STUDIES ON AUTOIMMUNITY IN RELATION TO MEDICINE

William James Irvine

VOLUME 3

Thesis submitted for the degree of D.Sc.,
University of Edinburgh
1970
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CHAPTER XI

REVIEWS

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THE IMMUNOPATHY OF THYROID DISEASE

W. J. Irvine, S. H. Davies and M. D. Sumerling

The Immunopathy of Thyroid Disease

W. J. Irvine, S. H. Davies and M. D. Sumberling

Clinical Endocrinology Research Unit (MRC) and Departments of Therapeutics, Haematology and Diagnostic Radiology, Royal Infirmary, Edinburgh, GB

Three lines of investigation have been followed in the attempt to determine the nature of the immunologic disturbance associated with certain forms of thyroid disease. These are:

1. The study of clinical disorders associated with thyroid disease.
2. The immunological response to thyroid damage induced in thyrotoxic patients by therapeutic doses of $^{131}I$.
3. The morphology of the thymus in thyroid disease.

1. The study of clinical disorders associated with thyroid disease

The most notable condition in this context is Addisonian (pernicious) anemia. In a study of 97 patients with primary hypothyroidism Tudhope and Wilson (1) found 10 who were suffering from Addisonian (pernicious) anemia. In a subsequent study, they found an incidence of histamine fast achlorhydria in 24 out of 52 patients with primary hypothyroidism (2). The histology of the stomach in pernicious anemia, characterized by atrophy of the specialized cells in the body of the stomach and a variable with often severe degree of lymphocytic infiltration, suggests that immunological factors may be important in its pathogenesis. In 1962 it was established that an immunological relationship exists between pernicious anemia and thyroid disease (3, 4, 5).

Autoantibodies against two constituents of human gastric mucosa have been described; the antigens are the microsomal fraction of gastric parietal cell cytoplasm (5, 6) and intrinsic factor (7, 8, 9). Three thyroid antigens are recognized (10). In the present study the incidence of antibodies to thyroid microsomal antigen (3), to thyroglobulin (11), to gastric parietal cell cytoplasm (6) and to intrinsic factor (12) was determined
in patients with thyroid disease, pernicious anemia and in control subjects. The results are shown in Table 1. There is a significantly high incidence of antibody to parietal cells in patients with thyroid disease and a comparable incidence of thyroid antibodies in patients with pernicious anemia. About half of the patients with pernicious anemia, some 3% of the thyroid patients, but none of the control subjects have antibody to intrinsic factor in the serum.

The gastric serology (3, 6) in 157 thyroid patients is correlated in Fig. 1 with the ability of the patient’s stomach to secrete HCl and intrinsic factor. In this figure there is some degree of bias in favor of patients with positive gastric serology in so far that the incidence of gastric parietal cell antibody in this group of patients is 40% as opposed to the 30% shown in Table 1 and the incidence of antibody to intrinsic factor is 8% as opposed to 3%. With few exceptions the presence of parietal cell antibody is associated with an impaired secretion of gastric acid in response to histamine. The reduction in the ability of the stomach to secrete acid may vary in different patients from a mild hypochlorhydria to complete

<table>
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<td>89</td>
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<td></td>
<td>Primary Hypothyroidism</td>
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<td>52</td>
<td>75</td>
<td>29</td>
<td>3</td>
<td>29</td>
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<tr>
<td></td>
<td>Pernicious Anemia</td>
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<td>28</td>
<td>41</td>
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<td>17</td>
<td>26</td>
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</table>

Table 1. The incidence of thyroid and gastric antibodies in the sera of patients with different forms of thyroid disease, Addisonian pernicious anemia and in control subjects

Methods:
Thyroid microsomal antibody - complement fixation titer $\geq 1:4$ (3)
Thyroglobulin antibody - tanned cell hemagglutination titer $\geq 1:25$ (11)
Gastric parietal microsomal antibody - indirect fluorescent antibody method (6)
Antibody to intrinsic factor - radioimmune assay (12).
immunopathy of thyroid disease

Histamine-fast achlorhydria. The high incidence of histamine-fast achlorhydria is to be noted in this population of thyroid patients (36%). Gastric parietal cell antibody was found in the serum of 75% of the thyroid patients with achlorhydria (pH not less than 6.0), 37% of patients with a gastric acid secretion of between zero and 5 mEq HCl in the post-histamine hour, 33% in those with an acid secretion of 5-10 mEq and in only 10% of the thyroid patients with an acid secretion of greater than 10 mEq HCl in the post-histamine hour. Antibody to intrinsic factor has so far only been found in patients with achlorhydria. Screening tests were done for pernicious anemia in all patients included in Fig. 1. This included a serum vitamin B₁₂ estimation and a Schilling test (13). The 12 patients with antibody to intrinsic factor all had malabsorption of vitamin B₁₂ correctable with intrinsic factor and an additional 8 patients were also found to have pernicious anemia but did not have antibody to intrinsic factor at the vitamin B₁₂ binding site.

