridostigmine or 1-2mg of neostigmine by nasogastric tube, repeated every eight hours. In my very limited experience the response has been disappointing and not comparable with that of adult myasthenia, to the extent that I had some scepticism about the diagnosis until recovery took place within the predicted period. (Circumstances did not permit electrodiagnostic studies.)

The transient nature of neonatal myasthenia is best accounted for by temporary presence in the infant of a noxious substance which has passed through the placenta from the mother. The rarity of this event makes it unlikely that an anticholinesterase drug passing from mother to child prevents normal endplate maturation. One of my patients had a myasthenic baby though she was not taking any drugs during the pregnancy.

It was formerly postulated that myasthenia gravis was caused by a circulating curare-like substance, possibly produced in the thymus, and that this might pass through the placenta from mother to child. A molecule resembling D-tubocurarine would soon be eliminated by the fetus and could not possibly account for a competitive neuromuscular block persisting for up to 12 weeks. Simpson (1960, 1964) pointed out that an antibody could survive for so long, drawing an analogy with rhesus antibodies. This, with the first recognition of multiple organ abnormalities and genetically linked disorders, was the clue to the autoimmune hypothesis of myasthenia gravis postulating a thymus-regulated production of antibody against the nicotinic acetylcholine receptors of skeletal muscle endplates (Simpson, 1960), a hypothesis now well validated. The original concept of a 'lock and key' occupation of receptors by antibody has not been fully confirmed and it seems that the major lesion is lysis of receptor structures by a complement-mediated antibody (Engel et al, 1977). Antibody is produced by B-lymphocytes because of defective immunoregulatory function, later shown to be controlled by the thymus. The exact nature of the thymic disorder in autoimmune disease is still uncertain and there is no adequate explanation
for the fact that thymectomy is beneficial if performed during the first 5-7 years of the illness (Simpson, 1958).

The titre of antireceptor antibody drops slowly in the two years following thymectomy but the explanation for the clinical benefit is not immediately apparent. Occurrence of neonatal myasthenia is not related to the severity of the disease in the mother. It does not occur with every pregnancy, even consecutive. Removal of the mother's thymus before or during pregnancy does not prevent the baby from having neonatal myasthenia (Levin, 1949; Nilsby, 1949; Geddes and Kidd, 1951). Olanow et al (1981) report neonatal myasthenia in the infant born to a thymectomised mother who had been without myasthenic symptoms for seven years. One of my patients had thymectomy three years before becoming pregnant. She was off all medication. The baby had transient neonatal myasthenia. Six years later the mother aborted another pregnancy and her myasthenia then relapsed (Simpson, 1964). It must be concluded that a certain antibody titre may harm the mother's endplates and vice versa. The lack of correlation between antibody levels and clinical effect in adult myasthenia is commonly discussed in terms of specificity of sub-classes of antibody. This argument could not account for these differences between mother and child, and the differences must be independent of ability to produce appropriate antibody.

Protective factors Two related problems immediately suggest themselves. Why does only a minority of babies at risk manifest neonatal myasthenia? Why does it not apparently affect fetal movements in utero?

The original theory was formulated at a time when myasthenia gravis was thought to be due to a "myasthenic toxin" and the thymus was classified as an endocrine gland. The seminal paper by Miller (1961) on the immunological role of the thymus had not been published. There had been many negative trials of human-human transmission by blood transfusion and of human to animal transfer without success. Simpson (1960) suggested that successful passive transfer might require tissue compatibility between donor and recipient, as in some other mother-child relationships. Many years later it was discovered that myasthenia gravis (except a group associated with thymic tumours) is closely linked with certain HLA antigens, B8 and DRW3 in Caucasians (Firskanen et al, 1972; Behan et al, 1973). In the rare familial type of myasthenia gravis we found that the association with HLA-B8 antigen is not a direct one nor a prerequisite for the disease (Dick et al, 1974). Of two identical twin sisters homozygous for HLA-B8 only one had the disease (Simpson, 1965; Dick et al 1974). It would be important to know the mother-child linkages in maternal and neonatal myasthenia but no such study has been reported.

In the last 10 years an experimental model of the disease has been produced
by sensitising laboratory animals with material rich in acetylcholine receptor and it has proved possible to transfer the myasthenic activity to recipient animals by serum from the inoculated animals or human patients (Toyka et al., 1975), usually apparent by reduced amplitude of spontaneous miniature endplate potentials recorded with a microelectrode, rarely by clinical disease in the recipient animal. We were unable to replicate these experiments (Rees et al., 1977) and considered the possibility that strain differences, equivalent to human HLA differences, might account for this. Studies on congenic strains of mice (genetically identical except for the H-2 locus, equivalent to the HLA gene locus of man) have demonstrated that susceptibility to induction of experimental myasthenia by inoculation of receptor antigen is controlled by genes at the Ir region of the appropriate chromosomes which code for molecules restricting immune recognition and the generation of T-cell helper and suppressor responses, but these congenic strains did not differ in their susceptibility to passive transfer using hybridoma monoclonal antibodies (Christadoss et al., 1979).

Presumably the conclusion that the major histocompatibility complex modifies the production of antibody and not the susceptibility of the endplate receptors also applies to the human disease, but the presence of a particular histocompatibility receptor on the membrane close to the acetylcholine receptor macromolecule can not be excluded from consideration. In similar experiments Herman et al. (1982) also showed H-2 locus control of antireceptor antibody levels, but no strain of mice failed to produce antibody and the occurrence of muscular weakness and paralysis did not depend on the antibody titre (not sub-classified). A similar lack of correlation between antibody titre and clinical weakness is found in human myasthenia gravis both adult and neonatal. Indeed personal experience in three cases shows that antibody is always detectable in cord blood if the mother is myasthenic, even if the child is apparently not affected. It is, therefore, necessary to examine the possible factors influencing the susceptibility of the endplate receptors, the hypothesis being that fetal endplates are 'protected' in the uterus but in some 12% of cases that protection fails after birth until maternal antireceptor antibody is degraded or excreted. Some possible mechanisms are listed in Table 1.

Acetylcholine released from the motor nerve terminal by a nerve action potential, in an appropriate environment of calcium and other divalent ions, combines with acetylcholine receptors (AChR) on certain parts of the muscle sarcolemmal membrane with a specialised folded structure and there evokes an endplate current by opening an ion-exchange channel in each AChR macromolecule. The total current through the simultaneously activated channels is normally in excess of
that required to produce a regenerative spike potential of the muscle membrane, the activator of the twitch mechanism. The excess current constitutes a "safety factor" for neuromuscular transmission. In myasthenia gravis the safety factor is reduced or abolished by antibody action on antigenic sites on or near the AChR structure.

The original postulate of an "immunopharmacological blockade" is still uncertain despite some evidence for its existence. The present evidence points to the important mechanisms being lysis and internalisation of receptors by a complement mediated antibody-antigen action which results in simplification of the folded subsynaptic membrane and loss and separation of receptors (Engel et al, 1977). Another mechanism, favoured by Drachmann (1981), is a permanent conformational change of the receptor structure by a process known as antigenic modulation. The deformed receptors are more rapidly degraded by the normal process of internalisation into the muscle cell. These disorders are countered by insertion into the subsynaptic membrane of new receptor structures which are synthesized by the Golgi apparatus underlying the endplate. Insertion and degradation are normal processes in substantial balance. Disturbance of this balance would modify the safety factor in one direction or the other. It is conceivable that insertion of receptors into the membrane could be dominant in the fetus.

Before the muscle cell is innervated the receptors are distributed all over its membrane. When a motor nerve terminal establishes contact, an endplate structure is induced and receptors become concentrated in the postsynaptic area of the muscle membrane. Extrajunctional receptors do not reappear unless the flow of acetylcholine from nerve to muscle is interrupted for more than a certain time, usually by denervation. To form an efficient synapse, the receptors must be clustered at a critical separation distance. The safety factor depends not only on the number of receptors but on their orientation (and of course on the release of sufficient acetylcholine from the terminal knob). At least two molecules of acetylcholine must bind to each receptor monomer and two receptors should be cross linked to promote the conformational changes which open and close the ion conductance pathway. The duration of occupation of receptor sites depends on diffusion of transmitter and on its hydrolysis by acetylcholinesterase. The latter may be inhibited by anticholinesterase drugs, with benefit to the myasthenic, but the diffusion mechanism is highly important and, of course, not amenable to chemotherapy. Clearly, it is important to know if the fetal muscle has a normal safety factor for transmission, whether its structure encourages retention of acetylcholine, and whether the insertion of receptor protein into the postjunctional membrane is efficient or not.
Neuromuscular transmission in the neonate. Anaesthetists have sometimes noticed that newborn infants tolerate relatively large doses of depolarizing relaxants and yet are very sensitive to the non-depolarizing ('competitive') type. In this respect the muscle of the newborn infant resembles that of myasthenia gravis in adults. A drug which cause depolarization block in the adult causes a competitive type of block in the newborn (Churchill-Davidson and Wise, 1963). Thus the neonate has a low safety factor. A suggestion by Keynes (1954) that the thymus might produce a 'myasthenic' substance to inhibit fetal movement cannot be taken seriously and the low safety factor is probably due to disproportion between presynaptic and postsynaptic structures (Diamond and Miledi, 1962) and the low gating time of immature receptors. It is necessary to elaborate on this because the low safety factor ought to make the fetus more rather than less sensitive to passively transferred antibody unless insertion of receptors is so vigorous as to counterbalance those destroyed by lysis or degradation.

The turnover rate of newly formed receptors is certainly fast in the developing neuromuscular junction (Burden, 1977) but I am not aware of any evidence that it is superior to the mature junction, and the half life of embryonic receptors is about 10% of that of the adult. Synthesis of receptors is an energy-dependent process which is stimulated by cyclic adenosine monophosphate. In summary, the immature neuromuscular junction of the fetus should, if anything, be more likely than that of the adult to have its safety factor critically reduced by anti-receptor substances. A possibility that can not be excluded is that fetal AChR is in some way sequestered from the circulating antibody, but it is more likely that the relative protection before birth is by prevention of immunological attack.

Immunosuppression in the fetus. It is now commonplace to regard the fetus as a single haplotype mismatched transplant on the mother, with the implication that it would be rejected by a host-v-graft reaction if this were not suppressed. The mother's contribution to this will be considered later. Four fetal components should be considered. (i) Thymic hormones controlling T-cell subsets in the fetus to induce tolerance to self proteins might be supposed to be particularly active in the embryo and to have the capacity to pass into the mother's circulation. (ii) Anti-idiotype antibodies may protect the fetus against passively transferred maternal antibodies. This would imply some procedure for recognizing tissue-destructive antibodies from the beneficial transfusion of protective immunoglobulins on which the neonate depends until able to develop its own protection. (iii) If the major myasthenogenic process is the complement mediated lysis of receptors rather than degradation, the low plasma complement of the fetus (about half of the adult level) would minimise destruction of receptors until after
birth and the liver increases its production of complement. (iv) Fetal-derived immunosuppression of the mother's lymphocytes is well authenticated and may be a factor in reducing the severity of the mother's myasthenia gravis which commonly occurs in the third trimester (Viets et al, 1942; Simpson, 1964a). To be an effective inhibitor of humoral antibody originating transplacentally it would be necessary for the fetus to cause significant suppression of maternal lymphocytes not only locally in the placenta but at the site where they develop immunocompetence, wherever that may be in the thymectomised myasthenic. Diffusible factors from fetal lymphoid tissue, lymphokines, are unlikely to be adequate but should be examined. Significant amounts of alpha-fetoprotein, produced in the fetal liver and yolk sac, are transferred from fetal to maternal serum and into the amniotic fluid. This glycoprotein is strongly immunosuppressive and is probably only one of a number of immunoregulatory substances. One of its major attractions in the context of transfer of immunological disease from mother to fetus is that it is produced from a very early stage of embryogenesis (first trimester) before lymphoid tissues have developed, giving the fetus early protection, but the concentration in maternal serum is low (under 10mg/ml) until the second trimester.

Although I consider that the low serum complement factors may be very important in protecting the fetus against maternal antireceptor antibody, there is no satisfactory evidence of their role. Abramsky et al (1979) suggested that alpha-fetoprotein inhibits binding of antibodies to AChR of both mother and fetus (Brenner et al, 1980, 1981; Donaldsen et al, 1981). The postulated effect on antibody-antigen binding is not accepted by some other workers. Certainly most of the evidence on immunosuppression by alpha-fetoprotein concerns a primary effect on antibody production.

Most obstetricians and paediatricians will rarely encounter neonatal myasthenia gravis and neurologists are not often present when their myasthenic patients deliver. It is hoped that this review will assist our colleagues to recognize and treat the affected babies which have an excellent prognosis, and at the same time encourage them to supply their immunology laboratories with appropriate maternal and cord blood, and possibly placental tissue, to solve a problem with significance for other types of immunological disease in infancy.


BRENNER T, BEYTH Y, ABRAMSKY O. 1980 Inhibitory effect of α-fetoprotein on the binding of myasthenia gravis antibody to acetylcholine receptor. Proceedings of the National Academy of Science, USA, 77, 3635-3639.


BURDEN SJ. 1977 Acetylcholine receptors at the neuromuscular junction: developmental change in receptor turnover. Developmental Biology, 61, 79-85.

CHRISTADOSS P, LENNON VA, DAVID CS. 1979 Genetic control of experimental autoimmune myasthenia gravis in mice I T-lymphocyte proliferative response to acetylcholine receptors is under H-2 linked Ir gene control. Journal of Immunology, 123, 2540-2543.


GEDDES AK, KIDD HM. 1951 Myasthenia gravis of the newborn. Canadian Medical Association Journal, 64, 152.


REJS D, BEHAN PO, BEHAN WH, SIMPSON JA. 1977 Myasthenia gravis: passive transfer from man to mouse. 7th Symposium on Current Research in Muscular Dystrophy, Birmingham, Abstract 16.


SIMPSON JA. 1964a Myasthenia Gravis. MD Thesis, University of Glasgow.


Myasthenia gravis is an autoimmune disease in which, owing to defective thymic control of lymphocytes, antibodies are produced against a number of tissues including the nicotinic receptor sites for acetylcholine. With the collaboration of complement, one or more of these antireceptor antibodies cause lysis or internalisation of the receptors at the endplates of skeletal muscle, with secondary distortion of the synaptic structure. The combined effect is to reduce the number of receptors available to the acetylcholine (ACh) released from the nerve terminal in response to a stimulus. Most of the packets of acetylcholine released are destroyed by the enzyme acetylcholinesterase (AChE) before they can link with a receptor and this lowers the safety factor for transmission which is normally provided by a surplus of ACh and receptors. The output of ACh from a train of nerve impulses normally decrements, but the safety factor is sufficient to provide enough transmitter-receptor linkages to open ion channels and cause the endplate membrane to depolarise to the extent required to trigger the action potential of its muscle fibre. When the safety factor is lost, the decrementing output causes more and more endplate potentials to become inadequate to trigger their muscle fibres and a myasthenic 'fatigue' results.

The safety factor can be improved to a limited extent by prolonging the life of ACh in the synaptic cleft. This is the role of anticholinesterase drugs such as neostigmine but full restoration of power will not occur if too many receptors are destroyed. This implies that optimum treatment may fail to restore full power.

The situation is not improved by further reduction of AChE, since prolonged occupation of receptors by ACh causes first prolonged depolarisation of the endplates and then desensitisation so that the receptors are blocked against further molecules of ACh. This depolarisation-densitisation block is the basis of the 'cholinergic state' which results from overdosage with anticholinesterase drugs. It is commonly caused by giving increasing dosage in an attempt to restore full
power when the limit has already been reached. The danger is particularly marked when using long-acting anticholinesterase drugs such as distigmine which have a cumulative action, since the overdose may not become apparent until the patient has left hospital. The other circumstance leading to overdosage is a temporary recovery of the safety factor with requirement for rapid reduction of dosage. This commonly happens, for unknown reasons, in the first two days after thymectomy.

I am convinced that unrecognised cholinergic state is the most common cause of death in myasthenics treated by the inexperienced. The most important emergency problems are the differential diagnosis between myasthenic crisis and cholinergic crisis, and other complications resulting from treatment.

**Myasthenic crisis**

This term is applied to rapidly deteriorating or end-stage weakness of skeletal muscles due to uncontrolled myasthenia gravis with severe loss of safety factor for neuromuscular transmission. For practical purposes this obsolete term refers to paralysis of respiration or swallowing, with actual or incipient ventilatory failure, a state easily identified at the bedside without special instrumentation or blood gas analysis. In the previously unidentified case there is no problem other than to recognise myasthenia gravis as one of the most common causes of 'bulbar paralysis'. In the patient under treatment there are three possibilities:

1. Anticholinesterase dosage is insufficient, usually being given too infrequently. Some patients require pyridostigmine only three times daily. Most require dosage at intervals of four hours or less. (For the method of establishing individual dosage schedules cf Simpson [1].)
2. Receptor destruction is increasing or neuromuscular demand is greater. This may be associated with any non-specific infection or (for reasons not understood) with emotional disturbance.
3. Concomitant administration of drugs which lower the safety factor at endplates or which add to respiratory embarrassment. These are listed in Table I and further discussed elsewhere [2].

Treatment requires:

1. Controlled ventilation by cuffed endotracheal tube, using a positive pressure respirator if necessary.
2. Nasogastric intubation for food, fluid and drug administration until adequate swallowing is restored.
3. Adjustment of dosage of anticholinesterase medication, giving crushed tablets by nasogastric tube. Parenteral administration is rarely needed, much more difficult to assess, and can be dangerous. Neostigmine should never be given intravenously.
TABLE I. Drugs which aggravate myasthenic weakness

1. Inhibitors of synthesis or release of ACh
   - Streptomycin
   - Dihydrostreptomycin
   - Neomycin
   - Kanamycin
   - Viomycin
   - Bacitracin
   - Polymyxin A and B
   - Colistin
   (Any aminoglycoside is suspect, but potentiation of neuromuscular block is not confined to that type of antibiotic.)
   - Corticosteroids (in first two weeks)

2. Blockers of ACh receptors
   - D-tubocurarine
   - Suxamethonium

3. Drugs reducing muscle response to endplate current
   - Hydantoinates
   - Quinine
   - Quinidine
   - Procainamide
   - Beta-blockers

4. Drugs which potentiate the autoimmune response
   - Thyroxine
   - Penicillamine
   - Oestrogens (?)

5. Drugs potentiated by myasthenic or cholinergic states
   - Respiratory depressants
   - Enema

4. Withdraw drugs listed in Table I if possible, but with discretion. If a particular antibiotic is essential it should be continued and compensated for by the adjusted dose of anticholinesterase.

   Emergency tracheotomy is very rarely required. Modern endotracheal tubes can be tolerated for at least 10 days and most crises should be controlled within that time, otherwise elective tracheotomy is necessary.

   It is sometimes necessary to give atropine to control bronchospasm, bronchorrhoea or diarrhoea but it is best avoided if possible as it will obscure signs of
overdosage with anticholinesterase drugs. Remember that a myasthenic state may be converted to a cholinergic state without an intervening period of normal muscle strength. One must be careful not to overdose the bulbar and respiratory muscles while attempting to strengthen the limbs. If reasonable control is not achieved when dosage has reached 20 tablets daily of neostigmine (15mg) or pyridostigmine (60mg) it is unlikely that further increments will be effective: treatment should then be concentrated on reducing the immunological attack on endplates by means of plasma exchange, with or without steroids or immunosuppressant drugs, if this can be done without compromise to the respiratory control.

An all too common situation, based on the belief that full power can always be restored by giving sufficient anticholinesterase medication, is that the patient struggling for breath or choking on saliva is injected with more and more neostigmine. Without taking account of the pharmacokinetics of previous oral and parenteral dosage, and in the absence of a readily available method for rapid estimation of blood levels, it is usual for toxic levels to be reached quite rapidly, converting the myasthenic state to a cholinergic one with depolarised or insensitive endplates. The same situation is reached more slowly by progressively increasing oral dosage of any anticholinesterase, but especially the long-acting ones which tend to be slowly cumulative. This risk is increased if there is renal insufficiency.

Cholinergic state

In former times the failure to improve with increasing dosage of neostigmine or pyridostigmine was attributed to 'neostigmine resistance', and some advocated a 'drug holiday' with ventilatory support. The cholinergic state, or depolarisation block, of receptor-deficient endplates cannot be recognised clinically and the electrophysiological methods need special expertise. The clue to the situation is the evidence for overstimulation of those neuromuscular junctions which have a relatively normal population of ACh receptors and of the constrictor muscle of the pupil which is not normally affected by antibody attack. The relatively spared skeletal muscles (e.g. distal lower limb muscles) show fasciculation of a coarse irregular type, and the pupil is constricted. It is a simple matter to measure the pupil diameter. If it is less than 3mm in normal room lighting there is no doubt that inhibition of cholinesterase has already passed the safety margin and any further increase will merely aggravate the cholinergic crisis and endanger the heart. (Although the effect is vagotonic the pulse rate is usually rapid and not slow as would be expected.) Bronchoconstriction, bronchorrhoea and hypersalivation further embarrass respiration and the increasing respiratory effort leads to a vicious circle situation which is likely to terminate in death if appropriate steps are delayed.

The warning signs in the pupils and bronchi are obscured if the other, less dangerous, signs of overstimulation of muscarinic ACh receptors (colic, diarrhoea,
sweating, salivation) have been treated with atropine. It should never be used until the situation has been fully assessed with a view to detecting cholinergic block of the nicotinic ACh receptors of skeletal muscle. Even this is not straightforward. Since endplate damage varies in degree in different muscles, it is possible for some muscles to be overstimulated (like the fasciculation described above) while others are still underdosed.

Fasciculation and small pupils are the warning signs that the cholinergic state is imminent in non-affected junctions. Attention must then be directed to the vital muscles of respiration and deglutition. The next clue is the time of onset of the respiratory crisis with respect to the last dose of an anticholinesterase drug. That is why it is advisable to give dosage ‘by the clock’ rather than on patient demand or doctor impulse. A well-managed patient should be having medication at intervals previously established by formal trial [1].

If muscular power has become weaker more than one hour after the last oral dose and temporarily improved by the next dose, it is highly probable that more drug is required. If respiration is secured by passive ventilation, there is no urgency. First shorten the dosage schedule to gaps of three hours then, if necessary increase alternate dosage by half tablet increments (i.e. 30mg of pyridostigmine, the preferred anticholinesterase). Wait for an adequate period (not less than nine hours) and then raise each dose to the new level. Continue in this way, gradually increasing until each dose is three tablets (180mg pyridostigmine) or until the pupils become smaller than 3mm. If this regime (three tablets three-hourly) is insufficient, nothing will be gained by further increments. It is now necessary to prepare for longer term ventilation and wait for spontaneous remission, or proceed to plasma exchange or steroid therapy.

The patient just described in myasthenic crisis was temporarily better in the first hour after a dose of anticholinesterase drug. If, on the other hand, the regularly dosed patient is weaker during the first hour and then improves a little and his pupils are constricted, it is highly probable that he is in a cholinergic state. Once again there is no need for panic reaction once he has been intubated. Stop all anticholinesterase drugs and wait for recovery of power. Give i.v. atropine 2mg every two hours until weakness is obviously myasthenic again or evidence of atropine toxicity develops.

If, on the contrary, after two doses have been omitted there is no improvement (preferably monitored by a Wright’s respirometer or similar device) the endplate status should now be assessed by injecting a short acting anticholinesterase, edrophonium (Tension). The question to be answered is ‘will extra inhibition of acetylcholine hydrolysis improve the patient (underdosed) or not?’ Further deterioration or fasciculation obviously indicates overdose (cholinergic state). A null response must always be interpreted in the same way. It is therefore extremely important to carry out the test properly and at the right time. (With experience, the endplate status can be assessed from time to time while the oral dosage is being increased. In this circumstance the best time is about one hour after a dose, when peak blood levels may be anticipated).
The edrophonium test

This test is simple and safe when used to confirm a diagnosis of myasthenia gravis in an untreated patient. When used to differentiate between myasthenic and cholinergic crises it is dangerous and may be the 'last straw' to precipitate severe respiratory paralysis or cardiac arrest. Resuscitation equipment must be at the ready. When the patient has already taken pyridostigmine or neostigmine, by whatever route, the injection of edrophonium should be preceded by intravenous injection of atropine sulphate 0.6mg some 10–15 minutes previously, or in emergency this should be added to the syringe containing edrophonium.