Gastric biopsies obtained by a Crosby capsule (14) showed that the presence of parietal cell antibody in thyroid patients is associated with the histological appearances of atrophic gastritis. In general, the severity of the gastritis paralleled the severity of the hypochlorhydria (15).

These findings indicate that in patients presenting with thyroid disease the pathology from which the patient is suffering may involve tissues other than the thyroid and that other organ-specific antibodies may occur in addition to those specific for thyroid.

2. The Immunological Response to Thyroid Damage

The immunological response to thyroid damage was studied in 101 thyrotoxic patients before and at intervals after treatment with therapeutic doses of ¹³¹I. In general patients who had no detectable level of thyroid antibody prior to treatment continued to give negative thyroid antibody tests following treatment, even if the treatment involved the use of repeated therapeutic doses of the isotope. Patients with thyroid antibodies prior to therapy showed a rise in antibody titer which was transient so that after a period of 6 months to a year the antibody titer was back to its pre-treatment level or lower. The change in titer of the thyroid complement fixing antibody was generally more pronounced than that of antibody to thyroglobulin (16). A typical response to a patient with a low titer of thyroid complement fixing antibody prior to treatment with 5mC ¹³¹I is shown in Fig. 2.
Fig. 1. Gastric acid secretion in the post-histamine hour correlated with the titer of gastric parietal cell antibody and the presence or absence of antibody to intrinsic factor in the sera of 157 patients with thyrotoxicosis, Hashimoto goiter or primary hypothyroidism. All patients were studied by the Schilling Test (13) for their ability to absorb oral $^{58}$Co-Vitamin B$_12$; patients with malabsorption of Vitamin B$_12$ are indicated.

▲ Pernicious anemia with antibody to intrinsic factor; △ Pernicious anemia without antibody to intrinsic factor; ○ Normal Schilling Test and no antibody to intrinsic factor; I.C. Indirect Coons fluorescent test for antibody to parietal cells.
Fig. 2. An example of immunological response to thyroid damage induced by $^{131}$I in a patient with thyrotoxicosis and a family history of thyroid disease. In contrast, thyrotoxic patients who do not have detectable levels of thyroid antibody before treatment with $^{131}$I generally show no immunological reaction.

These experiments indicate that the level of hypersensitivity or tolerance to thyroid antigen varies in different thyrotoxic patients. Thyrotoxic patients with a high degree of hypersensitivity to thyroid antigen were found to have a higher incidence of a positive family history of thyroid disease or pernicious anemia than thyrotoxic patients who appeared to be more immunologically tolerant to thyroid damage (16).

3. THE MORPHOLOGY OF THE THYMUS IN THYROID DISEASE

The histological finding of germinal centers in thymic biopsies from thyroid patients undergoing partial thyroidectomy is described in a separate paper (17). A correlation has been reported between the presence of germinal centers within the medulla of the thymus and the presence of lymphocytic infiltration within the thyroid (18).

As assessment of the size of the thymus in man can be achieved using the technique of pneumomediastinography, although this procedure will
not distinguish between the relative proportions of fat, connective tissue and lymphoid tissue within the thymus gland (19, 20). According to the thymic growth curves of Hammar (21) it would appear that an enlarged thymus gland, irrespective of its make-up, is indicative of thymic hyperplasia either at the present or some time in the past.

Fig. 3 compares a thymus shadow that is considered to be of normal dimensions with a thymus shadow that is considered to be enlarged. The size of the thymus shadow, recorded by planimetry in square centimeters, in 52 thyroid patients is shown in Fig. 4. The patients included 21 with histologically proven Hashimoto thyroiditis, 21 with thyrotoxicosis, 5 with primary non-goitrous hypothyroidism and 5 with simple goiter. All the patients were euthyroid at the time of the investigation as

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Fig. 3. Lateral radiographs (tomograms) of the chest using the technique of pneumomediastinography to demonstrate the thymus. The cross-sectional area of the thymus shadow in the central plane was estimated by planimetry.

a) Mrs. P. H. (aged 36 years). Thyrotoxic with negative serology. Thymus size, 2.6 cm² (normal). The two cornu of the thymus are demonstrated.

b) Mrs. C. F. (aged 42 years). Thyrotoxic with high titer of thyroid microsomal antibody (complement fixation titer = 256), a moderate titer of gastric microsomal antibody (complement fixation titer = 32), a low titer of antibody to thyroglobulin (tanned cell hemagglutination titer = 25) and a negative test for antibody to intrinsic factor.
IMMUNOPATHY OF THYROID DISEASE

Fig. 4. The cross-sectional area of the thymus shadow in the central plane was determined by pneumomediastinography in 52 patients with thyroid disease. The size of the thymus is compared in patients with positive thyroid or gastric serology and in patients with no serological evidence of autoimmunity.

a result of treatment when necessary. A comparison is made of thymus size in thyroid patients with positive serological tests for thyroid or gastric antibody and in a smaller group of thyroid patients with negative serology. It is apparent that enlarged thymus shadows tend to occur among the patients with serological evidence of autoimmunity.

It is known that the thymus is of functional importance in the development of the immunological system (22) and it is of interest to note its abnormality in adult patients with evidence of autoimmunity.
Conclusions

The three pieces of evidence presented are each in keeping with the hypothesis that the immunopathy associated with certain forms of thyroid disease is a consequence of a genetically determined disorder of immunological tolerance. It is tempting to speculate that the thymus may be primarily responsible, but before this can be said with any conviction a better understanding of the function of the thymus in the adult is required.

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AUTOIMMUNITY AND THE THYROID

W. J. Irvine

(1967) in Symposium of Thyroid Disease and Calcium Metabolism. Royal College of Physicians, Edinburgh. p. 31-50.
AUTOIMMUNITY AND THE THYROID

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Research Unit, Edinburgh

INTRODUCTION

Ten years have now elapsed since antibody to thyroglobulin was described in the sera of patients with Hashimoto thyroiditis (Roitt et al., 1956; Witebsky et al., 1957). It is therefore appropriate to review what developments have occurred in the field of thyroid autoimmunity in this interval.

Four cases of chronic thyroiditis with goitre were described by Hashimoto (1912) and it was from his pathological description that chronic goitrous thyroiditis became known as Hashimoto's disease. Clinically the patient is typically a middle-aged female with a firm, rubbery, diffuse goitre which may give rise to an aching discomfort in the neck and mild obstructive symptoms. The patient may be euthyroid but is often hypothyroid depending on the stage in the disease process at which the diagnosis is made.

The histology of Hashimoto thyroiditis (otherwise known as lymphadenoid goitre) is characterised by (1) Reduction in size of the thyroid vesicles, which contain little or no colloid but which may contain macrophages; (2) Askanazy cell change in the epithelial cytoplasm; (3) Lymphocytic infiltration; (4) Fibrosis. The relative severity of these
features varies in different goitres and often in the same goitre. Primary hypothyroidism without goitre is regarded as an atrophic variant of the same condition.

It is known from clinical observation that thyrotoxicosis is in some way related to Hashimoto goitre and to primary hypothyroidism, as if all three conditions were different manifestations of what was fundamentally the same disease process. In the older literature, before effective methods of treatment for thyrotoxicosis became available, a proportion of thyrotoxic patients who survived their illness subsequently went on to develop euthyroid goitre and eventually, often over a period of decades, became hypothyroid (Sattler, 1908). Furthermore, thyrotoxicosis not infrequently features in the family history of a patient with Hashimoto goitre or with primary hypothyroidism. As Professor McGirr has already mentioned, recent studies on the immunology of thyrotoxicosis go a long way to providing a unitary concept for this group of thyroid disorders.

At the present time four different thyroid antibodies are recognised (Table I).

**Table I**

*Thyroid Antibodies*

1. Antibody to thyroglobulin.
2. Antibody to a second component of colloid.
3. Antibody to insoluble component of thyroid cytoplasm.
4. Long acting thyroid stimulating substance (thyroid stimulating globulin).