First measure the time-to-fatigue of a muscle group known to be myasthenic, e.g. the outstretched hands, width of palpebral fissures on upward gaze, and, if possible, some measure of ventilatory function. Then inject 2mg edrophonium intravenously, retaining another 8mg in the syringe. Wait for 30 seconds and reassess the time-to-fatigue tests. If they improve or deteriorate the answer is clear (underdosed or overdosed) and no further injection is required. Fasciculation where none existed before (often periorbital) should be interpreted as a sign of maximal dosage. If there is no detectable change, slowly inject the remaining 8mg and repeat the observations. A small response or definite deterioration must be interpreted as evidence of overdosage. Never accept the patient's statement that he has benefited from the injection. Objective evidence is absolutely essential. Do not repeat the test within an hour. Although the major effect passes in a few minutes, some residual action persists.

Simulation of cholinergic crisis

I have stressed the importance of muscarinic (autonomic system) signs as indications of impending overdosage. The myasthenic patient is well known to be surprisingly resistant to these 'side effects' of anticholinesterase drugs and their appearance must always be considered ominous though there is certainly individual susceptibility. It is therefore important to recognise some common causes of hypersalivation, colic, diarrhoea, sweating, tachycardia and constricted pupils in the treated myasthenic. They may be due to: (i) drugs; (ii) hypoxia.

Hypersalivation

Hypersalivation with bronchorrhoea, when caused by anticholinesterase drugs is an excess of thin watery secretion. In true myasthenic crises, with severe dysphagia, normal saliva is not swallowed properly and a thick glairy, mucoid, secretion is often induced reflexly. Note that in the latter situation atropine makes matters worse and leads to bronchiolar plugging.
Sudden respiratory distress

Sudden respiratory distress in mild myasthenia usually indicates inhalation of food or a foreign body.

Colic and diarrhoea

Many physicians give supplements of potassium in myasthenia gravis. It is sometimes necessary during steroid therapy but is otherwise useless and commonly causes colic and diarrhoea. The pupils are not constricted. Intestinal hurry is also common with intragastric feeding with proprietary fluid diets, and antibiotic diarrhoea is well-known. The myasthenic patient is immunodeficient and often treated by immunosuppressants. Fungal oesophagitis and enteritis occur after prolonged intubation.

Sweating, tachycardia and constricted pupils

These cardinal signs of the cholinergic state also occur with severe hypoxia and may cause severe myasthenic crisis to be wrongly diagnosed as cholinergic crisis.

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Central</td>
</tr>
<tr>
<td>Air hunger</td>
</tr>
<tr>
<td>Dizziness</td>
</tr>
<tr>
<td>Confusion</td>
</tr>
<tr>
<td>Convulsions</td>
</tr>
<tr>
<td>Coma</td>
</tr>
<tr>
<td>Muscarinic</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Blood pressure</td>
</tr>
<tr>
<td>Pulse</td>
</tr>
<tr>
<td>Skin</td>
</tr>
<tr>
<td>Sweating</td>
</tr>
<tr>
<td>Salivation</td>
</tr>
<tr>
<td>Bronchorrhea</td>
</tr>
<tr>
<td>Bronchospasm</td>
</tr>
<tr>
<td>Miosis</td>
</tr>
<tr>
<td>Nicotinic or Tetanic</td>
</tr>
<tr>
<td>Twitching</td>
</tr>
<tr>
<td>Paralysis</td>
</tr>
<tr>
<td>Paraesthesia</td>
</tr>
</tbody>
</table>

202
In both states the sweating skin is usually warm and the patient is restless. Twitching may be present. Note, however, the signs of over-ventilation in the respirator patient. Some diagnostic criteria are listed in Table II.

**Syncope**

This is rarely caused by myasthenia or anticholinesterase drugs. (Remember that there is a recognised linkage between myasthenia gravis and epilepsy.) An important type, which may cause cardiac arrest is due to the use of an enema. I have not experienced this as I never use enemata in myasthenics since the risk was pointed out by Keynes [3]. The mechanism is unknown but could be due to a vagal reflex from distension of a bowel in tonic spasm due to anticholinesterases. Syncope is also a hazard of plasma exchange, not unique to myasthenics.

**Neuromuscular blockade from other drugs**

Some drugs which lower the safety factor for transmission have been listed in Table I. Only the temporary deterioration caused by steroids is likely to be significant. We have shown [4] that this is an endplate effect with lowered safety factor. Dosage of anticholinesterase drugs should be increased but it is then necessary to watch for the rebound improvement (usually in 10—14 days) as rapid reduction of pyridostigmine dosage may then be necessary. In careful hands, no emergency situation should arise.

**References**

John A. Simpson, Department of Neurology, Glasgow University and the Institute of Neurological Sciences, Southern General Hospital, Glasgow

It is a great honour to be invited to give the first Janet Reid Lecture. It is endowed for lectures on neuropsychiatric disorders and what could be more appropriate than a review of the first disease in which a disorder of synaptic transmission was fully worked out, with transformation of the prognosis for patients, for it may be a paradigm of other disorders of the central nervous system?

Following the classical descriptions by Erb, Goldflam and Jolly, myasthenia gravis (MG) became recognised at the beginning of the century. The favoured pathogenetic mechanism was a 'myasthenic toxin' acting either on the motor nerve terminals or on the endplates of muscles, though the only identified pathology was lymphorrhages in muscle. No morphological abnormality was detected at the neuromuscular junction. An association in 10-15 per cent of cases with thymomas suggested that the thymus was the source of a circulating toxin. The similarity to curare poisoning encouraged Mary Walker (1934) to treat a patient with physostigmine (an alkaloid of the Calabar bean known to be an antidote to curare) and then with a synthetic analogue, neostigmine (Walker, 1935). The wide publication of this 'wonder drug' coincided with confirmation of the role of acetylcholine in neuromuscular transmission (Dale and Feldberg, 1934) in an era when a biochemical basis for disease was the vogue. Anticholinesterase treatment with neostigmine transformed the outlook for myasthenic patients and its success was considered to establish three propositions. These were (1) that the pathogenesis must be either failure to produce or liberate sufficient acetylcholine (ACh), a pre-synaptic lesion, or competition by a curare-like substance for a supposed receptive substance on the endplate (circulating toxin), (2) that failure to reverse the transmission block by anticholinesterase drugs indicated either an insufficient dose or endplate resistance to neostigmine and (3) that pharmacologists should search for a long-acting form of neostigmine. For the next 20 years there were many reports of 'neostigmine-resistance' and of patients given up to 15,000 mg neostigmine daily, with atropine to block the muscarinic actions.

The quest for a 'long-acting neostigmine' led to the use of alkylphosphates, as a spin-off from war-time research on 'nerve gases' and a number of bis-neostigmine compounds were investigated in the 1950s. They have all been abandoned for two reasons. Either, like physostigmine, they crossed...
The striking but temporary remission in the first few days requires appropriate reduction of anticholinesterase dosage. A very strict protocol developed in Edinburgh is the basis for our very successful thymectomy programme in Glasgow (Fraser et al., 1978). ‘Surgical’ deaths have virtually disappeared; death from myasthenia is now rare and most deaths are due to associated autoimmune disease, tumour or other unrelated disease. At the same time the benefit has increased so that more than 90 per cent of non-thymoma patients had no symptoms or only mild disability after two years.

Fellows of the College who remember the Northern General Hospital, 1956–64 will agree that the striking drop in mortality cannot be attributable to availability of ventilators and intensive care units (Grob et al., 1981, and others). Ventilatory support in what used to be called a ‘myasthenic crisis’ is valuable, but tracheostomy is rarely required, and certainly not as a routine for thymectomy. The important advance was the recognition of the ‘cholinergic crisis’ and the use of edrophonium in differentiating it from myasthenic weakness (Osserman and Kaplan, 1953). Better prognosis was due to better management and avoidance of drug overdosage. Notice particularly that the major advances in treatment of a hitherto untreatable disease were based on a model of the disease which was probably wrong in every respect.

**AUTOIMMUNITY MODELS**

The survey for the thymectomy review gave me the opportunity to examine more than 400 patients or their records. This led to the formulation of a hypothesis that myasthenia gravis is an autoimmune disease, closely resembling systemic lupus erythematosus, with blockade of nicotinic receptors at muscle endplates by an antibody produced as the result of an abnormality of the thymus gland (Simpson, 1960). It was based on eight features (Table 1). Although most authors now attribute the hypothesis to recognition of associated autoimmune disorders, it must be remembered that most of the related disorders were not known to be immunological in origin at that time. The immunological role of the thymus was not established until the following year (Miller, 1961) and another autoimmune hypothesis published simultaneously (Strauss et al., 1960) had serious flaws. By the methods available at that time I was unable to isolate the proposed antibody. The theory was not taken seriously for the next 15 years until certain developments made it respectable.

The crucial finding was that a snake venom, alpha-bungarotoxin, was
the blood-brain barrier and caused headache, nightmares and personality disturbances, or they were cumulative and caused delayed overdosage with blockade of the neuromuscular junction. If this ‘cholinergic state’ was wrongly interpreted as increasing myasthenic weakness, the situation was fraught with danger, as further increments of dosage made the position worse and eventually blocked muscarinic receptors. The duration of action of pyridostigmine varied in different patients from 2–12 hours. It was safer to dose frequently than to risk cumulative poisoning.

It is my belief that many patients died from the anticholinesterase drugs and not from the disease itself. Nevertheless the availability of powerful anticholinesterase drugs altered the risk factors for the myasthenic patient. From 1934–56 there were 110 cases of myasthenia gravis treated without surgery at the National Hospital, Queen Square; 32 died from myasthenia and five from other causes. Most of the myasthenic deaths occurred two to six years after the onset of symptoms, regardless of whether they had a thymoma or not (Simpson, 1958). It was already recognised that the prognosis was worse in the presence of a thymoma, but many patients had a thymus with prominent germinal centres, often wrongly described as hypertrophied.

When Blalock (1941) introduced thymectomy, he considered that thymoma was the major indication for surgery. By 1955 three major American centres were abandoning thymectomy, whereas in London, Keynes increasingly advocated the operation. He had made the crucial observation that the best results were obtained in non-thymoma cases (Keynes, 1946, and later papers). His conclusions were amply confirmed by an independent review (Simpson, 1958) which also stressed that most benefit resulted from operation during the first five years. The age and sex of the patient were not relevant factors. In the non-tumour group the mortality was halved, even including a high peri-operative mortality. The poor prognosis for thymoma cases was largely attributable to an operative mortality of near 30 per cent.

For many years the operation was considered to carry an unacceptable risk, though this soon reached a low level in units with special experience and early thymectomy was accepted in the USA and elsewhere. My experience in Edinburgh (1956–64) managing the postoperative care (Mr Andrew Logan was the surgeon) convinced me that surgical complications were rarely responsible for postoperative deaths. Violent fluctuations in requirement for anticholinesterases are observed in the first three to four days after thymectomy. In an eight-year period, only one of 28 patients died postoperatively (from convulsions).
The striking but temporary remission in the first few days requires appropriate reduction of anticholinesterase dosage. A very strict protocol developed in Edinburgh is the basis for our very successful thymectomy programme in Glasgow (Fraser et al., 1978). ‘Surgical’ deaths have virtually disappeared; death from myasthenia is now rare and most deaths are due to associated autoimmune disease, tumour or other unrelated disease. At the same time the benefit has increased so that more than 90 per cent of non-thymoma patients had no symptoms or only mild disability after two years.

Fellows of the College who remember the Northern General Hospital, 1956–64 will agree that the striking drop in mortality cannot be attributable to availability of ventilators and intensive care units (Grob et al., 1981, and others). Ventilatory support in what used to be called a ‘myasthenic crisis’ is valuable, but tracheostomy is rarely required, and certainly not as a routine for thymectomy. The important advance was the recognition of the ‘cholinergic crisis’ and the use of edrophonium in differentiating it from myasthenic weakness (Osserman and Kaplan, 1953). Better prognosis was due to better management and avoidance of drug overdosage. Notice particularly that the major advances in treatment of a hitherto untreatable disease were based on a model of the disease which was probably wrong in every respect.

AUTOIMMUNITY MODELS
The survey for the thymectomy review gave me the opportunity to examine more than 400 patients or their records. This led to the formulation of a hypothesis that myasthenia gravis is an autoimmune disease, closely resembling systemic lupus erythematosus, with blockade of nicotinic receptors at muscle endplates by an antibody produced as the result of an abnormality of the thymus gland (Simpson, 1960). It was based on eight features (Table 1). Although most authors now attribute the hypothesis to recognition of associated autoimmune disorders, it must be remembered that most of the related disorders were not known to be immunological in origin at that time. The immunological role of the thymus was not established until the following year (Miller, 1961) and another autoimmune hypothesis published simultaneously (Strauss et al., 1960) had serious flaws. By the methods available at that time I was unable to isolate the proposed antibody. The theory was not taken seriously for the next 15 years until certain developments made it respectable. The crucial finding was that a snake venom, alpha-bungarotoxin, was
Table 1

EIGHT FEATURES OF MYASTHENIA GRAVIS SUGGESTING THAT IT IS AN AUTOIMMUNE DISORDER (AFTER SIMPSON 1960)

1. Multiple system disease?
   - Thyroid diseases (all types)
   - 'Rheumatoid' arthritis
   - Pericarditis
   - Pure red-cell aplasia
   - Reticuloses
   - Sarcoidosis


3. Age, sex, natural history resembling SLE.

4. Transmission to fetus.

5. Precipitation by infections or allergic reactions.

6. Possible genetic predisposition (HLA linked).

7. Lymphorrhages in muscles and other organs.

8. Germinal centres in thymus.

found to bind specifically and with high affinity to the ACh-binding site of the motor endplate. This made it possible to purify receptors by affinity chromatography. Patrick and Lindstrom (1973) used it to isolate receptor protein from the electric organs of Torpedo californica. To identify the protein they raised antibody against receptor substance. The injected rabbits developed a delayed neuromuscular syndrome with characteristics of myasthenia gravis. Quite soon the autoimmune theory of myasthenia gravis was accepted, and indeed it was soon regarded as the prototype autoimmune disease. Other consequences of the discovery of alpha-bungarotoxin were that it enabled detection of serum globulin which could prevent access of the toxin to receptors, and identification of receptor destruction at the myasthenic endplates. It is impossible in the time available to do justice to the new knowledge about the disorder at the neuromuscular junction. At least four abnormalities have been found, (1) reversible block of competitive type, (2) conformational changes of receptor-channel complexes, (3) lysis and internalisation of receptors and (4) atrophy of subsynaptic membrane with loss of secondary folds. Only the first of these changes is responsive to anticholinesterase drugs; complete restoration of power (as implied in chemical models) is not to be expected. On the other hand, rapid insertion of new receptors, development of new
synapses by terminal sprouting from the motor nerve terminal and a normal ‘turnover’ of endplates are important regenerative factors. The typical fluctuating clinical picture represents the changing balance between attack and repair, in addition to variations of the immunological attack. It is almost certain that more than one antibody is involved, some being complement dependent. Possibly for this reason, the antibodies detected by present techniques (recognising those acting at or near the site of action of alpha-bungarotoxin) do not correlate well with the clinical severity. Raised titres occur only in myasthenia gravis, but the titre is not a measure of severity. This is unfortunate because we have no good criteria for measuring the severity. I cannot accept the methods described in the literature based on the distribution of the disease (for reasons outlined in Simpson, 1958). In the section on treatment, the grading used represents a change of category. It will be shown that new forms of treatment do not improve survival and, as I am going to be very critical about them, I must stress that hazardous treatment may be justified if there is a major improvement in the quality of survival. This has not been demonstrated because of the lack of an agreed scale.

NATURAL HISTORY OF MYASTHENIA GRAVIS
To assess the various treatments it is necessary to recognise three clinical stages (Simpson, 1974). Stage 1 has the characteristic relapses and remissions as attack battles with repair. Most of the deaths occur two to four years after onset and thymectomy during this time is always beneficial. Stage 1 has a mean duration of about seven years. In Stage 2, there are fewer remissions but death is rare. (Impaction in the glottis is the major hazard.) Thymectomy is of little or no value in this stage. In Stage 3 the active disease is ‘burned out’. The endplates continue to have a low safety factor but with little responsiveness to anticholinesterases. Slow improvement is not uncommon.

Clearly hazardous treatment to prevent death may be justified in Stage 1. In the other stages the risks of treatment may be greater than the risk of death. Consider where we had reached before the autoimmune theory was validated. My review (Simpson, 1958) confirmed statistically the claims of Sir Geoffrey Keynes that thymectomy benefited most myasthenics, especially those without a thymoma. The analysis suggested that the best results followed early operation. Acting on this policy (with mean pre-operative time of two years) resulted in virtual recovery in more than 90 per cent of cases and confirmed the impression of a general shift towards
normality. This was coupled with a rigid protocol for avoidance of cholinergic crisis in the postoperative stage (Fraser et al., 1978) and at all stages of management. Peri-operation deaths are now exceptionally rare (two in 28 years, one from convulsions, the other from cardiac arrhythmia) and death from cardiorespiratory failure (presumed myasthenic) has been less common than death from unrelated diseases (report in preparation). Significantly in the period 1975–84, 10 patients (of whom four had thymoma) have died from uncontrolled infection or peritonitis. It is my belief that these were directly related to immunosuppressive treatment.

TREATMENT IN THE AUTOIMMUNE ERA

For ethical reasons I did not feel free to follow the therapeutic logic of the autoimmune hypothesis until it was generally accepted. By that time I had shown that early thymectomy virtually eliminated mortality due to myasthenia gravis. Any additional treatment must therefore be relatively free from risk and the advocates of new forms of treatment have an obligation to demonstrate the advantage. The most up to date review states that 'the firm evidence that myasthenia gravis is an antibody-mediated disease has encouraged greater use of immunological approaches to treatment and a consequent improvement in the prognosis for patients with this disease' (Newsom-Davis, 1984). If this is true, it is certainly not supported by published work which is conspicuously uncontrolled. Mortality had already become extremely low (in experienced units) and patients now die from the complications of immunosuppression who would otherwise have lived.

A second reason why I was slow to use steroids in the 1970s was my unfavourable experience of ACTH and cortisone in 1952-53. There was no severe deterioration but the ‘rebound improvement’ described at that time was not impressive. Two of the eight patients also had pernicious anaemia (the first time I had recognised the linkage). The report of Grob and Harvey (1952) discouraged further trials of these then rare and expensive drugs. Since 1971 long-term treatment with prednisone has been advocated. The best documented experience from T. R. Johns and his colleagues is generally favourable though one-fifth of cases had an unsatisfactory outcome and two-thirds had side effects or complications of prednisone therapy, cataract, metabolic bone disease and infection following in 26, 13 and 5 per cent of cases (Pascuzzi et al., 1984). It is difficult to reach a conclusion as more than half of these patients had had a thymectomy. There is little doubt that marked improvement or remission can be induced with
steroids but lack of a good criterion of severity makes it difficult to quantify the effect and the ratio of satisfactory responses has declined in each successive report. More seriously, Oosterhuis (1984) and others remark on the difficulty of weaning patients from steroids. This must be remembered in electively treating a severe relapse with steroids. Cohen et al. (1981) reported gastrointestinal haemorrhage in 22 per cent of patients given steroids for myasthenic crisis. A combination of steroids and anticholinesterases in high dosage is particularly dangerous. As there is strong evidence that steroids are more effective in patients who are over 50 years of age when symptoms first appear, steroid therapy is not the treatment of first choice in younger patients. If the decision is taken to use it for progressive disease unresponsive to pyridostigmine and early thymectomy, treatment should be started in hospital until the initial period of deterioration is past (10-30 days).

The reason for the deterioration and later improvement is unknown. There is little evidence that it is due to immunosuppression and we have good evidence for a pharmacological effect at the neuromuscular junction (Weir, 1982).

Antibody-mediated immunogenesis is supported by the finding that rapid remission may be induced by plasmapheresis (Pinching et al., 1976) and that globulin from the removed plasma induces some characteristics of myasthenia when injected into laboratory animals (Toyka et al., 1977). Although we have confirmed that plasmapheresis may be beneficial (Behan et al., 1979), the degree and duration of the induced remission are often disappointingly small and unpredictable. The authors of the original report later advised that decline in antireceptor antibodies and clinical improvement from immunosuppressive treatment were not greater when regular plasma exchange was added (Newsom-Davis et al., 1978). Plasmapheresis may still have a place in treatment of a myasthenic crisis.

Immunosuppression with azathioprine is having a vogue, though the early experience in Germany showed that any benefit was delayed for 4-15 months and commonly longer. It is usually necessary to continue with cytotoxic therapy; 90 per cent of patients who discontinued the drug within three years had relapses (Mertens et al., 1981).

Despite reassuring statements that infection is not a major hazard, I have had eight patients die from uncontrolled infection and two from 'silent' peritonitis since 1965; all have been on an immunosuppressant regime.

When I became interested in myasthenia gravis 30 years ago, patients were dying from overdosage with anticholinesterases as commonly as from inadequate treatment. In the last decade more have died from the results of
immunosuppression than from ventilatory failure. The best epoch (1956-64) was when treatment was based on early thymectomy and avoidance of overdosage with anticholinergics. A long-acting neostigmine is a dangerous myth. So is a safe immunosuppressant. The risk of treatment must be weighed against the risk of the disease. The risk of death falls rapidly after the third year. Justification for long-term immunosuppression must depend on clinical improvement which justifies risks which are considerable, especially during the growth and reproductive periods.

Contrary to received wisdom, discovering the main facts about the pathological mechanism of myasthenia gravis has not improved the life expectancy. I have been asked to forecast the future. Speculation is always exposed to criticism by those who will not move until conclusive data make speculation unnecessary. It has the positive advantage of providing a model against which observations can be tried out.

Most of the present research concerns the identification of a supposed critical antigen-antibody reaction from among the many which can now be identified with monoclonal techniques. I am less hopeful than formerly that this will lead to an antibody against a pathogenic antibody since in experimental myasthenia we have found considerable individual variations. If the same is true of the human disease, it would be necessary to raise a unique antibody for each patient (Barkas and Simpson, 1981). If the main membrane-lytic antibodies do not attack the toxin binding site, we are still a long way from identifying them in patients, and yet these should be associated with the poorest prognosis. The role of complement is still uncertain but inhibition of the cascade could be valuable. Secondly, what is the role of the thymus? If, as many contemporary workers advocate, it is both source of receptor antigen and site of antigen-presenting cells for transformation of T-cells, then is myasthenia an unique form of autoimmune disease or must we assume similar embryonic antigen sources for the associated autoimmune disease? What turns the thymus on if the antigen is there from the beginning, and what turns it off again, as suggested by the natural history? A thymic regulatory hormone could have a therapeutic role.

Finally, a major contribution to clinical management would be the discovery of methods for promoting re-insertion of receptors into the subsynaptic membrane, and other regenerative processes which I do not have time to discuss. I have been trying an androgenic steroid (stanozolol) but without obvious success so far. Discoveries in this area could revolutionise the prognosis for myasthenia. I am confident that complete control is a realistic possibility.
REFERENCES


8. Monoclonal antibody to human acetylcholine receptor: B cell hybrids established from thymus of mice with experimental autoimmune myasthenia gravis.

R. Vasquez, J. A. Simpson*, W. H. Stimson and D. Doyle**.
Division of Biochemistry, Department of Bio-science and Biotechnology, University of Strathclyde, Glasgow G4,
* Department of Neurology and ** Department of Neuropathology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51

Acetylcholine receptor (AChR)-specific B cell hybrids were obtained by somatic cell hybridization of lymphocytes from spleen and thymus of mice immunized with AChR from human muscle cells in tissue culture with the mouse myeloma X63-Ag8-653. The antibodies produced by the cell lines precipitated AChR from human skeletal muscle and bound to AChR of cultured human myotubes. In particular, the monoclonal antibody McAbT4 bound to AChR-antigenic determinants exposed extracellularly on the membrane of human myotubes in tissue culture. Competition between McAbT4 antibody with myasthenia gravis serum antibodies for binding to AChR showed that the two antibodies bound to the extracellular AChR, suggesting that the two antibodies play a pathogenic role in myasthenia gravis. The pathogenic activity of monoclonal antibody McAbT4 was demonstrated by an indirect method. Mice injected with 'AChR-depleted preparation', prepared by absorption of AChR from an AChR-rich preparation with McAbT4-hybridoma cells, failed to induce myasthenic clinical symptoms whereas 'unabsorbed AChR-preparation' induced the development of muscle weakness and paralysis.
Treatment of Myasthenia Gravis: An Audit

J. A. SIMPSON and T. THOMAIDES

From Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow

Accepted 23 March 1987

SUMMARY

Although treatment of myasthenia gravis with anticholinesterase drugs and thymectomy was based on wrong models of pathogenesis the resulting saving of life has not been improved by immunosuppressive treatment based on a current autoimmune model. Immunosuppression with steroids or azathioprine may reduce morbidity but as these drugs can rarely be withdrawn without causing relapse, the long-term hazards are serious, including increased mortality.

INTRODUCTION

Following the classical descriptions by Erb, Goldflam and Jolly, myasthenia gravis became recognized at the beginning of this century. The favoured pathogenetic mechanism was a 'myasthenic toxin' acting either on the motor nerve terminals or on the endplates of muscles, though the only identified pathology was lymphorrhages in muscle. No morphological abnormality was detected at the neuromuscular junction. An association in 10 to 15 per cent of cases with thymomas suggested that the thymus was the source of a circulating toxin resembling curare. The similarity encouraged Mary Walker to treat a patient with physostigmine (an alkaloid of the Calabar bean known to be an antidote to curare) and then with a synthetic analogue neostigmine [1, 2]. The wide publication of this 'wonder drug' coincided with confirmation of the role of acetylcholine in neuromuscular transmission [3] in an era when a biochemical basis for disease was the vogue. Anticholinesterase treatment transformed the outlook for myasthenic patients and its success was considered to establish three propositions: (i) the pathogenesis must be either failure to produce/liberate sufficient acetylcholine (ACh), a presynaptic lesion, or competition by a curare-like substance for a supposed receptive substance on the endplate (circulating toxin); (ii) failure to reverse the transmission block by anticholinesterase drugs indicated that the dose was insufficient or the endplate showed neostigmine resistance; (iii) the pharmacologists should search for a long-acting form of neostigmine. For the next 20 years there were many reports of 'neostigmine-resistance' and of patients given up to 15 000 mg neostigmine daily, with atropine to block the muscarinic actions.

The quest for a 'long-acting neostigmine' led to the use of alkyl-phosphates, as a spin-off from wartime research on 'nerve gases' and a number of bis-neostigmine compounds were investigated in the 1950s. They have all been abandoned for two reasons. Either, like physostigmine,
they crossed the blood–brain barrier and caused headache, nightmares and personality disturbances, or they were cumulative and caused delayed overdosage with blockade of the neuromuscular junction. If this ‘cholinergic state’ is wrongly interpreted as increasing myasthenic weakness the situation is fraught with danger as further increments of dosage make the position worse and eventually block nicotinic receptors. The duration of action of pyridostigmine varies in different patients from two to 12 hours and the dosage must be individualized [4]. It is safer to dose frequently than to risk cumulative poisoning.

It is probable that many patients died from the anticholinesterase drugs and not from the disease itself. Nevertheless the availability of powerful anticholinesterase drugs altered the risk factors for the myasthenic patients. Between 1934 and 1956 there were 110 cases of myasthenia gravis treated without surgery at the National Hospital, Queen Square: 32 patients died from myasthenia and five from other causes. Most of the myasthenic deaths occurred two to six years after the onset of symptoms, regardless of whether they had a thymoma or not [5]. It was already recognized that the prognosis was worse in the presence of a thymoma, but many others had a thymus with prominent germinal centres (often wrongly described as hypertrophied).

When Blalock introduced thymectomy, he considered that thymoma was the major indication for surgery [6]. By 1955 three major American centres were abandoning thymectomy whereas, in London, Keynes increasingly advocated the operation. He had made the crucial observation that the best results were obtained in non-thymoma cases [7]. His conclusions were amply confirmed by an independent review [5] which also stressed that most benefit resulted from operation during the first five years. The age and sex of the patient were not relevant factors. In the non-tumour group the mortality was halved, even though there was a high perioperative mortality. The poor prognosis for thymoma cases was largely attributable to an operative mortality of near 30 per cent, but late deterioration was more likely in patients who had a thymic tumour.

For many years the operation was considered to carry an ‘unacceptable’ risk though it soon reached a low level in units with special experience. It is comparatively recently that the policy for early thymectomy has been accepted in the United States and elsewhere. The senior author’s experience in Edinburgh (1956–1964) managing the post-operative care (Mr Andrew Logan was the surgeon) convinced us that surgical complications were rarely responsible for the deaths after surgery. Wide fluctuations in requirement for anticholinesterases are observed in the first three to four days after thymectomy. In an eight-year period, only one of 28 patients died after operation (from convulsions). The striking but temporary remission in the first few days requires appropriate reduction of anticholinesterase dosage. A very strict protocol developed in Edinburgh has remained the basis for our very successful thymectomy programme in Glasgow [8]. ‘Surgical’ deaths have virtually disappeared, death from myasthenia is now rare, most deaths are due to associated autoimmune disease, tumour, or other unrelated disease (Fig. 1). At the same time the benefit has increased so that more than 90 per cent of non-thymoma patients had no symptoms or only mild disability after two years.

The striking drop in mortality is often attributed to availability of ventilators and intensive care units [9]. Ventilatory support in what used to be called a ‘myasthenic crisis’ is valuable, but tracheostomy is rarely required, and certainly not as a routine for thymectomy. The important advance was the recognition of the ‘cholinergic crisis’ and the use of edrophonium in differentiating it from myasthenic weakness [10]. Better prognosis during the period 1956 to 1964 was due to better management and avoidance of drug overdosage.

AUTOIMMUNITY MODELS

The survey for the thymectomy review gave one of us the opportunity to examine more than 400 patients or their records. This led to the formulation of a hypothesis that myasthenia gravis is an
FIG. 1. Change of myasthenic status after treatment. The 'no thymectomy' group, with mean observation periods of 12 years is from ref. 5. The 1941–1956 thymectomy results are from the same source, before the importance of early operation was recognized. The 1965–1976 results (our experience) included those of Fraser et al. [8]. The categories are those of Simpson [5], based on change of status, not Osseman group: A: Full recovery: no anticholinesterase required. The state at review must represent a marked improvement from the fiducial date. B: Full life with minor myasthenic symptoms: requiring up to four tablets of anticholinesterase daily. Significant improvement from the fiducial date. C: Full life with few restrictions but (a) demonstrable myasthenia not requiring anticholinesterase or (b) still requiring it but at least 40 per cent less than before and with improved response. D: (a) Improved, but anticholinesterase requirements unchanged or increased, (b) unimproved, irrespective of dosage, (c) worse. Post-operative death: death occurring within three weeks of operation. Myasthenic death: death at a later date due to asphyxia or no other identifiable cause. Death other disease: death directly attributable to other significant disease (e.g. embolism, septicaemia, neoplasm).
autoimmune disease, closely resembling systemic lupus erythematosus, with blockade of nicotinic receptors at muscle endplates by an antibody produced as the result of an abnormality of the thymus gland [11]. It was based on eight features (Table 1). Although most later authors attribute the hypothesis to recognition of an autoimmune overlap syndrome, it must be remembered that most of the related disorders, recognized for the first time, were not known to be immunological at that time. The immunological role of the thymus was not established until the following year [12] and another autoimmune hypothesis published simultaneously [13] had serious flaws. By the methods available at that time the putative antibody could not be identified. Despite a series of papers adding supportive evidence, the theory was not taken seriously for the next 15 years until certain developments made it respectable. The myasthenic toxin model was given a new run as a secretion from the thymus which was now suggested as a target organ for immunological damage [14]. Physiologists recognized that spontaneous miniature endplate potentials were abnormally small at myasthenic endplates and postulated that the motor nerve released ‘small quanta’ of acetylcholine. Most workers favoured a presynaptic model for the failure of neuromuscular transmission.

The crucial finding was that a snake venom, alphabungarotoxin, would bind specifically and with high affinity to the ACh-binding site of the motor endplate. This made it possible to purify receptors by affinity chromatography. Patrick and Lindstrom [15] used it to isolate receptor protein from the electric organ of Torpedo californica. To identify the protein they raised antibody against receptor substance. The injected rabbits developed a delayed neuromuscular syndrome with characteristics of myasthenia gravis. Quite soon the autoimmune theory of myasthenia gravis was accepted and indeed it was soon regarded as the prototype autoimmune disease. A number of other animal models of ‘experimental autoimmune myasthenia gravis’ rapidly followed. They are not, of course, autoimmune responses but the basic mechanism is almost certainly that proposed in 1960.

The discovery of alphabungarotoxin also enabled detection of serum globulin which could prevent access of the toxin to receptors, and identification of receptor destruction at the myasthenic endplates. It is impossible to do justice to the new knowledge about the disorder at the neuromuscular junction. At least four abnormalities have been found: (i) reversible block of competitive type; (ii) conformational changes of receptor-channel complexes; (iii) lysis and internalization of receptors; (iv) atrophy of subsynaptic membrane with loss of secondary folds. Only the first of these changes is responsive to anticholinesterase drugs: complete restoration of power (as implied in chemical models) is not to be expected. On the other hand, rapid insertion
FIG. 2. Diagram of the immunological attack on nicotinic receptors of skeletal muscle endplates. A section of receptor membrane is shown with polymeric acetylcholine receptor (AChR) molecules traversing the membrane (receptor surface upwards). Antibody molecules (black) are bound to antigenic determinants of one or two receptor molecules, causing allosteric inhibition of ACh binding or conformational change of the ionophore (left). Fab fragments of IgG cross-linking adjacent receptors and bound to their main immunogenic sites activate complement C1-3 (centre) causing antigenic modulation, lysis and internalization of the AChR to autophagic vacuoles. Activation of the full membrane attack complex C5b-9 causes membrane lysis (right) with focal destruction of post-junctional membrane, probably irreversible ('simplification of post-synaptic folds'). New AChR polymers, synthesized in the Golgi apparatus of the sole plate are inserted into areas of membrane which escape lysis (lower left). Where the post-synaptic membrane is not interactive, the nerve terminal or its axonal sprout may move to an adjacent site and develop a new endplate.
of new receptors (Fig. 2), development of new synapses by terminal sprouting from the motor nerve terminal, and a normal 'turnover' of endplates are important regenerative factors. The typical fluctuating clinical picture represents the changing balance between attack and repair, in addition to variations of the immunological attack. It is almost certain that more than one antibody is involved, some being complement dependent. Possibly for this reason, the antibodies detected by present techniques (recognizing those acting at or near the alphabungarotoxin site) do not correlate well with the clinical severity. Raised titres occur commonly in myasthenia gravis, but the titre is not a measure of severity. This is unfortunate because we have no good criteria for measuring the severity. We cannot accept the methods described in the literature based on the distribution of the disease (for reasons outlined previously [5]). In the section on treatment, the grading used represents a change of category.

NATURAL HISTORY OF MYASTHENIA GRAVIS

To assess the various treatments it is necessary to recognize three clinical stages (Fig. 3). Stage 1 has the characteristic relapses and remissions as attack battles with repair. Most of the deaths occur between the second and fourth years but thymectomy during this time is always beneficial [5, 8]. Stage 1 has a mean duration of about seven years. In Stage 2, there are fewer remissions

![Diagram](image)

FIG. 3. The three stages of myasthenia gravis. The time scale is an average one. In the active stage (1) there are major relapses and remissions, most of the mortality, but also best response to thymectomy. In the inactive stage (2) there are fewer deaths or severe relapses but also fewer remissions and less response to thymectomy. In the 'burned-out' stage (3) there is no response to thymectomy and resistance to anticholinesterase drugs but improvement may occur spontaneously or with steroids (reproduced from ref. 35 with permission).
but death is rare [9, 16]. (Impaction in the glottis is the major hazard.) Thymectomy is of little or no value in this stage. In Stage 3 the active disease is ‘burned out’. The endplates continue to have a low safety factor but with little responsiveness to anticholinesterases. Slow improvement is not uncommon.

Clearly hazardous treatment to prevent death may be justified in Stage 1. In the other stages the risks of treatment may be greater than the risk of death from myasthenia gravis. Consider where we had reached before the autoimmune theory was validated. An independent review [5] confirmed statistically the claims of Sir Geoffrey Keynes that thymectomy benefited most myasthenics, especially those without a thymoma. The analysis suggested that best results were due to early operation. Acting on this policy (with mean time before surgery of two years) resulted in virtual recovery in more than 90 per cent of cases and confirmed the impression of a general shift towards normality (Fig. 1). This was coupled with a rigid protocol for avoidance of cholinergic crisis in the post-operative stage [8] and at all stages of management. Peri operation deaths are now exceptionally rare (two in 28 years, one from convulsions, the other from cardiac arrhythmia) and death from cardiorespiratory failure (presumed myasthenic) has been less common than death from unrelated diseases (report in preparation). Significantly, in the period 1975 to 1984 10 patients (of whom four had thymoma) have died from uncontrolled infection or peritonitis. We believe that these were directly related to immunosuppressive treatment.

**TREATMENT IN THE AUTOIMMUNE ERA**

For ethical reasons we did not feel free to follow the therapeutic logic of the autoimmune hypothesis until it was generally accepted. By that time we had shown that early thymectomy virtually eliminated mortality due to myasthenia gravis. Any additional treatment must therefore be relatively free from risk and the advocates of new forms of treatment have an obligation to demonstrate the advantage. The author of a recent review [17] states that ‘the firm evidence that myasthenia gravis is an antibody-mediated disease has encouraged greater use of immunological approaches to treatment and a consequent improvement in the prognosis for patients with this disease’. If this is true it is certainly not supported by published work which is conspicuously uncontrolled. Mortality had already become extremely low (in experienced units) and patients now die from the complication of immunosuppression who would otherwise have lived.

**CORTICOSTEROID THERAPY**

A second reason why we were slow to use steroids in the 1970s was our unfavourable experience of ACTH and cortisone during the year 1952 to 1953. There was no severe deterioration but the ‘rebound improvement’ described at that time was not impressive. Two of eight patients also had pernicious anaemia (the first time the linkage was recognized). The report of Grob and Harvey [18] discouraged further trials of these rare and expensive drugs at that time. Since 1971 long term treatment with prednisone has been advocated. It is complementary to treatment with anticholinesterase, not an alternative. (Anticholinesterases require functioning AChR (the left side of Fig. 2) whereas steroids may prevent AChR and membrane lysis (the right side of Fig. 2).) The best documented experience of steroid treatment from Johns and his colleagues in Virginia [19] is generally favourable though 20 per cent of cases had an unsatisfactory outcome whereas 66 per cent had side effects or complications of prednisone including cataract (26 per cent) metabolic bone disease (13 per cent) and infection (5 per cent). Steroid myopathy was an additional complication. It is difficult to reach a conclusion as more than half of these
patients had a thymectomy. There is little doubt that marked improvement or remission can be induced with steroids (the dosage regime makes no significant difference) but lack of a good criterion of severity makes it difficult to quantify the effect and the ratio of satisfactory responses has declined in each successive report from the Virginia group. More seriously only 14 per cent of their patients were able to discontinue treatment and others remark on the difficulty of weaning patients from steroids [20]. This must be remembered in electively treating a severe relapse with steroids. As there is strong evidence that steroids are more effective in late onset myasthenia gravis (over 50 years of age), treatment with steroids should certainly not be the first choice in younger patients. If the decision to use it is taken, for progressive disease unresponsive to pyridostigmine and early thymectomy, the treatment should be started in hospital until the initial period of deterioration is past (10-30 days).

The reason for the deterioration and later improvement is unknown. There is little evidence that it is due to immunosuppression and we have good evidence for a pharmacological effect at the neuromuscular junction [21].

The long-term antmyasthenic action of steroids is likely to be immunosuppressive and the relapse following steroid withdrawal to de-repression of the B-cell clones responsible for producing antireceptor IgG. The benefit is not questioned but unfortunately the risks are very significant, especially in the older group of patients who tend to gain the greatest benefit. The physician must decide for each individual the relative advantages of early response and long-term risks. We reserve steroids for two groups of patients: (i) those with life-threatening myasthenia, in the period between thymectomy and its delayed benefit, and (ii) late cases, usually elderly, with inadequate response to anticholinesterases. Despite these limitations to a comparatively small number of patients we have three cases with steroid cataract and vertebral collapse caused by steroid osteoporosis. Our patients on steroids are given calcium, stanozolol or vitamin D, and ascorbic acid to reduce the risk of bone softening. Hypertension has not been troublesome but 'silent abdominal emergencies' have been fatal in two cases. In one series gastrointestinal haemorrhage occurred in 22 per cent of steroid-treated patients in crisis [22]. A combination of steroids and anticholinesterases in high dosage is particularly dangerous. It is our practice to monitor faecal blood in such cases and to give alkaline or cimetidine to positive reactors.

PLASMA EXCHANGE

Antibody mediated immunogenesis is supported by the finding that rapid remission may be induced by plasmapheresis [23] and that globulin from the plasma removed will induce some characteristics of myasthenia gravis when injected into laboratory animals [24]. Although we have confirmed that plasmapheresis may be beneficial [25], the degree and duration of the induced remission are often disappointingly small, and unpredictable. The authors of the original report later advised that decline in antireceptor antibodies and clinical improvement from immunosuppressive treatment were not greater when regular plasma exchange was added [26]. There may still be a role in myasthenic crisis or in preparing a patient for thymectomy.

CYTOTOXIC IMMUNOSUPPRESSION

Azathioprine has been used in Germany for many years to treat myasthenia gravis. Although the favourable results were noticeable over a period of two years [27] they continued to use this form of immunosuppression as they were not impressed with the benefits from thymectomy and considered that myasthenia gravis continues to deteriorate in most cases [28]. Some improve-
ment may occur even after steroid failure in myasthenics treated with azathioprine for long periods [29]. It is usually necessary to continue with treatment. Less than 10 per cent of patients can discontinue the drug for periods of up to three years without relapse [30]. Azathioprine, a cytotoxic drug, is reported to produce less side effects than cytostatic drugs such as mercaptopurin, actinomycin and methotrexate, but in 64 cases of neuromuscular disease (mainly myasthenia gravis) azathioprine caused toxic effects in 42 per cent of patients which were sufficiently severe to require discontinuation of treatment in half of them [30]. As a compromise some physicians use azathioprine as a 'steroid-sparing'. No controlled trials have been reported comparing azathioprine treatment with others. The published results do not show demonstrable superiority of azathioprine alone or in combination over thymectomy plus anticholinesterases either with respect to mortality or to quality of life. The additional risks are on-going, requiring frequent monitoring of bone marrow and liver function. Consequently, we have only used azathioprine in a few patients so severely handicapped by muscular weakness that some risk seemed justifiable.

Despite reassuring statements that infection is not a major hazard, eight of our patients have died from uncontrolled infection and two from 'silent' peritonitis since 1965: all have been on an immunosuppressant regime (Table 2). There is some evidence that the risk is higher in thymoma-related myasthenia but the numbers are too small to be conclusive.

**DISCUSSION**

A fundamental tenet of scientific medicine is that effective treatment of a disease depends on an understanding of its basic biological mechanisms, followed by the application of drugs of known properties which will prevent or reverse the pathological processes. Acceptance of the autoimmune model has led to a large experimental literature (for a recent clinically-orientated review see ref. 31). It is salutary to review the development of knowledge and treatment of myasthenia gravis from these points of view. The clinical audit of treatment directed by one physician presented in this paper, in full awareness of scientific advances during three decades, suggests that the major advances in treatment of a hitherto incurable disease were based on a model of the disease which was probably wrong in every respect. Immunosuppressive medication has certainly improved the quality of life for many myasthenic patients (especially those older than 40 years of age), but at a considerable price. It has not affected mortality which was already negligible. It is necessary to be aware of the fluctuating course of the disease, even after thymectomy, and to adjust treatment accordingly. Unfortunately this requires considerable

---

**TABLE 2. Deaths in myasthenic patients**

<table>
<thead>
<tr>
<th>Period</th>
<th>Cardiorespiratory</th>
<th>Post-thymectomy</th>
<th>Infection/acute abdomen</th>
<th>Embolism</th>
<th>Myocardial/stroke</th>
<th>Tumour</th>
<th>Others</th>
<th>All deaths</th>
<th>Patients treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1934-55</td>
<td>65 (19)</td>
<td>29 (9)</td>
<td>5 (0)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>2 (1)</td>
<td>5 (0)</td>
<td>80 (29)</td>
<td>404 (47)</td>
</tr>
<tr>
<td>1956-64</td>
<td>14 (4)</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>4 (0)</td>
<td>21 (5)</td>
<td>98 (6)</td>
</tr>
<tr>
<td>1965-74</td>
<td>3 (1)</td>
<td>0 (0)</td>
<td>3 (1)</td>
<td>2 (0)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>10 (1)</td>
<td>101 (9)</td>
</tr>
<tr>
<td>1975-84</td>
<td>4 (0)</td>
<td>1 (0)</td>
<td>6 (4)</td>
<td>0 (0)</td>
<td>5 (0)</td>
<td>2 (1)</td>
<td>4 (0)</td>
<td>22 (5)</td>
<td>124 (11)</td>
</tr>
</tbody>
</table>

*Thymomas in parentheses, included in totals.*
experience, and the low mortality figures now reported from special centres are not achieved by physicians with only occasional myasthenic patients.

Thirty years ago, patients died from overdosage with anticholinesterases as frequently as from inadequate treatment. In the last decade more have died from the results of immunosuppression than from ventilatory failure. In our experience the best epoch (1956-1964) was when treatment was based on early thymectomy and avoidance of overdosage with anticholinergics (Table 2). A long-acting neostigmine is a dangerous myth. So is a safe immunosuppressant. The risk of treatment must be weighed against the risk of the disease. The risk of death due to neuromuscular weakness falls rapidly after the third year. Justification for long-term immunosuppression must depend on its producing clinical improvement to a degree which justifies the considerable risks, especially during the growth and reproductive periods.

Contrary to received wisdom, discovering the main facts about the pathological mechanism of myasthenia gravis has not improved the life expectancy. Is this situation likely to change as a result of current research? Speculation is always exposed to criticism by those who will not move until conclusive data make speculation unnecessary. It has the positive advantage of providing a model against which observations can be tried out.

Most current research concerns the identification of a supposed critical antigen-antibody reaction from among the many which can now be identified with monoclonal techniques. We are less hopeful than formerly that this will lead to an antibody against a pathogenic antibody since in experimental myasthenia gravis we have found considerable individual variations. If the same is true of the human disease, it would be necessary to raise a unique anti-idiotypic antibody for each patient [32]. We have demonstrated that inhibition of α-bungarotoxin binding can be mediated by antibodies which are not directed against the toxin-binding sites [33]. If the main membrane lytic antibodies do not attack the toxin binding site, we are still a long way from identifying them in patients, and yet these should be associated with the poorest prognosis. The role of complement is still uncertain but inhibition of the cascade could be valuable. Secondly, what is the role of the thymus? If, as many contemporary workers advocate, it is both source of receptor antigen and site of antigen-presenting cells for transformation of T-cells, then is myasthenia an unique form of autoimmune disease or must we assume similar embryonic antigen sources for the associated autoimmune disease? What turns it on if the antigen is there from the beginning, and what turns it off again, as suggested by the natural history? A thymic regulatory hormone could have a therapeutic role.

Finally, a major contribution to clinical management would be discovery of methods for promoting re-insertion of receptors into the subsynaptic membrane, and other regenerative processes. We have tried androgenic steroids (stanozolol and nandrolone) but without obvious success so far. In rats with experimentally-induced myasthenia gravis, De Baets [34] has recently shown that anabolic steroids increase receptor synthesis. Discoveries in this area could revolutionize the prognosis for myasthenia. We are confident that complete control is a realistic possibility. For the present it must be concluded that the addition of steroid or other immunosuppressant regimens does not diminish the already low mortality from myasthenia gravis. The quality of life of survivors may be improved in 80 per cent of patients but the final audit conclusion is — benefit now, pay later.