**Antibody to Thyroglobulin**

Antibody to thyroglobulin was first detected by the agar diffusion or precipitin method. In the agar diffusion test
Fig. 1
A positive agar diffusion test for antibody to thyroglobulin. A saline extract of human thyroid was placed in a central well. Three positive sera and one control serum are placed in the surrounding wells.
Fig. 2
Positive staining for antibody to thyroglobulin using methanol fixed sections of human thyroid in the indirect immunofluorescent technique.

Fig. 3
Positive staining for antibody to thyroid cytoplasm using unfixed sections of human thyrotoxic gland in the indirect immunofluorescent technique.
(Fig. 1) a layer of agar is placed on the bottom of a petri dish. Wells are bored in the agar equidistant from a central well. Purified human thyroglobulin, or a saline extract of human thyroid, is put in the central well and the test sera and control serum are placed in the peripheral wells. The antigen and antibody, if present, diffuse into the agar, and, where they meet in appropriate concentrations, precipitation occurs. This is a crude method for the detection of large amounts of antibody to thyroglobulin, but, as we shall see later, it remains a useful test in the differential diagnosis of thyroid disease.

A more sensitive and semi-quantitative method for detecting antibody to thyroglobulin is provided by the tanned cell agglutination test (Fulthorpe et al., 1961). Sheep red cells are treated with tannic acid to make them suitable for coating the surface of the red cells with human thyroglobulin. When these coated or sensitised red cells come into contact with antibody to thyroglobulin they agglutinate. The test is done in a series of cups moulded in a perspex tray. The test is semi-quantitative and it is common practice to titrate the sera in dilutions of $1 : 5$ to $1 : 2.5 \times 10^9$.

A third method of detecting antibody to thyroglobulin, and one which is also highly sensitive, is by the immunofluorescence technique. Sections of human thyroid tissue fixed in methanol are used. The section is incubated with the test serum, washed and then incubated with anti-human gammaglobulin conjugated with a fluorescent dye such as fluorescein isothiocyanate. After further washing the section is examined under ultraviolet light. The site of reaction between antibody in the test serum and antigen in the tissue section is shown by bright green fluorescent staining. Antibody to thyroglobulin gives a floccular staining pattern in the colloid (Fig. 2).
Antibody to Second Component of Colloid

The antigen involved in this reaction is distinct from thyroglobulin in so far as it does not contain iodine. In the fluorescent technique, using fixed sections of thyroid tissue, this antigen-antibody reaction gives a diffuse staining of the colloid (Balfour et al., 1961). As this antibody does not appear to have much clinical or pathological significance I will not describe it further.

Thyroid Cytoplasmic Antibody

This antibody is directed against a particulate, insoluble component of the thyroid secretory cells. The antigen is predominantly in the microsomal fraction of the thyroid cytoplasm as determined by ultracentrifugation studies.

Fig. 3 shows the detection of the thyroid cytoplasmic antibody by the immunofluorescent technique. Note that only the cytoplasm of the cells is stained and not the nuclei nor the interstitial tissue. This reaction is specific for human thyroid cells and will not occur with any other tissue and shows a high degree of species specificity. Antibody to thyroid cytoplasm can also be detected by the classical immunological technique of complement fixation (Belyavin and Trotter, 1959). The thyroid complement fixation test can be done manually or by an automated method that gives greater reproducibility with better standardisation (Irvine, 1966).

Thyroid Stimulating Globulin

Thyroid stimulating globulin is the new name for the substance that used to be known as long acting thyroid stimulator (LATS). The long acting thyroid stimulator
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was first recognised by Adams (1961) in New Zealand when he was using a bioassay technique for TSH. As currently used, this technique depends upon the thyroidal uptake of $^{131}$I given intraperitoneally to mice that have been fed a diet deficient in iodine. The animal’s own TSH secretion is then suppressed by giving thyroxine. Finally, the animal is injected with test or control sera. If the serum contains TSH the $^{131}$I is released from the animal’s thyroid into the blood stream. With TSH the rise in the level of radioactivity in the blood reaches a maximum at about 3 hours and then the level of radioactivity falls. The serum of a patient with primary hypothyroidism would give an identical response. The sera of certain patients with thyrotoxicosis, however, give a different type of response, being much more prolonged. The maximum level of blood radioactivity occurs at about 12 hours with thyrotoxic sera in contrast to the 3 hours characteristic of TSH. Hence the name long acting thyroid stimulator (LATS). LATS is an immunoglobulin of type IgG (Kriss et al., 1964; Dorrington et al., 1965) as are the other thyroid antibodies described above. LATS is not produced in the pituitary and there is some direct evidence to suggest that it is synthesised by the reticulo-endothelial system (McKenzie and Gordon, 1965). The levels of LATS in the blood fall in response to treatment with steroids (Kriss et al., 1964). Table II summarises the incidence of three of the thyroid antibodies in patients with thyroid disease and in the control subjects. The actual figures obtained for the percentage of patients positive depends on the sensitivity of the methods used. The main point is that antibodies to thyroglobulin and to thyroid cytoplasm are a common occurrence in lymphadenoid goitre, in primary atrophic hypothyroidism and also, though to a lesser extent, in thyrotoxicosis. The incidence of these
THYROID