ACKNOWLEDGEMENTS

We are grateful to the Muscular Dystrophy Group of Great Britain and the British Association of Myasthenics for regular financial support.
REFERENCES


Myasthenia gravis and related syndromes

HISTORICAL INTRODUCTION

Myasthenia gravis is a specific muscular disease characterised by the development of an abnormal amount of weakness in voluntary muscles following repetitive activation or prolonged tension, with a marked tendency to recovery of motor power after a period of inactivity or lessened muscular tension. Some authorities consider that a positive response to anticholinesterase drugs should also be included in the definition. It is important to agree on a definition because the literature contains many reports of cases of doubtful provenance. In particular, the term myasthenia has a less restricted meaning in the French language.

A case described by Thomas Willis (1672) is generally accepted as the first description of the disease. Later landmarks are the papers by Erb, Goldflam and Jolly in the nineteenth century (see Simpson 1983). The clinical picture was established by the review of Campbell & Bramwell (1900). Laquer & Weigert (1901) first noted a relationship with the thymus gland. Attention was concentrated on the concept of neuromuscular block by the demonstration by Walker (1934) of the beneficial effect of physostigmine. Twenty-five years of electrical and pharmacological studies ignored the pathogenesis until Simpson (1960) suggested that the link between thymus and muscle was immunological, with production of antibody against acetylcholine receptors (AChR) at the end-plates. The chance finding of Patrick & Lindstrom (1973) that animals used to raise antibody against acetylcholine receptor protein, purified from the electroplaques of the electric eel, became weak with an illness like myasthenia.
gravis, confirmed the feasibility of the mechanism. Since then this autoimmune hypothesis has been accepted and has become the rationale for modern treatment.

The molecular biology of nicotinic AChRs and of their ion channels is being disclosed, with clarification of the congenital myasthenias (Ch. 20). For a good review, see Engel (1984). Apart from showing that polyclonal antibodies are myasthenogenic only when they involve the main immunogenic region of the macromolecule (five subunits), detailed description has too little relevance to acquired myasthenia gravis to justify a full description here.

NATURAL HISTORY

Myasthenia gravis affects all races. Estimates of prevalence range from 1 in 50,000 to 1 in 10,000 of the population. Females are affected twice as often as males, the disproportion being 4.5:1 in the first decade, but reversing in later life (Fig. 19.1). The modal age of onset is about 20 years for each sex but because of the different distribution curves the mean age is a little lower for women (26 years) than men (31 years) (Simpson 1958, 1960). These ages refer to patients without a thymoma. A thymic tumour (Fig. 19.2) is found in 10–15% of cases, about 60% of whom are male (Schwab & Leland 1953, Simpson 1958). Myasthenia associated with thymic tumour (benign or malignant) tends to appear at a later age and is rare under the age of 30 years. Muscular weakness is usually severe and difficult to control with any form of treatment, including thymectomy.

If the thymus is not removed the prognosis is worse for women, according to Simpson (1958). The opposite findings, noted by Grob (1958) may have been due to the higher incidence, in females, of the ocular type of myasthenia in his series.

Onset of symptoms is usually insidious but may be sudden, apparently precipitated by an emotional upset or a febrile illness and less commonly by physical exertion. Symptoms may first appear during pregnancy or the puerperium. If myasthenia is already present before pregnancy it tends to remit at the end of the first trimester and to relapse soon after childbirth, but this is not invariable. An abnormal response to a relaxant drug used during anaesthesia may be the first indication of myasthenia gravis or of one of the symptomatic myasthenias.

The initial symptom, especially if it is ptosis or diplopia, may subside for months or years, and later remissions may follow relapses. Remissions
of more than a month occur in fewer than half of the cases, and usually only in the early years of the illness in patients treated without thymectomy or steroids, becoming less frequent and prolonged as time goes on. More than one long remission is uncommon, and if myasthenic symptoms return after an absence of a year or more and if muscles other than the extraocular are involved, the disease is usually progressive. Relapses are precipitated by the same factors as initial attacks, but additional causes are menstruation, extremes of cold or heat (especially a hot bath or a stuffy atmosphere), inoculation or vaccination and, occasionally, allergy. Bright sunlight may precipitate ptosis and blurred vision, and a few patients declare that it causes generalised weakness.

It is useful to divide the clinical course into three stages (Simpson 1969a). The clinical state is most labile during the first five to seven years (Stage I). Although the most significant remissions occur in Stage I, most of the deaths directly attributable to the disease occur in this period, particularly during the first year, with a second danger period at four to seven years, in progressive cases (Simpson 1958).

To be effective, thymectomy must be carried out in Stage 1 (Simpson 1958, 1960, Papastasss et al 1976). After 10 years (Stage 2) death from myasthenia per se rarely occurs, although the patient may be constantly at risk of asphyxiation from inhaled foreign bodies because of the diminished respiratory reserve. Although further progression is unlikely, there is little or no response to thymectomy. Relatively high titres of antireceptor antibody may persist and temporary improvement may be obtained from immunosuppressive treatment or plasma exchange, but the role of the thymus appears to be diminished. After 15 years or more, some cases enter Stage 3, with persisting weakness, a higher incidence of muscular atrophy, and a reduced response to anticholinesterase drugs. Steroids may still be beneficial in this 'burned out' stage, but thymectomy is of no value and other immunosuppressive regimes have not been assessed. Presumably, permanent morphological changes have occurred at the neuromuscular junctions.

Clinical staging, based on analysis of many cases, is of some value in selecting treatment but it remains difficult to prognosticate for the individual. Grading by 'severity' is less useful. The classification most commonly used (Osserman 1958) is as follows:

I Ocular myasthenia.

IIA Mild generalised myasthenia with slow progression; no crises; drug-responsive.

IIB Moderate generalised myasthenia; severe skeletal and bulbar involvement, but no crises; drug response is less satisfactory.

III Acute fulminating myasthenia; rapid progression of severe symptoms with respiratory crises and poor drug response; high incidence of thymoma; high mortality.

IV Late severe myasthenia; same as III, but takes two years to progress from Classes I or II; crises; high mortality.

These grades, reputed to be a progressive series from I, with good prognosis, to III and IV with poor prognosis, do not represent either discrete types or stages in a progression. It is a tautology to say that purely ocular myasthenia is 'safe' whereas weakness of respiratory muscles in a rapidly deteriorating disorder is dangerous to life. The future course cannot be predicted, although it is widely accepted that it is likely to be benign if signs remain confined to the extraocular muscles for two years. Grob (1953) and Ferguson et al (1955) report this type of history in 20–30% of cases, but it has been rare in my experience, except in males. Conversely, the prognosis is worse (with early death) if a thymoma is present, despite initial benefit from thymectomy.

SYMPTOMS AND SIGNS

The characteristic feature of myasthenia gravis is variability in the strength of affected muscles, rather than a generalised tiredness. It varies from day to day and even from hour to hour, classically increasing towards evening. Surprisingly, few patients comment on this until asked about it. It is not invariable; many patients are weakest on rising. Short-term weakness is often due to physical exertion but the emotional state is an important determinant. Affected muscles lose
further strength if contraction is maintained or repeated (so-called ‘pathological fatigability’). The contracting muscle may lengthen gradually if it is supporting a load, or a coarse tremor develops with increasing ‘rest periods’ until the attempt to sustain the contraction ceases. In the eye muscles this may cause a pseudonytagnus. Gradual ‘fatigue’ is not always seen and failure of contraction may be sudden, suggesting neurotic weakness to the inexperienced. Recovery after rest is often incomplete, even with optimal anticholinesterase dosage, especially in Stage 2. In Stage 3, there may be weakness which cannot be reversed with these drugs, and muscular atrophy is not uncommon, especially in males (Simpson 1958). The most commonly affected muscles are the extraocular muscles, triceps brachii, quadriceps femoris, and the tongue. Atrophy of the tongue is curiously selective, often giving triple longitudinal furrowing (Fig. 19.3) which, though rare, is very characteristic of myasthenia gravis (Wilson 1954). It seems to have been first described by Buzzard in 1905.

The symptoms associated with weakness of ocular, facial, and other muscles are readily appreciated. The distribution of clinically weak muscles in a large series of cases is shown in Figure 19.4, which also shows that the probability of weakness starting in a muscle group matches its overall probability of being involved. Without going into all possible variations, attention is drawn to the early and frequent involvement of the extraocular muscles and levatores palpebrarum.

Fig. 19.3 The triple furrowed tongue (there may be four furrows).

Fig. 19.4 Percentage of cases in which various muscle groups are affected at the onset (left of key) and at some time during the illness (right of key).
Tendon reflexes are surprisingly brisk and clonus may be present (but with a flexor plantar response). If a reflex is elicited repetitively, the jerk may decrease progressively until it disappears. Persistent absence of many tendon reflexes should suggest that weakness is due to carcinomatous myasthenia rather than to myasthenia gravis, but localised absence may be due to muscular atrophy. Differential diagnosis from the carcinomatous syndrome may be difficult because, in Stage 3, responsiveness to anticholinesterases is lost and some patients (usually men) find that muscle power is increased by exercise. Their muscles may show an incrementing response to tetanisation (Simpson 1966a, Schwartz & Stålberg 1975a, b).

Abnormalities of sensation

Normal sensation is the rule, although cases of myasthenia gravis have been reported with unexplained sensory loss, especially transitory trigeminal anaesthesia. Paraesthesiae of hands, thighs or face (Harvey 1948, Simpson 1960) and the common sensation of 'stiffness' probably have a mechanical explanation. Patients may complain of pain in weak muscles, especially in the neck, the back, and around the eyes. This is normally attributable to the extra effort required to maintain posture, but sometimes there appears to be a true myositis. Sudden substernal ache at the onset of the disease is an occasional and unexplained symptom.

Disorders of other organs

Disorders of the thyroid gland (including subclinical disease indicated by serum antibody studies) are more frequent in a myasthenic population but only a minority have abnormal thyroid function tests, and these patients are as commonly hypothyroid as hyperthyroid (Simpson 1966b, 1968). Milikan & Haines (1953) found that the incidence of hyperthyroidism before, during, or after detectable myasthenia gravis was about 5%. If all thyroid disorders are added, the incidence may be as high as 9% in males and 18% in females (Simpson 1958, Downes et al 1966). Many of these patients have thyrotoxic symptoms or signs.

Fig. 19.5 The myasthenic 'snarl' and ptosis

(more than 90% of cases), usually associated with weakness of orbiculares oculorum, an almost diagnostic combination. The facies is characteristic (Fig. 19.5). Speech and chewing weakness, difficulty in holding up the head, and proximal limb weakness (more in the upper than the lower limbs) are typical. Fortunately, the chest muscles and diaphragm are not involved until later. Nevertheless, any muscle or part of a muscle may be affected and the distribution is commonly asymmetrical. When the history or physical examination suggests the possibility of myasthenia gravis, the most commonly affected muscles should be subjected to a fatigue test and the response to edrophonium assessed. Some appropriate tests for use in doubtful cases are described below, but usually an appropriate performance test is readily improvised during the consultation.
for only a few months and these may precede myasthenic symptoms by many years. Thyrotoxic symptoms may be subsiding when myasthenia appears and vice versa. In a limited period of observation this may lead to the conclusion that the disorders have a 'see-saw' relationship to each other (McEachern & Parnell 1948).

It was clearly shown by Engel (1961) that myasthenia could not be caused by hyperthyroidism. The linkage between them is probably genetic and immunological rather than hormonal, and involves all non-tumour diseases of the thyroid including non-toxic goitre (Rowland et al 1956, Simpson, 1958, 1968, Bundey et al 1972), spontaneous myxoedema (Feinberg et al 1957, Sahay et al 1965), and Hashimoto's disease (Simpson 1960, 1964, 1966c, Becker et al 1964). Many myasthenics resemble thyrotoxic patients in other ways. Slight exophthalmos and thickening of the upper eyelids are common (Simpson 1960). The 'lid twitch sign' (transient upward overshoot of the upper eyelid on abruptly looking upwards, followed by a slower return to ptosis) described by Cogan (1965) is readily distinguished from thyrotoxic lid-lag. Many patients complain of excessive sweating, even without taking anticholineresterase drugs (Simpson 1960, Pirskanen 1976). There may also be a genetic as well as an immunological linkage between myasthenia gravis and other diseases. Primary adrenal insufficiency of autoimmune type is rare (Bosch et al 1978). An arthropathy resembling rheumatoid arthritis or ankylosing spondylitis is not uncommon in myasthenics or their relatives (Simpson 1960, 1966c, Oosterhuis 1964, Namba & Grob 1970).


Associated disorders which probably have an immunological basis or cause immunodeficiency are acrocyanosis, haemolytic anaemia, nephritis (Simpson 1960, Oosterhuis 1964), reticuloses (Alter & Osnato 1930, Symmers 1932, Simpson 1960, Cohen & Waxman 1967), diabetes mellitus (Simpson 1960), and herpes zoster (J A Simpson unpublished work).

Associated disorders which are relatively more common in myasthenics than those listed above, but in which no immunological pathology has yet been identified, are epilepsy (Hoefer et al 1958, Simpson 1960) and psychosis (Hayman 1941, Simpson 1960, Storm-Mathisen 1961). Emotional disturbances are common (Brolly & Hollender 1955, Meyer 1966).

**Neonatal myasthenia**

About one in seven live-born children of myasthenic mothers shows evidence of myasthenia at birth and, if the affected child survives, there is complete recovery in 1–12 weeks (mean 18 days) without later relapse. The literature was reviewed by Namba et al (1970). There is no correlation between the severity of the infant's symptoms and the duration of the mother's illness or the severity of the mother's myasthenia during pregnancy. It is extremely rare for a myasthenic mother to have more than one affected child. Previous thymectomy does not abolish the possibility that the baby will be myasthenic, but the transient nature of neonatal myasthenia suggests that the child is affected by a factor transmitted from the mother. Maternal anti-ACh receptor antibody is transferred to the infant (Lindstrom et al 1976b), but it is becoming evident that this probably occurs in all pregnancies, although only one in seven (Simpson 1960) results in clinical neonatal myasthenia. It does not depend on a high maternal titre of antibody or on non-specific inhibition by α-fetoprotein (Brenner et al 1980). Reactions of idiotypic antibody with anti-AChR antibodies of mother and child suggest that the clinically affected infant synthesises its own antibody,
possibly due to transplacental transfer of a cell clone from the mother (Lefvert & Osterman 1983). A myasthenic syndrome may be apparent in the new-born child of a mother without myasthenia, and may then persist throughout life. This congenital myasthenia is not autoimmune in type and is not related to myasthenia gravis (Ch. 20) although some children with congenital myasthenia have low blood levels of IgA (Bundey et al 1972, Behan et al 1976a).

GENETIC FACTORS

There is now clear evidence for a genetic factor in myasthenia gravis. Although a study by Jacob et al (1968) showed no secondary cases of myasthenia gravis in 448 relatives of 70 myasthenic patients, and Herrmann (1971) was unable to establish a definite genetic mechanism in his study of families containing more than one case, Simpson (1960, 1968) suggested that a genetic factor could have variable expression, accounting for the familial linkage with thyroid disease, arthropathy, pernicious anaemia and diabetes mellitus (p. 632). Jacob et al (1968) found no association between myasthenia gravis and the ABO rhesus blood groups or with secretor status or the ability to taste phenylthiocarbamide (PTC). Bundey (1972) recognised an early-onset form of childhood myasthenia with autosomal recessive inheritance of the trait. Her study gave limited support to the concept of alternative gene expression.

Linkage with human histocompatibility antigens, reported in earlier editions of this book, has been confirmed in many countries (Pirskanen et al 1972, Behan et al 1973, Fritze et al 1973, Dick et al 1974, Feltkamp et al 1974, Pirskanen 1976). There is a frequent association with HLA-A1-B8-DW3 haplotypes, but it is clear that the association is not a direct one and not obligatory (Dick et al 1974, Pirskanen 1976). Compston et al (1980) related these haplotypes to non-thymoma myasthenia of women with onset under age 40. They found an increased association with HLA-A3, B7, DRw2 antigens in the later onset, predominantly male group. An increased frequency of HLA-A2 or -A3 in patients with thymoma, reported by Feltkamp et al (1974) and Fritze et al (1974) was not noted by Dick et al (1974), by Pirskanen (1976) or by Compston et al (1980). Another histocompatibility antigen system, such as the LD antigens (Kaakinen et al 1975) situated near the SD loci bearing HL-A genes, may exhibit a stronger association with myasthenia gravis and with autoimmune disorders; both the SD and LD gene loci are close to some loci controlling immunological responsiveness (Ir genes). With respect to immunoglobulin (Gm) phenotypes, Garlepp et al (1984) found no significant linkage disequilibrium associated with anti-AChR antibodies, but the phenotype Gm (3;5) was associated with the development of antistriational antibody in males and older females. Genetic factors probably constitute risk factors for autoimmune diseases. (Adams (1977) suggests V genes.) The relative scarcity of familial cases of myasthenia gravis (3.4%, Namba et al 1971a; 7.2% in Finland, Pirskanen 1977) and involvement of only one of monozygotic twins (Simpson 1960, 1965, Namba et al 1971b) indicate that many aetiological factors may be involved. Twin studies are reviewed by Behan & Shields (1982).

CLINICAL CHEMISTRY

There are no consistent changes in blood chemistry in myasthenia gravis. Abnormalities of glucose tolerance (Cohen & King 1932, Simpson 1960, 1966b, Frenkel 1963), adrenocortical hypofunction (Simpson 1966b, Bosch et al 1978) and hyper- and hypothyroid function in the absence of clinical endocrinopathies (Simpson 1966b, 1968) are likely to be due to genetic and/or autoimmune linkage. Diminished pregnandediol excretion (Schrire 1959) has not been confirmed (Simpson 1966b).

Hypergammaglobulinaemia occurs in cases associated with other autoimmune diseases (Simpson 1960, 1966b) or with thymoma (Oosterhuis et al 1964) and some, but relatively few, of these patients have a raised erythrocyte sedimentation rate (ESR). Isolated instances of monoclonal gammopathy have been described (Rowland et al 1969). Hypogammaglobulinaemia sometimes occurs (Thévenard & Mende 1955, Simpson 1966b) and
may be associated with a thymoma (Te Velde et al 1966, Cohen & Waxman 1967).

Depressed levels of IgA were found in juvenile-onset myasthenia by Bundey et al (1972); Simpson et al (1976) found a selective decrease in IgA in 10 of 50 myasthenic patients, and this was not influenced by thymectomy. The lowest levels were in three patients with congenital myasthenia who had undergone thymectomy. Bramis et al (1976) confirmed that serum IgA concentration may be subnormal. In their series the lowest concentrations were associated with thymomas or other neoplasms. Decreased IgA concentrations tended to be associated with many or prominent germinal centres in the thymus and levels increased slowly after thymectomy. There were no congenital cases in the series of Lisak & Zweiman (1975) who found normal IgA levels in 19 myasthenic patients. These workers reported a slight but significant depression of the mean IgM level (raised in some subjects, especially with a thymoma). Serum IgG tended to be above normal. There was no consistent pattern and the immunoglobulin levels did not correlate with the clinical state.

Nastuk et al (1960) measured serum complement levels serially in myasthenic patients and stated that they were lower during active disease, rising to normal or super-normal levels in remission. Plescia et al (1966) found reduced levels of C2 and C4 and inhibitors to these components. Simpson et al (1976) found no abnormality of Clq, C3, C4, C7 and C3 proactivator. They also measured C3 conversion products with C3 activator levels, and CH50 units. All were normal although examined in active and remittent cases. Behan & Behan (1979) re-examined this material when Engel (1977a) demonstrated localisation of IgG and C3 at motor end-plates. Depression of C4 component was found in 34% of myasthenic patients and 29% had circulating immune complexes. The greatest immunological abnormalities were found in patients with mild disease.

Elevated globulin levels in the cerebrospinal fluid (CSF) of 10 patients were recorded by Simpson (1960, 1966b), who reviewed previous isolated reports and concluded that there was associated disease which may be of immunological type. Reports that anti-ACh receptor antibody may be present in the CSF (Lefvert & Pirskanen 1977) have not been confirmed.

**IMMUNOLOGICAL STUDIES**

The hypothesis by Simpson (1960) that a thymus-controlled lymphocyte-derived antibody against ACh receptor could be the proximate cause of myasthenia gravis could not be confirmed by immunofluorescence techniques then available (McFarlin et al 1966); however, using high-resolution electron microscopy, Rash et al (1976) found material ('fuzzy coats'), resembling IgG in its configuration and dimensions, in the region of the receptors in the neuromuscular junction. The presence of IgG and of the C3 component of complement at the postsynaptic membranes of human myasthenic neuromuscular junctions was convincingly demonstrated by Engel et al (1977a, b) by an immunoperoxidase method. These authors proposed that subsequent sequential activation of C5–C9 would complete the attack phase of the complement reaction sequence and set the stage for lytic destruction of the postsynaptic membrane. It remains possible that antibody or immune complex may block receptors competitively. The rapid beneficial effect of plasma exchange (p. 649) might support this concept but not necessarily so, because the regeneration of receptors is probably very rapid (Devreotes & Fambrough 1975) and there is usually a time lag of two days or more before muscle power is restored (Newsom-Davis et al 1978). Passive transfer of myasthenic immune complexes would help to differentiate between the three main possibilities: (1) lysis of postsynaptic membrane; (2) immunopharmacological blockade, and (3) IgG-induced modulation of AChR. Passive transfer of myasthenia from man to mouse by human myasthenic serum was demonstrated by Toyka et al (1975). It may be necessary to have previous reduction of the transmission safety factor in order to demonstrate the passive transfer (Stahlberg et al 1978) or to use animals of specific H2 haplotype (Fuchs et al 1976). Tissue culture experiments suggest that receptor blockade (Drachman et al 1977) and accelerated degradation
of ACh receptors (Anwyll et al 1977, Kao & Drachman 1977b) may both be involved. Synthesis of AChR appears not to be affected by immunoglobulin from myasthenic patients (Drachman 1978).

Serum antibody acting directly on acetylcholine receptor sites has not been identified by standard immunological techniques, but its existence is strongly indicated by a number of methods demonstrating the presence in myasthenic serum of an IgG which will prevent access of α-bungarotoxins (α-Bgtx) to nicotropic receptors, indicating high affinity of the immunoglobulin for receptor or closely adjacent material. Early assays, based on measuring the inhibition of binding of α-Bgtx to extrajunctional receptors of denervated rat muscle (Almon et al 1974), or to junctional receptors of human muscle (Bender et al 1975), were of low sensitivity. Indirect evidence of an antibody in myasthenic serum which cross-reacts with AChR from electrogenic tissue of Torpedo californica is provided by complement fixation (Aharonov et al 1975a).

More sensitive assays are based on the binding of antibody to AChR linked to isotope-labelled α-Bgtx. The antibody-AChR-α-Bgtx complexes are then precipitated, together with carrier immunoglobulin, by adding anti-immunoglobulin and the radioactivity of the resulting pellet is measured (Appel et al 1975, Lindstrom 1977). The globulin detected by this method blocks the access of α-Bgtx to the AChR site by binding the receptor complex at a site different from, but in close proximity to, the acetylcholine site which, apparently, is not usually the target for the antibody. Most of the monoclonal antibodies isolated by recent studies bind to the main immunogenic region of the alpha subunits of the receptor macromolecule (Tzartos et al 1983). Lefvert (1982) reports antibody reaction outside the toxin-binding site of myasthenic and denervated muscle in higher titre than against normal muscle (suggesting presentation of unusual epitopes on extra-junctional and myasthenic end-plate receptors). It is increasingly clear that the anti-AChR antibody response is polyclonal, but not all antibodies are pathogenic — only those to the exposed area of the receptor macromolecule, but α-Bgtx binding may be inhibited by antibodies not directed against the toxin-binding sites (Barkas et al 1982). In some reports (Lindstrom et al 1976b) the titre of antibody measured by this radioimmunoassay correlates reasonably well with the severity of the disease, but only if AChR from human muscle is used. In other reports (Lefvert et al 1978, Newsom-Davis et al 1978) the correlation is rather low. No significant titre of antibody is found in serum from congenital myasthenia (Newsom-Davis et al 1978). Antibody has been detected in sera from infants with neonatal myasthenia, as well as from their mothers (Lindstrom et al 1976b, Keeseey et al 1977, Lefvert et al 1978), but some infants with receptor antibodies have no detectable weakness, possibly because of receptor antigenic difference between mother and child (Simpson 1960, Keeseey et al 1977). Lefvert et al (1978) have suggested that IgG antibody detected by radioimmunoassay may not be a primary cause of the disease, because it is sometimes preceded by IgM antibodies in the early stages of myasthenia. Anti-AChR antibodies are predominantly of the IgG1 and IgG2 subclasses, rich in k-light-chains. IgG3 antibodies, even richer in k-light-chains, and reacting better with receptors from ocular muscles than limb muscles, have been significantly more prominent in sera from patients with ocular myasthenia.

The titre of IgG antibody against AChR is higher in myasthenia gravis associated with thymoma and falls with all the procedures listed later as immunosuppressive (p. 647).

Antimyosin antibodies are found in sera from some myasthenics, the titre being highest in those with a thymoma (Strauss et al 1960, Beutner et al 1962, van der Geld et al 1964, Djanian et al 1964). Using an immunofluorescence technique, binding of antibody to A bands of skeletal muscle is seen only with myasthenic sera and is highly correlated with the presence of a thymoma (Vettens 1965). The A-band antibody also reacts with myoid cells in the thymus (Feltkamp-Vroom 1966). Antibodies to other antigens of skeletal and cardiac muscle occur in myasthenic sera and are usually associated with a thymoma. Aarli has studied an acid-extractable antigen (CA antigen) which is considered to be specific for thymoma-associated
myasthenia (Aarli & Thunold 1981, Gilhus et al 1984). It is probably a membrane antigen unrelated to the cross-striational pattern of the muscle fibre. Antisera to the CA extract react strongly with neoplastic epithelial cells of the human thymoma. Other antibodies commonly found in sera from myasthenic patients are antinuclear factor, antithyroid and antigastric substances. Rheumatoid factor may also be present in the blood. It does not usually correlate closely with a history of arthritis (Simpson 1964, 1966b), but may do so (Aarli et al 1975). Patients with pemphigus vulgaris have antiepithelial antibody (Noguchi & Nishitani 1976). Specific antineuronal and antispermatogonia nuclear antibodies in sera of patients with myasthenia gravis have been reported by Martin et al (1974). Clearly, there is a wide spectrum of autoantibodies (Simpson 1983) and they are also more frequent in sera from relatives of myasthenics (Feltkamp et al 1974), supporting the suggestion of genetic predisposition to autoimmune diseases which may include myasthenia gravis when the anti-AChR antibody titre is raised.

It is premature to attribute a pathogenetic role to the 'receptor antibody', although changes in titre closely follow the clinical severity of myasthenia and the passive transfer studies described above are impressive. Protection may be provided by development of anti-idiotypic antibodies. They occur in myasthenia gravis (Dwyer et al 1983) but their biological role is uncertain. Experimentally raised anti-idiotypic antibodies against anti-AChR globulin are unique to the individual (Barkas & Simpson 1982, Waldor et al 1983).

There is also uncertainty about the role of cell-mediated immunity (CMI). There are now many reports of minor abnormalities of T-cell subsets (too numerous to list, see reviews by Behan et al 1975, Vincent 1980, Clementi & Conti-Tronconi 1983). Many indicate a deficit of T-suppressor cells, often antigen-dependent and it is probable that abnormality of regulatory T cells precedes the production of anti-self antibodies. The term CMI is now restricted to immunological reactions involving autoaggressive lymphocytes and macrophages without antibody or complement dependence, as in delayed-type hypersensitivity. This type of immune response is minimal in human myasthenia gravis and in the animal model.

Experimental autoimmune myasthenia gravis

Animal models of myasthenia evoked by inoculation of nicotinic receptors and termed experimental autoimmune myasthenia gravis (EAMG) are described in Chapters 9 and 12.

The successful production of EAMG depends on there being some interspecies cross-reactivity of AChR: at first, the sera of animals immunised with electrophoretic AChR have antibodies recognising the electric organ in much higher concentration than that of antibodies recognising the AChR of the animal's muscle. At a later stage of the immune response the absolute amount of antibody against recipient AChR is high (Lindstrom et al 1976a) suggesting true autoimmunity. Passive transfer of EAMG by lymph node cells in the guinea pig (Tarrab-Hazdai et al 1975a) and rat (Lennon et al 1976), and by γ-globulin in the rat (Lindstrom et al 1976b) supports the theory that the proximate mechanism of myasthenia is autoimmune. But the human disease does not result from inoculation of foreign AChR and there is evidence that syngeneic AChR released from damaged muscle does not evoke an antibody response in the human or rat (Lindstrom et al 1976b). It has been suggested that a source of antibody-stimulating AChR may be the myoid cells of the thymus. Myoid cells have long been known to cross-react with antimyosin antibody (van der Geld & Strauss 1966) and have been shown by Kao & Drachman (1977a) to have surface ACh receptors which may be separate but immunologically related target organs in myasthenia gravis (Aharanov et al 1975b). It is unlikely that myoid cells provide a triggering antigen stimulus for the autoimmune reaction. Goldstein & Whittingham (1966) reported a myasthenic syndrome in guinea pigs inoculated with calf thymus, muscle, or lymph node and attributed this to 'autoimmune thymitis' with liberation from the thymus of a neuromuscular blocking substance. Other workers did not confirm their findings (e.g. Veters et al 1969). The conflicting experimental results and conclusions were critically reviewed by Simpson
The Goldstein hypothesis would make it difficult to account for myasthenia originating after thymectomy, or would require ad hoc explanations for associated autoimmune disorders if the primary source of autoantigen were the thymic myoid cell.

THYMUS GLAND

There are pathological changes in 70–80% of patients with myasthenia gravis. The most consistent finding is lymphoid hyperplasia of cortex and medulla with T lymphocytes in both parts, not mainly in the cortex as in normal subjects (Aarli et al 1979). It is commonly associated with numerous germinal centres in the medulla (Castleman & Norris 1949) (Fig. 19.6). (The term 'hyperplasia' is criticised by Levine 1979.) The normal gland is not involuted, as suggested by earlier observers. Germinal centres are characteristic of several autoimmune disorders and are not unique to myasthenia gravis. Their significance is uncertain because the prevalence of germinal centres in the thymus bears no clear relationship to the duration or severity of the disease, or to the clinical response to thymectomy (Vetters & Simpson 1974). Levine (1979) points out that germinal centres are separated by basal lamina from the true thymic parenchyma. These extra-parenchymal centres have venules with high endothelium, like lymph nodes. Bofill et al (1985) agree that germinal centres are reactive and stress the importance of hyperplasia of medullary epithelium. Preponderance of B cells in thymic germinal centres (Abdou et al 1974) has been interpreted as an expression of antibody formation against inappropriate T-cell clones, or excess antibody production attributable to lack of suppressor cells. Some thymic lymphocytes synthesise anti-AChR in culture, but usually from thymuses of patients with long duration of disease (Scadding et al 1981). Intrathymic production of antibody is unlikely to be a major mechanism. Thymic cells

![Fig. 19.6 Germinal centre in thymus, H & E, ×42](image-url)
increase production of anti-AChR antibody by autologous peripheral blood lymphocytes (Newson-Davis et al 1981). This is not T-cell dependent and may result from rare antigen-presenting cells in germinal centres (Willcox et al 1984). Papatestas et al (1976) found lower counts of peripheral blood lymphocytes in patients with many germinal centres and suggested that the latter may indicate a state of immunosuppression. A. L. Goldstein et al (1976) suggested that a thymic hormone, thymosin, influences precursor T cells, possibly via an adenylate cyclase-dependent process, and that genetic factors and/or viral infection may lead to a deficiency of suppressor or regulatory T cells which in turn removes the mechanisms controlling B-cell function (including formation of autoantibodies). The source of thymosin is unknown; it would be premature to identify it with the granules described in the thymus by Vetter & MacAdam (1972, 1973). Immunoreactivity for thymosin \( \alpha_1 \) is localised on thymic epithelial cells at the periphery of Hassall's corpuscles and on some circulating T cells (Dalakas et al 1981). The concept of the role of the thymus in autoimmune disease is more plausible than the alternative that the thymus is itself a target for immunological attack ('thymitis') with consequent release of a neuromuscular blocking substance, as in the theories of Strauss et al (1966) and G. Goldstein (Goldstein & Whittingham 1966). (The 'thymin' of G. Goldstein must not be confused with the 'thymosin' of A. L. Goldstein.)

Wekerle et al (1981) propose that B cells in the thymus and peripheral lymph nodes are instructed by anti-AChR helper T-cell lines whose antigen-reactivity critically depends on presentation of antigen (AChR on myoid cells) and that this interaction is restricted by the HLA phenotype of the presenting cells which are probably epithelial. Their hypothesis would isolate myasthenia gravis from the other autoimmune diseases with which it is associated clinically and genetically. An alternative hypothesis, which I prefer, is aberrant expression of HLA-DR antigens on the antigen-presenting thymic epithelial cells. An idiotypic mimicry model, as in the original Simpson (1960) hypothesis, remains possible (Cleveland et al 1983).

Whatever the mechanism, there is general agreement that anti-AChR and antimitosin antibody titres are higher in the presence of a thymoma and that this correlates with clinical severity. A thymoma is usually encapsulated and may be cystic and calcified, but it is sometimes malignant. Invasiveness is limited, spread being usually confined to the thorax and occasionally to the lymph nodes of the neck. An account of the histological types described has been given by Iverson (1956). The thymoma which occurs in myasthenia gravis shows a predominance of lymphocytes and epithelial cells which may have an acinar structure. The spindle-celled thymoma has no special association with myasthenia gravis. Indeed, the benign neoplasm may be an associated disorder, the important tissue being the adjacent non-tumour thymus which commonly contains germinal centres (Castleman 1955, Alpert et al 1971).

MUSCLE AND NERVE

Despite frequent statements in the past that myasthenia gravis is a disease without morbid anatomy, the lymphocyte infiltrations of muscle described by Weigert (1901) and termed lymphorrhages (Fig. 19.7) by Buzzard (1905) are found repeatedly in such cases, but not invariably. According to Oosterhuis & Bethlem (1973) they are seen particularly in patients with a thymoma. On rare occasions they occur at the end-plates (Wiesendanger & D'Alessandri 1963). Buzzard (1905) also reported degenerative changes of muscle fibres which were re-investigated and classified by Russell (1953). These changes were regarded as non-specific. Simpson (1960) suggested that they may indicate cell-mediated immunological damage, a concept supported by Rule & Kornfeld (1971).

In some muscles of some patients, denervation atrophy has been reported, the criteria being groups of small muscle fibres with angular cross-sectional contours, and 'target' fibres (Fenichel & Shy 1963, Brody & Engel 1964, Oosterhuis & Bethlem 1973 and others). The histochemical pattern is usually normal but type II fibre atrophy is not uncommon (Brooke & Engel 1969). These changes are not uniquely associated with denervation due to presynaptic pathology: Coers &
Telerman-Toppet (1976) rarely found the increased terminal innervation ratio which they regard as a sensitive index of denervation with reinnervation.

The peripheral nerve trunks are histologically normal in human myasthenia gravis (Oosterhuis & Bethlem 1973) and in EAMG (Hill et al 1977). Collateral sprouting, a hallmark of conventional denervation, is rare in myasthenia and occurs mainly in older patients (Coers et al 1973). By intravital staining with methylene blue, Coers & Desmedt (1959) demonstrated two types of abnormality of the terminal arborisation of motor nerves (Fig. 19.8): a ‘dystrophic’ type, considered to be a reaction to muscle fibre degeneration, and a ‘dysplastic’ type highly characteristic of myasthenia gravis. In the latter there are few terminal knobs and these are arranged serially along a scanty number of terminal branches ending in a remarkably elongated end-plate region, especially in young patients (Coers & Telerman-Toppet 1976). Bickerstaff & Woolf (1960) and MacDermot (1960) confirmed these findings and also described prolific ultraterminal sprouting, important evidence of vigorous but aberrant regeneration.

Ultramicroscopic studies of the end-plate region show poor development of junctional folds and of secondary clefts (Zacks et al 1962, Woolf 1966, Engel & Santa 1971, Fardeau et al 1974). These changes are accompanied by abnormal reduction of the subneural apparatus (Engel & Santa 1971) (Fig. 19.9). Bergman et al (1971) also noted abnormalities of muscle and nerve fibres, damage to Schwann cells, and grossly thickened basement membrane of capillaries but these findings are inconsistent, as are minor changes described in nerve terminals such as dense bodies, myelin figures, mitochondrial abnormalities, and decreased numbers of synaptic vesicles (see review by Engel...
Fig. 19.8 Methylene blue preparations of human motor nerve terminals. a. Normal end-plate. b. Elongated end-plate from a case of myasthenia gravis. c. Motor end-plate with shrunken terminal expansions from a case of myasthenia gravis resistant to neostigmine. d. Axonal sprout with diminutive ending in a case of myasthenia gravis with prominent lymphorrhages — myositic response, previously termed 'dystrophic' (Simpson 1969b, with permission from the publishers and by courtesy of the late Dr A. L. Woolf).

& Santa 1971). Fardeau et al (1974) reported that synaptic vesicles had a normal diameter (about 60 nm) but that the vesicular stacks (at the presumed release sites of ACh) were rarely visible. Nevertheless, they agreed with Engel & Santa (1971) that the changes predominate in the subneural apparatus, which is grossly elongated although the mean nerve terminal area is reduced (measured into the folds). It is generally agreed that the postsynaptic membrane disorganisation is not caused by anticholinesterase medication and that it is of functional significance, because the use of labelled α-Bgtx to identify receptor sites has shown reduced amounts of AChR to a degree which correlates linearly with the decreased miniature end-plate potential amplitude (Engel et al 1977b). Localisation of the IgG and C3 component of complement to receptors of end-plates obtained only from myasthenic patients was identified by Engel et al (1977a) (Fig. 19.10). From morphometric analysis of electron micrographs, these authors found that immune complexes are more abundant in the less severely affected patients than in those with more severe myasthenia (who have less AChR remaining in their end-plates). They interpret this as evidence for a destructive autoimmune reaction involving the postsynaptic membrane. There is functional evidence that anti-AChR antibody increases the degradation and internalisation of junctional (and extrajunctional) receptors of muscle (Reiness & Weinberg 1978) and induces modulation of AChR (Heinemann et al 1978) without involving complement. Fambrough et al (1973) demon-
CAUSATION OF MYASTHENIA GRAVIS

The clinical, pathological, and immunological data now available, with the model provided by EAMG, provide strong support for the Simpson hypothesis of myasthenia gravis as a genetically predisposed autoimmune disorder, the proximate mechanism being destruction of end-plate ACh receptors by a complement-fixing antibody reaction. It is probable, although not proved, that cell-mediated immunity and immunopharmacological blockade of receptors are also important. It is still not known what starts the immunological reaction, although a viral attack on thymus or muscle is an attractive hypothesis. This would account for the occasional finding of IgM antibodies, supplanted by the IgG type in early myasthenia (Lefvert et

strated that the number of ACh receptors is decreased by 70–90%, assuming absence of a population of receptors inaccessible to α-Bgtx (Fambrough 1979). Immunopharmacological blockade, as suggested by Simpson (1960), remains a possibility which is supported by the prompt relapse of myasthenic symptoms noted after retransfusion of homologous cell-free lymph (Matell et al 1976), and by the indication that serum from myasthenic patients reduces the mep amplitude of rat end-plates, an effect which can be reversed by washing with a control solution (Shibuya et al 1978). The relative importance in the human disease of modulation and internalisation, complement-mediated lysis and blockade of receptors is discussed by Engel (1984) and may vary from case to case.

Fig. 19.9 End-plate regions of external intercostal muscles. Acetylcholine receptor sites are stained by α-Bgtx conjugated to horseradish peroxidase, × 22 300. In (a), from a normal subject, AChR is associated with terminal expansions and deeper surfaces of the postsynaptic folds; the presynaptic membrane (arrowhead) stained by leaching, and Schwann cell membrane (arrow) facing crest of folds are lightly stained. In (b), from a case of moderately severe myasthenia gravis, the postsynaptic regions are simplified, only segments of it react for AChR (Engel et al 1977b)
Fig. 19.10 Semi-thin sections of motor end-plates from two cases of myasthenia gravis showing localisation of IgG (a and c) and C3 component of complement (b and d). Reaction at the end-plates is more intense in the case of mild myasthenia gravis (a and b) than in the more severe case (c and d). One of two end-plate regions in c (*) displays only a trace of IgG. Background staining is absent. Unstained sections photographed with a green filter, ×1425 (Engel et al 1977a).

Tindall et al (1978) report elevated titres of complement-fixing antibody to cytomegalovirus (CMV) in myasthenics not treated with thymectomy or steroids. Their suggestion that there is persistent viral antigenic stimulation in the myasthenic thymus, arising from incorporation of viral protein into myoid cells, and subsequent induction of anti-AChR antibody, would be consistent with the thymitis theory reviewed above; the same criticisms would be valid. Nevertheless, virus-induced breakdown of immune surveillance with lack of recognition of self — a strong possibility in all autoimmune diseases — is more likely to be due to infection of cells of the lymphoid system, as a mutagenic agent, than to infection of target organs. Immuno-deficiency is a possible background to myasthenia gravis (Dawkins et al 1975a, Simpson et al 1976) but also to CMV infection. Some important biological aspects of myasthenia gravis are discussed by Rule & Kornfeld (1971) and Drachman (1981).

**DIAGNOSTIC TESTS AND CLINICAL PHYSIOLOGY**

**Performance tests**

The abnormal fatiguability of skeletal muscles shown by bedside tests (p. 495) may be documented in many ways, providing objective data which may be important in the assessment of the pharmacological tests described below, or in evaluating treatment. Diaphragmatic movement, or the ability to swallow a mouthful of barium before and after intramuscular injection of edrophonium, may be observed on the fluoroscope; the objective measurement of diplopia, e.g. with prisms, may be valuable in demonstrating the changing extent...
of weakness. A recording dynamometer or ergogram (Greene et al 1961) is useful and is readily improvised. Other techniques for recording the amount of muscular contraction are ocular tonometry (Campbell et al 1970), nystagmography (Spector & Daroff 1976) and audio-impedance measurement of the stapedius reflex (Blom & Zakrisson 1974). These tests are all occasionally useful when muscular weakness is confined to the appropriate sites.

Electrophysiology

In 1895, Jolly showed that the pathological fatigability of myasthenia could be reproduced by faradic stimulation of a motor nerve while the 'fatigued' muscle would still respond to locally applied galvanism. Electromyographic recording shows that the loss of power when the motor nerve is supramaximally and repetitively stimulated is accompanied by a decrement of the evoked action potential of the muscle, while the antidromically conducted nerve action potential is unchanged in amplitude. In view of the ultrastructural and immunological data, it is unnecessary to present all the facts of neurophysiological observation relevant to the nature of the functional abnormality. For this purpose the reader is referred to reviews by Slomie et al (1968), Simpson (1969b) and Kim (1982). It is now accepted that the essential lesion is degeneration of ACh receptors on the postsynaptic membrane, with or without immunopharmacological blockade, but it is clear from these studies and from computer analysis of evoked motor unit potentials (Ballantyne & Hansen 1974) that additional, if less important, prejunctional and muscle fibre lesions may coexist. The essential electrical features are: (1) subnormal amplitude of spontaneous miniature end-plate potentials (mepps), (2) transmission failure due to lowered safety factor, and (3) intact prejuncional potentiation mechanisms. It has been assumed that a lowered safety factor uncovers a physiological decrement of quantal release following a train of nerve impulses; contemporary studies challenge this. Prejuncional nicotinic receptors may also be involved in the disorder. Subnormal amplitudes of mepps, identified by Elmqvist and colleagues, were at first attributed to production of small quanta of ACh following a prejuncional lesion (Elmqvist 1965), although on theoretical grounds a postjuncional morphological abnormality was at least as probable (Simpson 1971). The evidence of a receptor lesion reconciles the electrophysiological and morphological findings. Decreased amplitude of spontaneous mepps in biopsied intercostal muscle can be used as a diagnostic test because this finding is probably unique to myasthenia gravis, but meticulous technique is necessary for reliable results (Elmqvist & Lambert 1968). Spontaneous negative discharges recorded with macroelectrodes at the end-plate zone (motor point) of human muscle are considered to be miniature end-plate potentials. Lovelace et al (1970) reported reduction in frequency of firing and of burst duration, but the amplitude was only slightly reduced. Grob (1971) found no significant difference between these values and those from normal subjects but he had greater difficulty in locating the negative spontaneous discharges in myasthenic subjects. The value of analysis of spontaneous end-plate activity (not to be confused with 'end-plate noise') has not been proved.

Tests of reduced safety factor for transmission

Electromyographic signs of transmission failure at the neuromuscular junction (Fig. 19.11) are fundamentally: (1) evidence of a decremental muscular response to slow repetitive supramaximal per-neural stimulation in the presence of a normal antidromically conducted motor nerve potential and normal direct muscular excitability; (2) increased 'jitter' and blockings of single muscle fibre responses to repeated nerve impulses (voluntary or evoked). Both are due to subnormal end-plate potentials, summed from subnormal miniature end-plate potentials. The practical applications of these principles are described in Chapter 30 but some riders must be added. Certain electrophysiological phenomena point to additional presynaptic and muscle fibre abnormalities. The decrementing response and increased 'jitter' are non-specific indicators of a reduced safety factor for transmission at the neuromuscular junction. Their presence supports a clinical diag-
nosis of a myasthenic reaction but does not indicate the type of lesion. Normal findings do not exclude a diagnosis of myasthenia gravis. Their role is to support a tentative clinical diagnosis and they do not strengthen the evidence if 'fatiguable' weakness is demonstrable at the gross level.

Pharmacological tests

The reduced safety factor also increases susceptibility to neuromuscular blocking drugs of competitive type such as α-tubocurarine (Ch. 30) and quinine bisulphate. Agonist-competitors, mainly quaternary ammonium drugs such as decamethonium and suxamethonium, have increased agonist and decreased blocking effect. Their use in diagnosis is of historical interest only but should be known to anaesthetists as the paralysant effect is unpredictable in myasthenics and recovery may be prolonged. The myasthenic end-plate has reduced sensitivity to acetylcholine (Engbaek 1951) because of the loss of receptors in the postsynaptic membrane. Acetylcholine is more effective if its hydrolysis is slowed by inhibiting the acetylcholinesterase at the end-plates. Neostigmine, pyridostigmine and other anticholinesterase drugs given orally or parenterally cause temporary restoration of muscle power but the response is delayed and persists for one to six hours, which is inconvenient. The prolonged action and muscarinic stimulation are unpleasant if the subject does not, in fact, have myasthenia. The short-acting anticholinesterase, edrophonium, is more suitable on both counts although it is necessary to inject the drug intravenously and to adopt a strict protocol.
Edrophonium test. The short antimuscarinic effect of edrophonium chloride (Tensilon®) makes it very suitable for a diagnostic test. A syringe is loaded with 1 ml (10 mg) for intravenous injection. Initially 2 mg should be injected to detect sensitivity but if there is no response the remaining 8 mg is injected after 30 s (Osserman & Kaplan 1953). Within 0.5–1 min there is improvement if weakness is due to myasthenia gravis, but weakness returns in 4–5 min. Some normal subjects experience no obvious effects, while others feel a tight sensation around the eyes and fasciculation may be seen for a few seconds. If weakness is due to cholinergic crisis in a patient under treatment it is transiently increased and fasciculation may occur. Unfortunately, respiratory weakness may be increased to an extent endangering life.

The positive responses described are valuable and reliable when present, but failure to obtain improvement or fasciculation does not indicate either that weakness is not myasthenic or that it is cholinergic. Negative responses are sometimes found, but false positive results are very rare provided that only objective criteria are used. Subjective ‘improvement’ should never be relied on, especially when the test is used to assess the adequacy of treatment. The test is best performed at the time of greatest activity of the therapeutic drug: where pyridostigmine is being used, it should be performed one hour after a dose.

Because of the differing degree of involvement of different muscles, it is important to test respiratory and bulbar but not ocular muscles when the test is carried out to differentiate between myasthenic and cholinergic weakness. These vital muscles may be overdosed while the ocular muscles are still underdosed, and failure to recognise this may lead to fatal overdosage. An equivocal or ‘adequate’ response should always be taken to mean that further dosage may be dangerous.

Neostigmine test. Although the latency is greater, a favourable response to injection of neostigmine remains the most convincing evidence of myasthenia gravis because its duration is sufficient to permit repeated testing of all muscles (Viets & Schwab 1935). The duration makes it unsuitable when cholinergic crisis is suspected. Neostigmine methylsulphate is injected intramuscularly (1.5 mg) alone or combined with 0.6 mg of atropine sulphate. Improvement begins in 10–15 min but is most obvious after 30 min. The same preparation may be used intravenously (0.5 mg), when the response is more rapid but the danger of ventricular fibrillation or arrest is greater. The drug should never be given by this route unless accompanied or preceded by atropine. The response to 15 mg of neostigmine bromide orally may be sufficient to make the diagnosis clear. If any of the parenteral tests are equivocal and the diagnosis of myasthenia gravis seems highly probable on clinical grounds, it is worth carrying out a therapeutic trial with oral medication for a week.

The Walker effect

The report by Mary Walker (1938) that exercising a myasthenic limb muscle induced extraocular paralyses has not been confirmed by experienced observers (Johns et al 1956) but was supported by Tsukiyama et al (1959). The test is usually conducted with exercise distal to a tourniquet and Patten (1975) postulated that under hypoxic conditions the myasthenic muscle releases lactic acid which adversely affects other muscles when the cuff is released, possibly by lowering the serum calcium levels. I have never witnessed a convincing Walker effect. There is no acceptable evidence of a blocking substance in serum (Nastuk et al 1959). Cell-free lymph re-injected into a myasthenic patient precipitated weakness which had been reduced by thoracic duct drainage (Matellet al 1976) and passive transfer from man to mouse has been described above. These effects are attributed to immunoglobulin. There is no convincing evidence for a role of other ‘myasthenic toxins’ originating in muscle or thymus.

RADIOLOGY OF THYMUS

The thymus gland is not seen in chest radiographs unless there is a thymoma. Small tumours localised to part of the gland may remain invisible, but those seen at surgery before the gland is sectioned
can almost always be detected on straight films of the chest (posteroanterior and lateral) (Fig. 19.2) assisted by tomography if necessary. Pneum mediastinography or thymic venography are unnecessary and are not recommended. Isotopic scanning of the mediastinum with selenomethionine-75 (Cowan et al 1971) or gallium-67 citrate (Swick et al 1976) gives good imaging of thymomas. As the radiopharmaceutical agent accumulates in numerous tissues, false positive studies are reported, but negative studies are considered to be of value in ruling out the presence of a thymoma. Gallium-67 has become the preferred isotope. It may be valuable in detecting recurrences of malignant thymoma after operation. A large thymus is visible on computed tomography and may be wrongly assumed to be a thymoma (Brown et al 1983).

TREATMENT

There are three facets of treatment: (1) immuno suppression; (2) elevation of the safety factor for neuromuscular transmission; (3) avoidance of factors which lower the safety factor or further embarrass respiration.

Immunosuppressive measures

In principle, the autoimmune disorder may respond to removal or suppression of the thymus, ultrathymic immunosuppression, or removal of antibodies and immuno-aggressive cells.

Thymectomy. For a long time the value of thymectomy was obscured by failure to appreciate that results are different in cases with a thymoma (Keynes 1955). In cases without a thymoma there is a clear benefit in favour of thymectomy, regardless of age or sex of the patient, provided that the disease is still in Stage 1 (Simpson 1958). The contrary view of Papatestas et al (1971) was later reversed (Papatestas et al 1976). Published series since 1960 are too numerous to review but all support these conclusions (Oosterhuis 1984).

There is no clear indication as to which patients are most likely to benefit, apart from those in whom the disease has been of short duration. Failure of thymectomy to prevent myasthenic death occurs more often in patients requiring a large dose of anticholinesterases drugs, but if they do survive (which is now the rule) the ultimate state is not, apparently, correlated with preoperative severity. Patients with severe bulbar weakness are most likely to die, despite operation. On the other hand, if myasthenia remains confined to the extraocular muscles for two years, the prognosis for life is so good that thymectomy has not been considered justified (Grob 1953, Ferguson et al 1955): nevertheless, I believe that it is unusual for weakness to remain so restricted and the operation is now entirely safe, regardless of the surgical method (Fraser et al 1978). The surgical technique (sternal split or transcervical incision) is a matter of choice by the surgeon. Operative morbidity is less with the transcervical approach, but complete removal of the gland is less certain and this is considered desirable if possible. Prejudice against the sternal splitting operation is due to the belief that respiratory support by endotracheal intubation is usually necessary. It is generally unnecessary if anticholinesterase medication is not discontinued (Fraser et al 1978). Undoubtedly, sites of aberrant thymic tissue are easier to reach with the sternal incision.

Even in Stage 1 (active stage), thymectomy is not a cure: it arrests deterioration, promotes remission and makes the further course more benign; this is increasingly evident with the passage of time (Simpson 1958, Perlo et al 1966, Papatestas et al 1976). It is still controversial whether the pathology of the thymus is important. It is generally agreed that the long-term outlook is much poorer if there is a thymoma, although for five to ten years the results of thymectomy may be excellent. Indeed, there are many records of myasthenia becoming first clinically apparent months or years after removal of a thymoma (Koch et al 1970). There are conflicting reports about the correlation between the presence of germinal centres and the response to surgery: some reported series are too small for analysis; others have assessed the histology subjectively. Not surprisingly, they disagree with each other. Differing conclusions are: (1) there is no relationship between thymic histology and postoperative response (Castleman & Norris 1949, Seybold et al
follow thymectomy with immunosuppressive drugs aimed at these cells, and not merely in the most seriously affected patients. It might be argued that adequate chemical immunosuppression would remove the need for thymectomy. This should certainly be investigated but the proof will have to be convincing now that thymectomy carries no operative mortality.

Suppression of the thymus. Alternative means of suppressing the thymus have not been shown to be as effective. Carotid sinus denervation, claimed to cause adrenocortical hypertrophy and thymic atrophy, has been abandoned. Radiotherapy as a method of destroying thymic function is less certain than thymectomy and may cause initial deterioration. Preoperative radiotherapy has been advised before removal of a thymoma (Keynes 1955) and also for non-tumour cases (Schulz & Schwab 1971). The additional benefit has not been proved.

Ultrathymic immunosuppression. Whole-body irradiation reduces the total pool of immune cells and all subsets are equally at risk. Thiopurines and nitrogen mustards depress immune responses by suppression of T-cell-mediated humoral responses, most effectively at the time of antigen presentation and proliferation of antigen-specific T cells. They therefore have the potential of selective suppression of T/B-cell interactions leading to production of IgG class antibodies, but in practice have an appreciable risk of side effects which endanger life. Despite 20 years of clinical trials, cytotoxic drugs have not been established as being superior to thymectomy alone.

Azathioprine. Even in a dosage of 100 mg/day, significant reduction of myasthenia gravis is delayed for 2–24 months, control is inadequate (Hertel et al 1979) and relapse follows withdrawal of treatment after prolonged suppression of symptoms (Hohlfeld et al 1985). When used as a ‘steroid sparer’ with low-dose prednisolone, the metabolic complications of both drugs are reduced but the risk of uncontrolled infection is not, and the clinical advantage has not been established.

Cyclophosphamide. This alkylating immunosuppressant has greater activity than azathioprine
against B cells and other replicating cells. It is more toxic to the gonads. Lymphomas and leukaemias occur more commonly and enhancement of infection is greater. Clinical and neurological remission of myasthenia may be obtained with cumulative dosage of 30 g over a period of one to two years but relapse is common when treatment is stopped (Perez et al 1981).

No cytotoxic immunosuppressive regime has been shown to induce permanent remission, and all are hazardous, but they may have a limited role in treatment of patients who have not been offered thymectomy during clinical stage I.

Corticosteroids; adrenocorticotrophin. Early reports of ‘rebound’ remission after initial deterioration of myasthenia treated by ACTH were not followed up because of early fatalities, until the management of crisis situations improved and intensive therapy units became commonplace. It was then possible to use larger doses of ACTH or prednisolone with striking benefit. In the first seven to ten days of treatment there is often clinical deterioration which is associated with a rise of receptor antibody preceding a sustained fall (Lefvert et al 1978), with marked remission of symptoms. As intubation and supported respiration may become necessary, the treatment should be started in hospital. It appears that any corticosteroid or timing regime may be used (Brunner et al 1976) at any clinical stage including the most advanced. Serum concentration of anti-AChR antibody decreases and anticholinesterase dosage may be reduced progressively (but not abruptly withdrawn). A recommended regime is prednisone 100 mg daily or on alternate days for a month or more. Subsequent withdrawal of prednisone must be very gradual, by no more than 10 mg decrements at six to eight week intervals. Pascuzzi et al (1982) reported no satisfactory improvement in 20% of 116 patients. The median time to maximal improvement was five to six months. Only 14% of patients could discontinue steroid treatment and this was not improved by previous thymectomy. Most benefit is experienced by patients over the age of 50 years, but individual responsiveness is not predictable. Results of treatment are impressive, but the side effects and hazards are not negligible (Brunner et al 1976, Mann et al 1976). It is not known how long steroid treatment must be continued. In my opinion it is not a treatment of first choice but should be reserved for patients not responding to thymectomy and anticholinesterases and preferably not until three years or more after operation. Some early reports were certainly over-enthusiastic and not supported by controlled trial (Howard et al 1976). Indeed, it is by no means certain that the benefit from steroids is due to immunosuppression. A direct neuromuscular action is possible, perhaps on regeneration of end-plates or on acetylcholine release (Weir 1982).

Antilymphocyte and antihymocyte sera. It is possible that cytotoxic and steroid immunosuppression may act on immunocompetent cells in division, presumably in the reticuloendothelial system, and so the phase of activity may be critical. This limitation may not apply to these sera. Antilymphocyte serum acts to deplete paracortical lymph node areas where recirculation of antigen-sensitive lymphocytes occurs. An additional action may be to coat the receptor sites of the lymphocytes. Antihymocyte serum has been used in myasthenic patients with varying degrees of clinical improvement, especially when used after thymectomy (Pirofsky et al 1971, Roux et al 1974). Aureggi et al (1970) also used antilymphocyte serum. Although of theoretical importance, these antisera appear to be of limited practical value.

Removal of antibodies and immunooaggressive cells. Drainage of lymph from the thoracic duct, with removal of 0.5–2.0 l daily, to a total of 4–5 l, causes improvement of myasthenic weakness within 48 h. Although the lymph volume increases again in a few days, there is maintained improvement for a year or more (Matell et al 1976). This treatment has been supplanted by plasmapheresis.

Plasma exchange (plasmapheresis) is an effective method for reducing serum anti-AChR antibody. Newsom-Davis et al (1978), who first used the treatment, reported progressive improvement in strength, commonly after two days. We have had good results with six exchanges of 4 litres. Plasmapheresis followed by immunosuppressive therapy may give long remissions. In most cases, however, relapse occurs in one to three weeks.
Some authors recommend repeated courses of plasmapheresis (Newsom-Davis et al 1981).

Unfortunately, relapse at five to six months is sometimes severe. Susceptibility to infection is temporarily increased by antibody depletion, the role of which is still being assessed. There is no direct relationship between the magnitude of fall in antibody titre and improvement. If it merely removed an immunopharmacological block, antibody depletion would be restricted to the management of myasthenic crises or for preoperative preparation, but it may also permit synthesis of new receptors if followed by long-term immunosuppression (Newsom-Davis et al 1978). The rapid changes in the safety factor for neuromuscular transmission require careful adjustment of anticholinesterase dosage. The efficacy of lymph duct drainage and plasmapheresis depends on the fact that the antireceptor antibody regenerates more slowly than the main pool of immunoglobulin. Nevertheless, increased susceptibility to infection is a hazard, and feedback overproduction of autoantibodies (Bystryn et al 1971) may be dangerous. A method of inhibiting their production selectively would be ideal.

**Induction of anti-idiotypic antibodies.** A second-best procedure would be selective destruction of autoantibody. Preliminary experimental induction of anti-idiotypic antibodies by immunisation against AChR-educated lymphocytes has been reported (Schwartz et al 1978). If confirmed and extended to the human, this would provide a highly effective treatment for myasthenia gravis, but Barkas & Simpson (1982) found that anti-idiotypic antisera do not cross-react between individuals or species. Favourable response to polyclonal antibody mixtures has been reported (Fatch-Moghadam et al 1984). Meanwhile, although treatment of the immunological basis of myasthenia gravis must be a prime consideration, it is always necessary to raise the safety factor for neuromuscular transmission. Anticholinesterase medication should be continued in an appropriate dosage throughout all immunosuppressive treatment.

**Choice of treatment.** Protagonists of thymectomy, immunosuppression and plasmapheresis vigorously support their favoured regime in the literature. I believe that treatment should be based on certain principles: (1) Anticholinesterase drugs must be used with discretion to avoid cholinergic poisoning: there is no evidence that long-term use in therapeutic dosage is harmful in man. (2) Thymectomy is safe and of proven value although rarely curative. (3) Death from myasthenia gravis is rare after the second year if choking is avoided. (4) Immunosuppressant drugs have serious side effects and can rarely be withdrawn without relapse resulting. I therefore recommend that all myasthenics should take pyridostigmine in adequate but not excessive dosage (see below). Anti-immunological therapy is a parallel, not an alternative treatment.

Every patient should be offered thymectomy if there is clinical evidence that myasthenia is not confined to the extraocular muscles and symptoms have not been present for more than seven years. Sex and age do not influence the results if account is taken of the appropriate natural history. If myasthenia is generalised, the severity is not relevant because the future course is not predictable. Late spontaneous remissions are rare. With longer duration before diagnosis, thymectomy is still indicated if there is radiological or serological evidence of thymoma as the tumour is potentially invasive. Late cases failing to respond to pharmacological treatment have less to gain but nothing to lose from thymectomy supervised by an experienced neurologist.

No special preparation is required if the policy of early thymectomy is followed (Fraser et al 1978). It should be delayed until a 'crisis' (see below) is controlled by plasmapheresis or steroids if necessary. Otherwise preoperative steroid therapy is unnecessary. Preparation for operation and treatment of 'crisis' are the only indications for plasmapheresis. Steroids undoubtedly suppress autoimmune reactivity and would be strongly endorsed but for the serious and irreversible side effects. I use them (usually prednisolone) only for patients with life-threatening weakness or where there is a contraindication to surgery. I consider that cytotoxic drugs have little advantage and greater risk. After using them to gain experience
I have abandoned azathioprine and cyclophosphamide. If newer immunoregulatory drugs prove less toxic, the audit will be different.

**Insertion of new receptors.** The rate of insertion of AChR receptors is increased by anti-AChR antibody in EAMG and is highest in denervated muscle. It appears to be indirectly related to the concentration of receptors already present in the postjunctional membrane. Anabolic steroids have no significant clinical effect (J. A. Simpson, unpublished work). De Baets (1984) found a protective effect in rat EAMG but this was related to lower antibody titres in the hormone-treated animals.

**Elevation of the safety factor for transmission**

**Anticholinesterase drugs.** Inhibitors of end-plate acetylcholinesterase raise the safety factor for neuromuscular transmission by preventing hydrolysis of ACh and so prolonging the occupancy of receptor sites by ACh. Anticholinesterases do not increase production of ACh and so are effective for as long as the transmitter is released at motor nerve endings and the ACh receptors of the end-plates are intact. In stage 3 myasthenia this may not be so, at least in some muscles (‘neostigmine resistance’). On the other hand, if inhibition of cholinesterase is carried to excess, so that ACh persists at receptor sites, the end-plate remains depolarised or becomes desensitised (‘cholinergic blockade’) (see p. 653).

Anticholinesterases which have been used previously include physostigmine (eserine), galanthamine, ambenonium, bis-quaternary compounds such as distigmine bromide, and alkyl phosphates. They have been abandoned, either because they cross the blood–brain barrier, with central actions, or because their prolonged action leads to cumulative poisoning.

**Edrophonium chloride (Tensilon®).** This hydroxy-anilinium salt, administered intravenously (2–10 mg) has a peak action in 2–3 min, which rapidly subsides. Although some effect is still apparent 20–30 min later, this is too brief for therapeutic purposes. It is used to confirm the diagnosis of myasthenia or to differentiate between underdosage and overdosage of anticholinesterases (p. 653).

**Neostigmine bromide (Prostigmin®).** The 15 mg tablet of neostigmine has a cholinergic activity which evokes a surge of muscular power for 30–60 min, followed by continued activity at a lower level for 2–6 h. Subsequently, strength is rapidly lost, making it difficult to adjust the timing of dosage. Most myasthenics prefer pyridostigmine for this reason. The ‘boost’ effect of neostigmine is valuable if taken 30 min before a meal or a special physical effort.

**Pyridostigmine bromide (Mestinon®).** Pyridostigmine has less peak effect than neostigmine and its plateau of activity is very little longer but it wanes more slowly, allowing a sustained blood level to be achieved by judiciously timed dosage: this varies from 2–8 h and the frequency must again be established by trial. The 60 mg tablet of pyridostigmine is approximately equivalent to the 15 mg tablet of neostigmine. Each of these drugs should be given by mouth (crushed tablet by nasogastric tube if necessary) in preference to parenteral injection, but in some patients absorption is erratic and it is then necessary to rely on subcutaneous or intramuscular injection of neostigmine methylsulphate (1 mg having an effect equivalent to 15 mg neostigmine or 60 mg pyridostigmine given orally). It should rarely be given intravenously as bradycardia may be dangerous.

**Potentiation of ACh release and muscular responsiveness.** A number of drugs raise the safety factor for transmission by potentiating release of ACh presynaptically and/or by sensitisation of ACh receptors or muscle contraction. The major action is uncertain and they are inferior to anticholinesterases in treatment of myasthenia gravis. Guanidine and the aminopyridines are discussed on p. 657.

**Adrenaline; ephedrine etc.** Adrenaline and its amine analogues have exceedingly weak anticholinesterase activity. They are of no practical value for myasthenia gravis but ephedrine may be
beneficial by combating the bronchoconstriction caused by anticholinesterases (Ringvist & Ringvist 1971). The oral dose is 10–25 mg thrice daily.

Veratrum alkaloids; germin esters. Drugs of this group 'amplify' the muscle response by causing repetitive firing of nerve endings and of the stimulated muscle. Their potential value is insufficient to compensate for important side effects such as hypotension, cardiac arrhythmias and sensory symptoms, although Flacke et al (1966) found these negligible with germine in short-term studies.

Potassium; aldosterone inhibitors. Potassium was once used extensively as an adjuvant in myasthenia gravis. The rationale was obscure and the benefit not documented. It may cause nausea and diarrhoea resembling cholinergic crisis. Spiro- lactone, given to conserve potassium (Gottleib & Laurent 1961), is of no proven value although it gives a sensation of well-being. Provision of potassium to counteract loss of intracellular potassium during steroid therapy is quite another matter and its use is rational (Critchley et al 1977).

Theophylline; caffeine etc. Phosphodiesterase inhibitors (which increase intracellular cAMP) have been reported to increase the strength of myasthenic patients by increasing Ca+ flux at the presynaptic membrane (Dretchen & Standaert 1981). Appel et al (1981) reported stimulation of receptor synthesis of myotubes in culture from calcium and cyclic nucleotides.

Drugs which may lower the safety factor

Enemas may cause sudden death in myasthenics (Keynes 1950). The mechanism is unknown, but may involve stretching of a bowel rendered tonic by anticholinesterases. Corticosteroids, adrenocorticotropic and thyroxin may cause temporary deterioration. Respiratory depressants, including morphine and sedatives, must be used with care, but diazepam is relatively safe. Myasthenic syndromes are very occasionally caused by penicillamine and b-adrenergic blocking drugs (p. 655), but there is no evidence that these remedies aggravate spontaneous myasthenia gravis. Several drugs regularly lower the safety factor for transmission at the neuromuscular junction and should be used (with appropriate adjustment of anticholinesterase dosage) only if the indication is clamant (see Ch. 29).

Inhibitors of production or release of ACh. A number of aminoglycoside antibiotics have this action, including streptomycin, dihydrostreptomycin, neomycin, kanamycin, gentamycin, viomycin, bacitracin, polymyxin A and B, and colistin, especially with renal insufficiency (Hokkanen 1964). Low ionised serum calcium may be implicated in a presynaptic action (Wright & McQuillen 1971).

Blockers of ACh receptors or of muscle response. Any neuromuscular blocking drug must be used with caution in myasthenia gravis. Despite the increased sensitivity to curare (p. 645), D-tubocurarine is the best if relaxation for surgery is required, because its mode of action is consistent and is antagonised by neostigmine. The anomalous responses to depolarising drugs (decamethonium, and suxamethonium and others) are dose-dependent and vary in different muscles (Churchill-Davidson & Richardson 1952).

Membrane stabilisers (hydantoines, quinine, quinidine, procainamide) are, in principle, harmful but rarely cause significant deterioration.

MYASTHENIC CRISIS

There is little justification for this traditional term, which implies sudden spontaneous exacerbation of disease activity. Unquestionably, most cases in the past were examples of unrecognised cholinergic crisis or asphyxia. It is, of course, true that weakness is increased by unusual physical exertion, emotional upset, an infection or childbirth, but it responds to management of the stressful situation. The drugs listed above, which lower the safety factor for transmission or depress respiration, should be used with caution. Myasthenic crisis is rare in well-managed patients.

The absence of cholinergic signs and the presence of a favourable response to the edrophonium test (p. 646) indicate the cause of the severe
weakness. If it is necessary to increase the dose of anticholinesterase medication, the only suitable method in emergency is intramuscular injection of neostigmine. Endotracheal intubation is necessary if pulmonary ventilation is failing, or there is severe dysphagia. Thick, glairy bronchial secretion must be aspirated by bronchosopic suction. Tracheostomy is not required unless the need for passive ventilation continues for more than one week. Once respiration is safeguarded, treatment can proceed methodically without panic measures, the dose of neostigmine or pyridostigmine being regulated by repeated edrophonium titration (Osserman & Kaplan 1953). It is possible that plasma exchange may produce rapid improvement but it cannot be relied on.

CHOLINERGIC CRISIS

Mild muscarinic effects of anticholinesterase medication (colic, diarrhoea, belching, nausea) are not uncommon in myasthenic patients, although less prominent than in normal subjects taking the same dose. More severe muscarinic signs such as vomiting, sweating, hypersalivation, lachrymation, miosis and pallor are less common and indicate that the dose is nearing a dangerous level. The most valuable indication of impending danger is the size of the pupil. It should not be allowed to contract to less than 2 mm diameter in normal room lighting. Bradycardia is very unusual with oral medication, but may be prominent and lead to cardiac arrest with intravenous medication. Hypotension occurs with severe cholinergic poisoning. In the most severe cases, confusion and coma indicate block of cerebral synapses. The use of an antagonist such as atropine sulphate (0.3–0.6 mg) is obligatory with intravenous dosage, but need not be given if the cholinergic drug is administered orally or by subcutaneous injection, unless colic is intolerable. The disadvantage of suppressing the muscarinic symptoms is that more serious nicotinic signs may be overlooked (Schwab 1954). There is, however, no evidence that atropine inhibits the nicotinic signs, the earliest of which is fasciculation of muscles. This need not be a serious sign as it will first appear in muscles unaffected by myasthenia. Persistent fasciculation in the leg muscles is consistent with excellent clinical control.

Conversely, a depolarisation block may be reached without previous fasciculation, or the latter may be transient and therefore overlooked. A muscle may pass from myasthenic weakness to cholinergic block without passing through a stage of normal strength. Poisoning has reached a dangerous level ('cholinergic crisis') when weakness increases because of depolarisation block. This may be difficult to recognise and undoubtedly accounts for most cases of 'neostigmine resistance' not attributable to muscular atrophy. It must be emphasised that different muscles will reflect their degree of myasthenic involvement. Thus, some muscles may suffer cholinergic block while others still require further anticholinesterase medication. As the muscles of respiration are often relatively spared by myasthenia, they may be blocked by a dose of neostigmine which is insufficient for the ocular or limb muscles. It is extremely important to measure the effect of a test dose of edrophonium on the respiratory and bulbar muscles as well as on the more easily tested muscles. Even though short-acting, the additional cholinergic effect of edrophonium may be fatal in cholinergic crisis. In these circumstances atropine should be injected first and there should be facilities for immediate assisted respiration. The test is described on page 464.

Cholinergic paralysis requires urgent treatment. A cuffed endotracheal tube should be passed at once and positive-pressure respiration started. Tracheostomy may be necessary if this has to be prolonged. Atropine sulphate should be injected intravenously (2 mg/h) until signs of atropine toxicity develop. Specific antidotes for anticholinesterase poisoning are not satisfactory in clinical practice. Drugs of the oxime group have some effect on overdosage of quaternary ammonium anticholinesterases (Grob & Johns 1958). Personal experience is limited to pyridine-2-aldoxime (2-PAM) and methane sulphonate (P₂S) but their latency has been found to be too long and their potency and duration of action inadequate for satisfactory treatment. Physiological antagonism can be obtained by the use of d-tubocurarine if respiration is artificially controlled. In these circumstances there is little need for an antidote.
other than atropine to protect the cardiovascular system. Controlled respiration and repeated atropine injection pending the recovery of the neuromuscular response is the most satisfactory form of treatment available at present. Anticholinesterase medication should not be resumed until there is a clear 'myasthenic' type of response to edrophonium on two successive occasions at intervals of 1 h. On resumption, neostigmine should be given by injection and an adequate dose discovered by trial, guided by edrophonium testing. Only when this has been done should oral medication be resumed, at first with neostigmine and then with longer-acting drugs by cautious substitution and prolongation of dose-interval.

DIFFERENTIAL DIAGNOSIS

Myasthenia gravis has to be differentiated from the symptomatic myasthenias described below. More commonly, the problem is to fail to recognize the existence of a myasthenic disorder; once considered, the diagnosis is rarely in doubt. Positive EMG tests (p. 644) are confirmatory but negative tests do not invalidate the diagnosis. A properly conducted edrophonium test, with objective response, is very reliable and a raised titre of anti-AChR antibody in the blood is diagnostic of myasthenia gravis. A normal titre does not, however, exclude the diagnosis.

Myasthenia gravis is commonly mistaken for hysteria because it is so often precipitated by emotional disturbances, and physical signs may be absent if the patient has rested before examination. The intermittent nature of the symptoms and the frequent occurrence of diplopia and dysarthria or other bulbar symptoms may suggest multiple sclerosis. Motor neurone disease, parkinsonism, peripheral neuropathy, and endocrine disorders, particularly thyrotoxicosis, may cause weakness which increases with effort; hypokalaemic states, periodic paralysis, paroxysmal myoglobinuria, botulism, craft palsies and other disorders causing transient paralysis may be confused with myasthenia gravis.

The most difficult disorders to differentiate from myasthenia gravis are the condition termed ‘pseudoptosis’, mitochondrial myopathy and the congenital syndromes with facial and extraocular palsies including congenital ptosis, ocular myopathy and the Von Graefe–Moebius syndrome. None of these conditions responds favourably to anticholinesterase drugs.

OTHER MYASTHENIC SYNDROMES

The syndrome of progressively decreasing muscular power during continuous or repeated contraction, which is relieved by rest, will result from any disorder which lowers the safety factor for neuromuscular transmission, short of complete block. Many of the types described in the literature are detected only during EMG or pharmacological studies and do not show pathological fatiguability on clinical testing. The lesion may be either pre- or post-junctional. Thus Churchill-Davidson & Wise (1963) examined children under the age of six months and found that successive muscle responses to repetitive stimulation of the motor nerve showed a decrement of amplitude, followed by post-tetanic facilitation. Furthermore, such infants were remarkably resistant to high doses of depolarising drugs such as decamethonium. At birth, human motor end-plates are immature, many consisting of terminal clubs, and a terminal arborisation (if present) is simple. Many immature end-plates are seen in children up to the age of two years (Coers & Woolf 1959). It is possible that maturation arrest might account for the unusual case of benign congenital myopathy with myasthenic features described by Walton et al (1956) and for the congenital myasthenic syndrome in a 15-year-old boy described by Engel et al (1976a). Neither of these patients had a worthwhile response to neostigmine. In the case described by Engel et al (1976a) there was acetylcholinesterase deficiency in the subneural apparatus of the motor end-plates and no increase in anti-AChR antibody. The congenital myasthenias are described more fully in Chapter 20.

Cholinesterase deficiency

Deficiency of pseudocholinesterase may be genetic or acquired. In normal life there is no muscular weakness, but prolonged apnoea occurs if the
affected person is given a depolarising relaxant drug (e.g., suxamethonium) and this disorder must then be distinguished from carcinomatous myasthenia. The genetic variety has a number of phenotypes which may be identified by measuring the inhibitory effect of dibucaine or fluorine (Lehmann & Liddell 1969).

Acquired deficiency of pseudocholinesterase is caused by liver disease, pregnancy, and use of certain drugs, phenelzine (a monoamine oxidase inhibitor) and ephedriophate (an organophosphorus compound used as eye drops for glaucoma) (Pantuck & Pantuck 1975). The most important and the only necessary treatment for prolonged response to suxamethonium is adequate pulmonary ventilation, continued for several hours if necessary.

Nutritional, metabolic and toxic myasthenia
A disease named kubisagari in Japan was described in the late nineteenth century. This was an outbreak of paralysis with ptosis and bulbar symptoms. Similar epidemics occurred in prisoner-of-war camps in the Far East. Denny-Brown (1947) reported that parenteral administration of thiamine caused the symptoms to disappear in one week but Japanese authorities now consider that kubisagari was synonymous with myasthenia gravis.

In rare cases of peripheral neuropathy a decrementing response to serial stimulation may be seen, as in diabetic neuropathy, Guillain–Barré syndrome and post-zoster motor neuropathy (Simpson & Lenman 1959, Simpson 1966a). It is also found in other lower motor neurone diseases including polymyelitis, syringomyelia and motor neurone disease. This statement refers to the electrophysiological findings: clinical myasthenia is exceedingly rare in disorders of the lower motor neurones. One condition in which it has been described is acute idiopathic porphyria, in which the myasthenic weakness is said to respond to neostigmine (Gilliespy & Smith 1954).

Muscular weakness of 'myasthenic' type in chewers of tobacco which had fermented as the result of contamination by Clostridium perfringens was described by French authors (Coulonjou & Salaun 1952). This organism usually causes severe myositis (gas gangrene) and it is possible that minimal muscular damage was responsible for the symptoms described, but it is interesting to consider the possibility of an exotoxin such as that produced by Cl. botulinum.

Treatment of patients with a number of β-adrenergic-blocking drugs has resulted in a clinical syndrome described as resembling myasthenia gravis by Herishanu & Rosenberg (1975) who attributed it to a neuromuscular-depressant effect described by previous workers. It should be remembered that serious adverse reactions to some β-blockers are immunological in nature (Behan et al 1976b).

A number of patients have developed typical myasthenia gravis while taking D-penicillamine for rheumatoid arthritis or Wilson’s disease (Bucknall 1977). Marked falls of serum IgA and other immunoglobulins have been reported with penicillamine treatment for non-immunological diseases (Stephens & Fenton 1977) and a lupus reaction has been induced (Golding & Walshe 1977). It therefore seems likely that myasthenia results directly from the drug (and not from association with rheumatoid arthritis) and that it has an immunological pathogenesis. It clears up when D-penicillamine is withdrawn. Nevertheless, as only two cases of myasthenia have occurred during treatment of Wilson’s disease (Dawkins et al 1975b), existing immunological abnormality or genetic constitution may be important predisposing factors. Penicillamine-induced myasthenia gravis has been found to be associated with HLA haplotypes A1, A8 by Bucknall (1977) and Bw, DR1 by Garlepp et al (1983).

Polymyositis and related disorders
A myasthenic type of weakness is commonly present at some stage of polymyositis and dermatomyositis (Walton & Adams 1958). The decrementing neuromuscular response is similar to that of myasthenia gravis and there is usually an initial favourable response to edrophonium or neostigmine. Typically, the ‘fatiguability’ is transient or the response to anticholinesterase drugs is not maintained after the first few doses. A similar transient myasthenic syndrome occurs in systemic lupus erythematosus but classic myas-
Myasthenia gravis also occurs in that disease (Harvey et al 1954). As interstitial myositis may occur in myasthenia gravis, and the diagnostic status of anticholinesterase responsiveness is debatable, it is clear that the diagnosis will often give rise to disagreement. The most reasonable interpretation is that all are autoimmune diseases which often show clinical and serological overlap and association with thymic tumours. Dermatomyositis is particularly related to carcinoma. The EMG findings were described by Simpson (1966a) who drew attention to a marked facilitation during rapid stimulation, and occasional slow augmentation of tension and EMG on voluntary contraction (Simpson & Lenman 1959). These authors and Simpson (1966a) described a number of patients in whom the incrementing response was the major reaction. None of their cases had a malignant tumour or developed one subsequently and some had other EMG or histological characteristics of polymyositis. This intermediate group, which constitutes an important link between polymyositis and the carcinomatous myasthenic syndrome, is now classified as an autoimmune form of Lambert–Eaton syndrome.

Lambert–Eaton syndrome

The occasional occurrence of a myasthenic type of muscular weakness associated with malignant tumours was first recognised when Anderson et al (1953) reported prolonged apnoea after administration of succinylcholine to a patient with bronchial neoplasm, and similar patients were reported in the next two years. As the neuromuscular block was reversed by edrophonium, these authors recognised an abnormal end-plate responsiveness resembling that occurring in myasthenia gravis (p. 646). Croft (1958) found abnormal responses to relaxant drugs in patients with carcinomatous neuropathy, not all of whom had symptoms of muscular fatiguability, and drew attention to the absence of the tendon jerks. The clinical syndrome was first clearly defined by Lambert et al (1961). They found later that the syndrome is usually related to malignant tumour, either contemporaneously or preceding it by several years, but mainly in men over the age of 40, whereas most women with this syndrome do not develop a recognised neoplasm. The tumour is usually a small-cell or oat-cell carcinoma of bronchus; Greene et al (1968) drew attention to possible histological differences in the tumour cells, suggesting a secretory function. Less commonly the syndrome has occurred with intrathoracic reticulum cell sarcoma (Rooke et al 1960) and with carcinoma of breast, colon, stomach, prostate and other organs (Adams 1975).

Association of the non-carcinomatous type with autoimmune diseases was noted by Gutman et al (1972) and Lang et al (1981). Patients with both types of Lambert–Eaton syndrome have a linkage disequilibrium for HLA-B8 and DRw3 antigens and the IgG heavy chain marker Gm(2) (Willcox et al 1985).

The principal symptoms are weakness and fatiguability of proximal muscles of the extremities, particularly of the pelvic girdle and thighs. Careful manual testing often reveals a delay in development of strength at the onset of maximal voluntary contraction. Ptosis may be present, but striking differences from myasthenia gravis are that symptoms of involvement of ocular and bulbar muscular weakness either do not occur, or are mild and transient, and that the tendon reflexes are depressed or absent. Common complaints are aching of the lower limbs, peripheral paraesthesiae, dryness of the mouth and loss of potency.

The significance of these symptoms is commonly apparent in retrospect after the patient has had prolonged apnoea following administration of a muscle-relaxant drug during surgical procedures. The diagnosis is then readily confirmed by characteristic EMG responses to repetitive supramaximal motor nerve stimulation. The rested muscle shows a pronounced depression of the response to a single stimulus. Low rates of stimulation evoke further decrement: stimulation rates above 10/s evoke markedly incremental responses (Lambert et al 1965, Lambert 1966). For further details see Chapter 30. Intracellular recordings from the end-plate region of single muscle fibres reveal normal miniature end-plate potentials but subthreshold end-plate potentials which vary greatly, indicating that a very low number of ACh quanta are released from the nerve ending until it is stimulated repetitively (Elmqvist & Lambert 1968). Pharmacological studies on biopsied muscle have shown a reduction in the number of quanta of
acetylcholine released from the nerve terminal and also of non-quantal release ('molecular leakage') (Mokenaar et al 1982). The transmission characteristics are similar, but not identical, to those of a normal neuromuscular junction exposed to a high Mg$^{2+}$ concentration. The blood level of Mg$^{2+}$ is normal. The quantum content of the end-plate potential is raised by increasing the external calcium ion concentration and with the addition of guanidine.

Pathology. The clinical neurophysiology and pharmacology indicate a prejunctional abnormality of ACh release. In light microscopic specimens, stained intravitaly with methylene blue, Wise & MacDermot (1962) noted irregularity of calibre and abnormal swelling of axons of intramuscular nerve fibres, increased preterminal branching, and abnormally large and complex end-plates which they regarded as consistent with a mild peripheral neuropathy. Engel & Santa (1971) found no significant abnormality in the mean area of motor nerve terminals, and a normal number of synaptic vesicles, but there was possibly a decrease in the mean diameter of the vesicles and of the mean area of mitochondria in the nerve terminals. Freeze-fracture studies of the neuromuscular junction showed loss and disorganisation of presynaptic active zone particles, possibly representing distorted calcium channels (Fukunaga et al 1983a). Engel & Santa (1971) also drew attention to an overdevelopment of the post-junctional region, highly complex secondary clefts and folds, and the sacroplasmic folds contained numerous pinocytotic vesicles. At present it is not understood why the post-junctional region is altered in this way or how it contributes to the functional deficit.

There is indirect evidence for antibody-mediated attack on the presynaptic active zone particles (see below). Serum antibody against ACh receptors is not increased (Lindstrom et al 1976a). There is no report on anti-axon antibody.

Pathogenesis. Speculation about the possible causes of the cancer-related myasthenic syndrome has postulated tumour-derived neurotoxin (Ishikawa et al 1977), polypeptide hormone (Simpson 1982) and immunological disorder (cf previous editions). Immunogenesis is virtually certain, as Newsom-Davis and colleagues have demonstrated that the IgG fraction of plasma from affected patients when injected into mice evokes a similar syndrome (Lang et al 1983, 1984). It is probable that an IgG antibody binds to nerve terminal determinants concerned with release of ACh, causing the distortion of active zones observed by Fukunaga et al (1983a, b). The London and Mayo Clinic groups in collaboration showed that paucity and disorganisation of active zone particles and reduction of quantal content of ACh could be induced in the nerve terminals of mice injected with IgG from patients with the myasthenic syndrome. Late components of complement are not required (Prior et al 1985). Voltage-dependent calcium channels necessary for quantal release of ACh may be damaged. Presumably the postsynaptic changes which are so distinctive morphologically must be secondary. The mechanism of induction of the apparent antibody is unknown. As in most autoimmune disorders, a reduction of circulating suppressor T cells (as marked by the OK T8 monoclonal antibody) has been reported (Robb et al 1985) but, surprisingly, only in the tumour-associated type.

Treatment. Evidence for humoral autoimmunity has encouraged treatment with corticosteroids (previously used empirically), cytotoxic immunosuppressants and plasma exchange as for myasthenia gravis (Newsom-Davis & Murray 1984). The most favourable treatment regime has not yet been established. Pending recovery of nerve terminal function, release of acetylcholine may be potentiated by several drugs. Anticholinesterase drugs (e.g. pyridostigmine) are temporarily beneficial. Guanidine hydrochloride is strikingly effective on prolonged administration. Lambert (1966) recommended 20–30 mg/kg/day in divided dosage: the effect is superior to that of 4-amino- pyridine or 3-4-diaminopyridine (Lundh et al 1977, 1982), both of which lower the threshold for seizures. Where tumour has not been identified but may be occult, immunosuppressant treatment may accelerate growth of oat-cell carcinoma and Newsom-Davis & Murray (1984) advise caution in heavy smokers with the Gbm(2) heavy chain phenotype.
REFERENCES

Alter N M, Osnato M 1930 Myasthenia gravis with status lupus and multiple thymic granulomas. Archives of Neurology and Psychiatry 23:345
Barkas T, Simpson J A 1982 Lack of inter-animal cross-reaction of anti-acetylcholine receptor antibodies at the receptor-binding site as demonstrated by heterologous anti-idiotypic antisera: implications for immunotherapy of myasthenia gravis. Clinical and Experimental Immunology 47:119
Bickerstaff E K, Woolf A L 1960 The intramuscular nerve endings in myasthenia gravis. Brain 83:10
Bosch E P, Reith P E, Granett D K 1978 Myasthenia gravis and Schirmer syndrome. Neurology (Minneapolis) 27:1179
Brenner T, Beyth Y, Abramsky O 1980 Inhibitory effect of α-lactoprotein on the binding of myasthenia gravis antibody to acetylcholine receptor. Proceedings of the National Academy of Sciences USA 77:3635
Brolley N, Hollender N H 1955 Psychological problems of patients with myasthenia gravis. Journal of Nervous and Mental Disorders 122:178
Brooke M H, Engel W K 1969 The histographic analysis of human muscle biopsies with regard to fiber types. 3. Myotonia, myasthenia gravis, and hypokalemic periodic paralysis. Neurology (Minneapolis) 19:469
Bundey S, Donach D, Soothill J T 1972 Immunological studies in patients with juvenile-onset myasthenia gravis and in their relatives. Clinical and Experimental Immunology 11:321
Buzzard E F 1965 The clinical history and post-mortem examination of five cases of myasthenia gravis. Brain 88:438
Castleman B, Norris E H 1949 The pathology of the thymus in myasthenia gravis. A study of 35 cases. Medicine (Baltimore) 28:27
Cohen S J, King F H 1932 Relation between myasthenia gravis and exophthalmic goitre. Archives of Neurology and Psychiatry 28:1338
Cohen S M, Waxman S 1967 Myasthenia gravis, chronic lymphocytic leukemia, and autoimmune hemolytic anemia. Archives of Internal Medicine 120:717
Croft P B 1958 Abnormal responses to muscle relaxants in carcinomatous neuropathy. British Medical Journal 1:181
De Baets M H 1984 Autoimmunity to cell surface receptors. Leiter-Nyrels, Maastricht
Denny-Brown D 1947 Neurological conditions resulting from prolonged and severe dietary restriction. Medicine (Baltimore) 26:41
Dianian A Y, Beutner E H, Witebsky E 1964 Tanned-cell haemagglutination test for detection of antibodies in sera of patients with myasthenia gravis. Journal of Laboratory and Clinical Medicine 63:60
Elmqvist D 1965 Neuromuscular transmission with special reference to myasthenia gravis. Acta Physiologica Scandinavica (64 suppl) 249:1
Engback L 1951 Acetylcholine sensitivity in diseases of the motor system with special regard to myasthenia gravis. Electroencephalography and Clinical Neurophysiology 3:155
Engel A G 1961 Thyroid function and myasthenia gravis. Archives of Neurology 4:663
Engel A G, Santa T 1971 Histometric analysis of the ultrastructure of the neuromuscular junction in myasthenia gravis and in the myasthenic syndrome. Annals of the New York Academy of Sciences 183:46
Engel A G 1984 Myasthenia gravis and myasthenic syndromes. Annals of Neurology 16:519
Fambrough D M, 1979 Control of acetylcholine receptor in skeletal muscle. Physiological Reviews 59:165
Fambrough D M, Drachman D B, Satyanarumi S 1973 Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. Science 182:293
Golding D N, Walshe J M 1977 Arthopathy of Wilson's
The endocrine thymus: potential role for thymosin in the
Academy of Sciences 274:390
Goldstein G, Whittingham S 1966 Experimental autoimmune
thymitis. An animal model of human myasthenia gravis.
Lancet 2:215
Gottlieb D, Johns R J 1958
Small cell carcinoma of
the thymus: potential role
of thymosin in the
Academy of Sciences 183:248
Green P 1958 Aplastic anaemia associated with thymoma.
Report of two cases. Canadian Medical Association Journal
78:419
Small cell carcinoma of lung. Observations on four
patients including one with a myasthenic syndrome.
Archives of Internal Medicine 122:333
Greene R, Rideout D F, Shaw M L 1961 Ergometry in the
diagnosis of myasthenia gravis. Lancet 2:281
Grob D 1953 Course and management of myasthenia gravis.
Journal of the American Medical Association 153:529
Grob D 1958 Myasthenia gravis. Current status of
pathogenesis, clinical manifestations, and management.
Journal of Chronic Diseases 8:536
Grob D 1971 Spontaneous end-plate activity in normal
subjects and in patients with myasthenia gravis. Annals of
the New York Academy of Sciences 183:248
Grob D, Johns R J 1958 Use of oximes in the treatment of
intoxication by anticholinesterase compounds in patients
with myasthenia gravis. American Journal of Medicine
24:512
Gutman L, Crosby T W, Takamori M, Martin J D 1972
The Eaton-Lambert syndrome and auto-immune disorders.
American Journal of Medicine 53:854
Harvey A M 1948 Some preliminary observations on the
clinical course of myasthenia gravis before and after
thymectomy. Bulletin of the New York Academy of
Medicine 24:505
Harvey A M, Shulman L E, Tumulty P A, Cowley C L,
Review of the literature and clinical analysis of 138 cases.
Medicine (Baltimore) 33:291
Hausmanowa-Petrusewicz I, Chorzelski T, Strugalska H 1969
Myasthenia gravis and psychosis. Report of a case with
observations on its psychosomatic implications.
Psychosomatic Medicine 3:120
Heinemann S, Merlie J, Lindstrom J 1978 Modulation of
acetylcholine receptor in rat diaphragm by anti-receptor
sera. Nature 274:62
Herchamru V, Rosenberg P 1975 Ig-blockers and myasthenia
gravis. Annals of Internal Medicine 83:834
Herrmann C 1971 The familial occurrence of myasthenia
gravis. Annals of the New York Academy of Sciences
183:334
Hertz G, Mertens H G, Reutterer P, Ricker K 1979 The
treatment of myasthenia gravis with azathioprine. In: Dau
P C (ed) Plasmapheresis and the immunobiology of
myasthenia gravis. Houghton Mifflin, Boston, p 315
Experimental autoimmune myasthenia gravis: no
morphometric abnormalities of nerve trunks. Neurology
(Minneapolis) 27:200
gravis and epilepsy. Archives of Neurology and Psychiatry
80:10
Hohlfeld R, Tovka K V, Besinger V A, Gerhold B.
Heininger K 1985 Myasthenia gravis; reactivation of
disease and of autoimmune factors after
discontinuation of long-term azathioprine. Annals of
Neurology 17:238
Holkanen E 1964 The aggravating effect of some antibiotics
on the neuromuscular blockade in myasthenia gravis. Acta
Neurologica Scandinavica 40:346
Howard P M, Duane D D, Lambert E H, Duube J R 1976
Alternate day prednisone. Preliminary report of a double-
blind controlled study. Annals of the New York Academy of
Sciences 274:596
Ishikawa K, Engelhardt J K, Fujisawa T, Okamoto T,
Katuki H 1977 A neuromuscular transmission block
produced by a cancer tissue extract derived from a patient
with the myasthenic syndrome. Neurology (Minneapolis)
27:140
Iversen L 1956 Thymoma. A review and reclassification.
American Journal of Pathology 32:695
Jacobi A, Klick E R, Emerick A E H 1968 Genetic study of
sample of 70 patients with myasthenia gravis. Journal of
Medical Genetics 5:257
Johns R J, Grob D, Harvey A M 1956 Studies in
neuromuscular function. 2. Effects of nerve stimulation in
normal subjects and in patients with myasthenia gravis.
Bulletin of the Johns Hopkins Hospital 99:125
Joseph B S, Johns T R 1973 Recurrence of non-neoplastic
thymus after thymectomy for myasthenia gravis.
Neurology (Minneapolis) 23:109
Kazakos A, Pirsckanen R, Tsilkanen A 1975 LD antigens
associated with HL-A8 and myasthenia gravis. Tissue
Antigens 6:175
Kao I, Drachman D B 1977a Thymic muscle cells bear
acetylcholine receptors: possible relation to myasthenia
gravis. Science 195:74
Kao I, Drachman D B 1977b Myasthenic immunoglobulin
accelerates acetylcholine receptor degradation. Science
196:527
Keesey J, Lindstrom J, Cokely H, Herrman C 1977 Anti-
acetylcholine receptor antibody in neonatal myasthenia
gravis. New England Journal of Medicine 296:1
Keynes G 1950 Thymectomy for myasthenia gravis. In:
Maingot R H (ed) Techniques in British surgery.
Saunders, Philadelphia, p 126
Keynes G 1955 Investigations into thymic disease and
neuromuscular blockade. British Journal of Surgery 42:449
Kim Y I 1982 Neuromuscular transmission in myasthenia
gravis. Seminars in Neurology 2:199
Koch F, Regli F, Reimle W 1970 Myasthenia gravis nach
Thymektomie. Schweizerische medizinische Wochenschrift
100:65
Laquer L, Weigert C 1901 Beitrag zur Lehre von der
Erb'schen Krankheit uber die Erb-sche Krankheit
(Myasthenia Gravis). Neurologisches Centrallblatt 20:594
Myasthenic syndrome occasionally associated with
bronchial neoplasm: neurophysiologic studies. In: Viets
H R (ed) Myasthenia gravis. Thomas, Springfield, p 362
Lambert E H, Okhio M, Rooske E D 1965 Clinical
physiology of the neuromuscular junction. In: Paul W M,


Lefvert A K 1982 Differences in the interaction of acetylcholine receptor antibodies with receptor from normal, denervated and myasthenic human muscle. Journal of Neurology, Neurosurgery and Psychiatry 45:70

Lefvert A K, Pirskanen R 1977 Acetylcholine receptor antibodies in cerebrospinal fluid of patients with myasthenia gravis. Lancet 2:351


Lefvert A K, Osterman P O 1983 Newborn infants to myasthenic mothers: a clinical study and an investigation of acetylcholine receptor antibody in 17 children. Neurology 33:133


Lindstrom J 1977 An assay for antibodies to human acetylcholine receptor in serum from patients with myasthenia gravis. Clinical Immunology and Immunopathology 7:36


MacDermot V 1960 The changes in the motor end-plate in myasthenia gravis. Brain 83:23

Meecham D, Parnell J L 1948 The relationship of hyperthyroidism to myasthenia gravis. Journal of Clinical Endocrinology 8:342


Nambo T, Grob D 1970 Familial concurrence of myasthenia gravis and rheumatoid arthritis. Archives of Internal Medicine 125:1056


Nastuk W L, Osserman K E 1962 Search for a neuromuscular blocking agent in the blood of patients with myasthenia gravis. American Journal of Medicine 36:294


Newsom-Davis J, Willcox N, Scadding G, Calder L, Vincent A 1981 Anti-acetylcholine receptor antibody synthesis by


Osmer K E, Kaplan L I 1953 Studies in myasthenia gravis: use of edrophonium chloride (Tensilon) in differentiating myasthenia from cholinergic weakness. Archives of Neurology and Psychiatry (Chicago) 70:385


Patrick J, Lindstrom J 1973 Autoimmune response to acetylcholine receptor. Science 180:871

Patten B M 1975 A hypothesis to account for the Mary Walker phenomenon. Annals of Internal Medicine 82:411


Reineke C G, Weinberg C B 1978 Antibody to acetylcholine receptor increases degradation of junctional and extrajunctional receptors in adult muscle. Nature 274:68


Russell D S 1953 Histological changes in the stripped muscles in myasthenia gravis. Journal of Pathology and Bacteriology 65:279


Schwab R S 1954 Belladonna drugs in cholinergic poisoning
during treatment of myasthenia gravis. Journal of the American Medical Association 155:1445
Schwartz M S, Stalberg E 1975a Myasthenia gravis with features of the myasthenic syndrome. An investigation with electrophysiologic methods including single-fiber electromyography. Neurology (Minneapolis) 25:80
Shibuya N, Mori K, Nakazawa Y 1978 Serum factor blocks neuromuscular transmission in myasthenia gravis: electrophysiologic study with intracellular microelectrodes. Neurology (Minneapolis) 28:804
Strom-Mathisen A 1961 Myasthenia gravis. Archehong/Arimiuz and Witkell, Oslo/Stockholm
Strupper P 1954 Elektromyographische Studien zum Wirkungsmechanismus Endplatten blockierender Stoffe. Aerzteliche Forschung 8:564
Symmers D 1952 Malignant tumours and tumour-like growths of the thymic region. Annals of Surgery 95:544
Te Velde K, Huber J, van der Slikke L B 1966 Primary acquired hypogammaglobulinemia, myasthenia and thyromma. Annals of Internal Medicine 65:534
Tindall R S A, Cloud R, Luby J, Rosenberg R N 1978 Serum antibodies to cytomegalovirus in myasthenia gravis:


Weigert C 1901 Pathologisch-anatomischer Beitrag zur Erb-Ucht Krankheit (Myasthenia Gravis). Neurologisches Zentralblatt 20:597


Wilcox H N A, Newsom-Davis J, Calder L R 1984 Cell types required for anti-acetylcholine receptor antibody synthesis by cultured thymocytes and blood lymphocytes in myasthenia gravis. Clinical and Experimental Immunology 58:47

Wilcox N, Demaine A G, Newsom-Davis J, Welsh K I, Robb S A, Spiro S G 1985 Increased frequency of IgG heavy chain marker Glm (2) and of HLA-B8 in Lambert-Eaton myasthenic syndrome with and without associated lung carcinoma. Human Immunology 14:29

Wills T 1672 De anima brutorum. Oxford, p 404


MYASTHENIA GRAVIS IN PREGNANCY:

John A. Simpson
Glasgow University Department of Neurology, Institute of Neurological Sciences
Southern General Hospital, Glasgow.

The highest incidence of myasthenia gravis is in women of reproductive age. Keynes (1952) pointed out that many women with myasthenia gravis (MG) are weaker at some time in the menstrual cycle. In my experience the timing is individual, being in the first 3-4 days of menstruation in some women whereas in others weakness is greater in the premenstrual week and muscular power improves when bleeding starts. Schrire (1959) investigated pregnandiol metabolism in myasthenic women. He reported that there was an abnormally low urinary excretion of pregnandiol during the proliferative and luteal phase of the menstrual cycle. Schrire injected myasthenic women with progesterone and reported a low recovery of pregnandiol from their urine. Injection of adrenocorticotrophin resulted in recovery of very large amounts of pregnandiol. His suggested explanations do not carry conviction and Dr K. Fotherby (MRC Clinical Endocrinology Unit, Edinburgh) was unable to confirm these results in my patients (Simpson, 1960). The latter paper introduced the autoimmune hypothesis of MG, now validated, and endocrine studies have lapsed since then. Occasional attempts to influence MG by progesterone or oestrogens are referred to by Rowland et al (1966).

In MG, a life-threatening disease, muscular weakness is precipitated by emotional as well as physical stress. It might be anticipated that pregnancy and parturition would be hazardous. On the contrary, most myasthenic women have some degree of remission during pregnancy but, as with menstruation, there is great individual variation. Simpson (1964) described two patients, one of whom improved at the putative time of conception (defined accurately as it occurred during her husband's return on short leave during the Second World War). Myasthenia recurred just before the first post-parum period at three months. The other patient was symptom free for two years after thymectomy when she relapsed. She later relised that she was pregnant. When pregnancy was terminated at the fifth month the myasthenic symptoms disappeared on the same day. Onset of MG immediately following conception was previously recorded by Tilney (1907).

Before thymectomy and immunosuppression became established treatments, all authors agree that the effect of pregnancy on myasthenic weakness is very variable (Kennedy and Moersch, 1937; Viets et al, 1942; Harvey, 1948; Fraser and Turner, 1953). Some patients have a relapse, others a remission. From a study of only eight patients, Viets et al (1942) concluded that there was
commonly a moderate relapse in the first trimester and often a remission, sometimes complete, during the last six months. The pattern may be diametrically opposite in different pregnancies in the same patient. Fraser and Turner (1953) described 15 pregnancies in 14 myasthenic women. They agreed with Harvey (1948) that the course was most variable but were unable to confirm the late-pregnancy remission described by Viets et al (1942). They reported that labour was relatively normal but advised that the mother should be admitted to hospital 2-3 weeks before the expected date to anticipate or avoid premature onset of labour. All the authors cited agree that a normal labour can be expected, other circumstances being equal, and my experience concurs. It is acknowledged that prolonged labour may precipitate myasthenic exhaustion and that there is a danger of relapse post partum, especially during the first three weeks. Fraser and Turner (1953) noted post-partum relapse in half of their patients.

Simpson (1964) recorded the course of 120 pregnancies as judged by retrospective interviews and a study of contemporary case records. In 30% of cases there was no significant change of myasthenic status during or after pregnancy. Most of these patients felt fitter than usual, as in many normal pregnancies, but could not be said to show genuine remission of MG. Of the remainder, as many improved as relapsed during pregnancy, but if this group was tabulated as a 'balance chart' (Fig 1) it could be seen that in general there is a tendency to relapse in the first trimester, to remit in the second and third, and to relapse again after the child is born. Similar findings were reported by Seitz (1966) with the additional observation of of first manifestation of MG by the mother in the first trimester. In my cases the late relapse often started during or immediately after labour which sometimes required instrumental assistance. Fraser and Turner (1953) probably underestimated the dangers of labour, but with good neurological supervision a normal labour can usually be expected and MG does not normally constitute an indication for termination of pregnancy (Osserman, 1956; Simpson, 1964). Five of the pregnancies in Fig 1 were aborted or terminated (exact records are no longer available) in the second trimester.

It has been assumed that the fluctuating state and individual differences reflect hormonal status of the mother but no biochemical basis has been established. Simpson (1964, 1971) drew attention to the patient's emotional state as an important factor which correlated highly with the 'balance chart'. A wanted pregnancy sometimes seems to be associated with remission and so is a desired termination. An illegitimate pregnancy or an accidental miscarriage may precipitate a relapse. The curve of Fig 1 parallels the emotional responses
of many normal women to their pregnancy, labour, and the fatigue of the puerperium.

The psychological factor may, however, be fortuitous. These observations were made before it was possible to identify the antiacetylcholine receptor (antiAChR) antibody which damages the nicotinic receptors of the endplates of skeletal muscle, and it would be valuable to make serial clinical, serological, and psychological studies throughout pregnancy. It is interesting to note that I have no records of pregnancy occurring in a myasthenic patient who is known to have had a thymic tumour, but thymoma-related MG is rare in the reproductive age group and otherwise fertility appears to be normal.

Other clinical observations which should still be recorded concern the relationship between the putative pregnancy effect on the myasthenic woman and the probability that the child will develop neonatal MG. For instance, are those women who fail to improve in the second trimester more likely to have a myasthenic child? In the writer's 1964 series, most abortions were in the group which improved in the second trimester but there are alternative possible immunological explanations for this (Simpson, 1991) which were not known when the earlier data were gathered. In the original hypothesis (Simpson, 1960) it was suggested that the phenomena of neonatal MG compared with the failure of transference of MG by interadult blood transfusion might be due to tissue compatibility factors. There are now a few HLA studies and peri-partum antibody assays in neonatal MG but none on the mother-child relationship in those pregnancies where the child is not affected. These data are essential to determine whether the risk-benefit balance to both mother and child are due to pregnancy related fluctuations in the mother's production of antiAChR antibody, because the alternative possibility that pregnancy or the products of conception induce protective factors is of fundamental importance for future work on the suppression of MG and other autoimmune diseases. A hormonal factor is not necessarily excluded since clinical studies suggest a hypothalamo-pituitary influence on susceptibility to MG (Simpson, 1960) and there is increasing evidence for a role of the neuroendocrine system in the regulation of the immune system (Ader, 1981; Martin, 1984) and a feedback effect on the hypothalamo-pituitary axis could account for the occasional reports of a 'see-saw' relationship between thyrotoxicosis and MG (McBachern and Parnell, 1948). A more probable mechanism would be that the mother and fetus are both protected from attack by the maternal immunological system by immunoprotective products of the conceptus.
Alpha-fetoprotein has been investigated in this context and shown to influence the myasthenic status of animals with experimental MG (Brenner et al, 1980) but seems unlikely to be the sole or the major factor. Possible pregnancy sustaining inhibitory immunological mechanisms are still uncertain (Pavia et al, 1987). Further developments in this field would have considerable implications for the control of MG in both sexes. The main purpose of this paper and related papers (Simpson, 1991a,b) is to invite the collaboration of obstetricians and paediatricians throughout the whole course of pregnancy in myasthenic women in addition to any clinical assistance the neurologist may provide. Women who improve in pregnancy and produce an unaffected child are even more important biologically than their unfortunate sisters.
BRENNER T, BEYTH Y, ABRAMSKY O. Inhibitory effect of α-fetoprotein on the binding of myasthenia gravis antibody to acetylcholine receptor. Proc Nat Acad Sci USA 1980;77,3635-3639.


SIMPSON JA. Recurrent intrauterine death associated with the anticardiolipin antibody in myasthenia gravis. Submitted for publication 1991a.

SIMPSON JA. Neonatal myasthenia gravis. Submitted for publication 1991b.

TILNEY F. A case of myasthenia gravis pseudoparalytica with adenoma of the pituitary body. Neurographs (Brooklyn), 1907;1,20.

Fig 1  Balance chart of numbers of pregnancies in which myasthenic status improved ('remission') or deteriorated ('relapse') in the three trimesters of pregnancy and two post-partum trimesters (shaded). The majority of pregnancies with no significant change of myasthenic status are excluded from the chart.
RECURRENT INTRAUTERINE DEATH ASSOCIATED WITH THE ANTICARDIOLIPIN ANTIBODY IN MYASTHENIA GRAVIS

John A. Simpson MD, FRCP, FRSE
Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow.

If the relative remission of myasthenia gravis (MG) in the course of pregnancy and the proposed in utero protection of the fetus are related (Simpson 1991a,b), it might be supposed that fetal survival would be within the normal range, but Kosotsky et al (1955) reported a high rate (27%) of spontaneous abortion in myasthenic women. In 1964 Simpson recorded 15% fetal loss in 81 known pregnancies in 59 women. This is presumably a minimum figure. As it did not indicate a special tendency to spontaneous abortion, no detailed study was made in later patients. My interest in the matter was renewed in 1983 when recurrent abortion became a major concern of a patient with comparatively mild MG.

Case 1. AG, a young woman from the north of Scotland, had early symptoms of myasthenia gravis in the spring of 1980 when she was 29 years of age. Starting in the muscles of both hands, it rapidly became generalised with involvement of bulbar and ocular muscles. She was referred to the author when 14 weeks pregnant, having had four previous pregnancies, all of which miscarried, at 23, 7, 32 and 23 weeks of gestation. In one case the fetus (male) was reported as normal and a placental infarction was considered to be the cause of abortion. She complained of joint pains in wet weather but had no clinical or serological evidence of rheumatoid arthritis and no histoty indicative of any other recognized autoimmune disease, but she had a skin allergy to penicillin. Her mother had had rheumatoid arthritis and pernicious anaemia.

Clinical, electrophysiological and pharmacological examination confirmed the diagnosis of MG. During the pregnancy, treatment was restricted to pyridostigmine, 660mg/day in 2-hourly oral doses. The pregnancy continued normally until mid-term when she aborted. The resulting depression was associated with temporary exacerbation of myasthenia, followed by improvement with reduction of pyridostigmine to 420mg/day within three months. Trans-sternal thymectomy was carried out in October 1981 (Mr H I Tankel, Glasgow) with complete removal of the thymus which was large with many germinal centres (Dr D Doyle, Glasgow). The post-operative course was uneventful.

The severity of myasthenia fluctuated in the next two years. Steroids were not used as the general trend was towards improvement. She was still
within the latent period for benefit from thymectomy (Sjöpse, 1958) and was determined to try another pregnancy. In June 1983 she became pregnant for the sixth time. She remained in good health with good myasthenic control and was able to work part-time as a home-help. At 10 weeks the obstetrical findings were normal but at 14 weeks an ultrasonic scan showed a cyesis appropriate to 14 weeks gestation with no obvious heart action (Dr D A R Lees, Inverness). The pregnancy was terminated by an extra-amniotic infusion of prostaglandin on 7 October 1983 when a high level of alpha-fetoprotein in the blood and a repeat scan confirmed fetal death. The aborted fetus appeared grossly normal.

Up to that time biochemical and immunological studies (Table 1) were indicative of uncomplicated MG, but in the same month a leading article in the British Medical Journal drew attention to an association between recurrent intra-uterine deaths and lupus anticoagulant, anticardiolipin and other lupus related antibodies. By courtesy of Drs G R V Hughes and L E Hart of Hammersmith Hospital, London the anticardiolipin antibody (ACA) was assayed by the method of Harris et al (1983a). The titre was 18 standard deviations above normal control levels.

Mrs AG's blood group was 0 Rh positive (CC De). Her HLA phenotype (Prof H Dick, Glasgow) was A1, A2, CW4, B6, B12(4), Bw4, Bw6. Prof M Ferguson Smith FRSS (Glasgow) made a chromosome analysis by Giemsa banding. Mrs AG and her husband were normal female and male karyotypes respectively.

Being in very substantial remission of myasthenia, the patient wanted to try one more pregnancy. In view of the probable immunological basis for the previous abortions it was agreed, after discussion with her family doctor and obstetrician to try to carry another pregnancy to term with immunosuppression started before conception. Increasing arthralgia suggested active systemic lupus erythematosus (SLE) but the DNA binding capacity was 20%. In February 1985 studies of tissue and blood thromboplastin inhibition showed very mild lupus-type inhibition (Dr B Benett, Aberdeen) and a negative test for lupus anticoagulants (Prof J Vermylen, Leuven). Although these findings suggested decreasing lupoid activity it was agreed to start her on prednisolone along with aspirin and pyridostigmine (with cimetidine to minimise the high risk of gastric bleeding on this regime).

Myasthenia went into full remission and all treatment was gradually withdrawn in April 1986. In June she became pregnant. Prednisolone and aspirin were restarted. In August the anticardiolipin titres were very high (Hammersmith Hospital) but dropping, she had no clinical myasthenia, and measures of complement activity (C3, C4, Factor B and C1 inhibitor) were normal. Cyesis was normal until severe proteinuric pre-eclampsia developed at 32 weeks gestation. A normal baby was delivered by Caesarean section (Dr K S Stewart, Stirling).
The mother had a very high titre of anti-AChR antibody ($4624 \times 10^{-12} \text{M}$) but the cord blood had none.

In the puerperium the mother had occasional myasthenic symptoms but she developed pleurisy with haemoptysis at five weeks. At 10 weeks she was found to have active endocarditis with aortic incompetence. Post partum, levels of ACA rose from 120 to 310. She is now under treatment for active SLE with only occasional myasthenic symptoms.

**Case 2** AE, a young woman with slight right ambyopic strabismus, had no diplopia until she was 20 years old when she developed intermittent diplopia and ptosis, worse in the evening. It was relieved by edrophonium and pyridostigmine. A clinical diagnosis of ocular MG was supported by a high serum titre of anti-AChR antibody (Table 1). One year previously a thyroid cyst was excised from a non-toxic nodular goitre. Her mother and brother had thyrotoxicosis and a grat-aunt had pernicious anaemia.

Thymectomy was not advised as myasthenia remained confined to ocular muscles. After one year on pyridostigmine she had complete clinical remission for eight years although anti-AChR antibody persisted. In the following six years she had occasional relapses, well controlled without steroid therapy.

Her first pregnancy, at age 26 years, was uneventful. A healthy female child was born by spontaneous labour at 36 weeks. A second pregnancy, at age 29 years, was aborted at 12 weeks. A third pregnancy, at age 31 years, appeared to be proceeding normally until vaginal bleeding started at 24 weeks. Emergency section was required for abruptio placentae at 27 weeks. The 1.1kg infant died two days later from intraventricular haemorrhage. Mrs AE then decided to have tubal ligation.

In February 1985, at age 34 years, a blood sample obtained for measurement of anti-AChR antibody titre ($75 \times 10^{-7} \text{M}$) did not clot. (As she had left the Institute, no coagulation screen was obtained.) In view of the obstetric history an aliquot was sent to Hammersmith Hospital (Dr L E Hart). Lupus anticoagulant was not assayed but anti-cardiolipin IgG was detected with a binding index of 3.44 (upper limit of normal, 3.0).

When last seen in 1986 the patient was clinically well with no myasthenic signs, euthyroid and without obvious pathology of other organs. She refused further investigation as she menstruated regularly and had been sterilised.

**DISCUSSION**

Escobar et al (1982) reported a gonadotrophic resistant ovary syndrome in myasthenia gravis which they suggested may have an autoimmune mechanism, but this would not account for recurrent intrauterine deaths. The cases reported here are believed to be the first recorded in MG associated with an anti-cardiolipin antibody. The mechanism of abortion could be similar to
that described by Firkin et al (1980). Case 2 had defective coagulation. The lupus anticoagulant is an immunoglobulin which reacts with the phospholipid component of the prothrombin activator complex (Conley and Hartman, 1952). It has been found in blood samples of women with connective tissue disease who have experienced recurrent spontaneous intrauterine deaths and may precipitate thrombosis in placental vessels by its interference with prostacyclin (Carreras et al, 1981). Derve et al (1985) found marked elevation of ACA levels in a majority of women with SLE and other autoimmune disorders who experienced one or more intrauterine deaths. They found infarction in four placentae from patients with connective tissue disease. In Case 1 of the present report only one of the five placentae was examined histologically and it was considered that infarction was the probable cause for intrauterine death. Lockshin et al (1985) proposed that antibody to cardiolipin may be a better predictor than other lupus-associated criteria of fetal distress or death in patients with SLE and others, and that placental infarction may be less relevant. Elevated ACA levels are associated with a number of clinical disorders including venous and arterial thrombosis (Harris et al, 1983, 1984). The successful pregnancy in Case 1 supports the suggestion of Lubbe et al (1983) that suppression of antiphospholipid antibodies with oral corticosteroids may promote fetal survival.

The prevalence of ACA in MG has not been investigated. A definite correlation between the presence of the lupus anticoagulant (LA), raised serum ACA levels and biological false positive tests for syphilis has been established (Boey et al, 1983; Harris et al, 1983) and the false positive tests for syphilis may result from the cross reaction of LA with cardiolipin (Johansson and Lassus, 1974). In an earlier study before the introduction of the VDRL precipitation test, Simpson (1964) noted anticomplementary reactions in seven of 15 cases of MG.

Certain similarities between MG and SLE were among the original reasons for postulating an autoimmune basis for MG (Simpson, 1960). Myasthenia gravis is characterised by the development of antibodies against numerous tissue antigens additional to polyclonal antibodies to nicotinic acetylcholine receptors of the endplates of skeletal muscle, pointing to a loss of immunological tolerance (Simpson, 1981, 1983). Myasthenia gravis and SLE share a common histocompatibility antigen HLA-DW3 (Celada et al, 1979). Michalski et al (1978) reported monozygotic twins with Klinefelter's syndrome discordant for SLE and symptomatic MG.

A myasthenic syndrome is well recognized in SLE (Harvey et al, 1954) and there are now many reports of SLE developing in patients with MG. Chan and
Britton (1980) considered that it is rare to find both diseases active simultaneously. The onset of myasthenic weakness in SLE is related to the appearance of anti-AChR antibody in the blood (Valesini et al, 1983). Conversely the vasculitis and nephropathy of SLE are related to deposition of DNA-antiDNA and other immune complexes. In our patients with MG Behan and Behan (1979) and Barkas et al (1980) found circulating immune complexes in 42% with depressed C4 component of complement, but the antigenic component of the complexes has not been characterised. The immunoregulatory reasons for these differences are speculative. Are MG and SLE different in kind or only quantitatively different breakdowns of immunological tolerance? A study of MG in the pregnant woman and in the neonate may be definitive.

Table 1

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AntiAChR</td>
<td>$56.2 \times 10^{-10}$ M</td>
<td>$2300 \times 10^{-10}$ M</td>
</tr>
<tr>
<td>Antinuclear factor</td>
<td>1:256 homogeneous</td>
<td>negative</td>
</tr>
<tr>
<td>DNA binding capacity</td>
<td>15.7% (normal)</td>
<td></td>
</tr>
<tr>
<td>Thyroglobulin precipitin</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>Thyroid microsome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric parietal</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>VDRL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid function tests</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Urine and electrolytes</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Liver function tests</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>Serum B12</td>
<td>500 ng/l</td>
<td></td>
</tr>
<tr>
<td>Red cell folate</td>
<td>282 µg/l</td>
<td></td>
</tr>
</tbody>
</table>


SIMPSON JA. Neonatal myasthenia gravis. Submitted for publication 1991b.