Table II

Incidence of Thyroid Antibodies in Thyroid Disease

<table>
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<th>Conditions</th>
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<td>Primary atrophic hypo-thyroidism</td>
<td>70</td>
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<td>Thyrotoxicosis</td>
<td>42</td>
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<tr>
<td>Simple goitre</td>
<td>19</td>
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<td>Malignant goitre</td>
<td>15</td>
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<td>Controls</td>
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* Titre > 1:4 in complement fixation test using 2MHD complement.

antibodies in simple goitre and malignant goitre is only slightly higher than in the controls. On the other hand, LATS is found exclusively, or virtually exclusively, in thyrotoxicosis. It has been detected in the sera of 80 per cent. of patients with thyrotoxicosis when methods of concentrating the sera have been used (Carneiro et al., 1966a).

VALUE OF THYROID AUTOANTIBODIES IN DIFFERENTIAL DIAGNOSIS

A euthyroid patient with goitre may have a Hashimoto thyroiditis, simple goitre, or malignant goitre. Figure 4a shows the incidence of antibody to thyroglobulin in these three conditions of the thyroid gland. For a positive result in the tanned cell test to be clinically significant the titre must exceed 1:2,500, but even then the value of the tanned cell
method is limited by not being accurately quantitative. On the other hand, a positive agar diffusion test shows a high correlation with Hashimoto thyroiditis, but only about one-half to two-thirds of the patients give a positive reading. The complement fixation test gives a better correlation with the histology of the thyroid gland (Fig. 4h). The patients with Hashimoto thyroiditis are more clearly distinguished from those with simple or malignant goitres. A titre of $\geq 1 : 32$ is of clinical significance.
When these three tests are used in combination, the sera of 90 per cent. of patients with lymphadenoid goitre will give a significant thyroid complement fixation titre, a really high titre of antibody to thyroglobulin in the tanned cell test or a positive diffusion test. Equivalent findings by any of these three tests is rare in patients with malignant goitre and has not occurred in my experience in patients with simple goitre. Strongly positive thyroid serology may occur when Hashimoto thyroiditis and thyroid malignancy are both present within the same gland. Hirabayashi and Lindsay 38
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(1965) have studied a large series of thyroid malignancies and consider that a thyroid malignancy may induce a chronic thyroiditis.

The clinical and serological diagnosis of Hashimoto thyroiditis should be confirmed histologically by needle biopsy of the goitre under local anaesthetic. The patient can be treated either with thyroxine alone or a short 10-day course of prednisolone in a dose of 60 mg./day, followed by thyroxine. So far 7 patients with Hashimoto thyroiditis with goitres estimated to be greater than 60 g. in size prior to treatment have been given such a course of prednisolone. The reduction in size of the goitre is very rapid and is apparent within a period of 7 days. The prednisolone is then tailed off and thyroxine started in a dose of 0.2 or 0.3 mg./day. The response to thyroxine alone is often rather slow and may be incomplete and give rise to diagnostic uncertainties. The goitre may tend to increase a little after the course of prednisolone in spite of thyroxine therapy. However, partial thyroidectomy is rarely required in patients with Hashimoto goitre.

I have mentioned that thyroid antibodies occur not only in patients with Hashimoto thyroiditis but also in patients with thyrotoxicosis and to a lesser extent in control subjects without overt thyroid disease. The incidence in the controls rises with age and is higher in females than in males. In such subjects the presence of thyroid antibodies, particularly thyroid cytoplasmic antibody, shows a statistical correlation with focal thyroiditis (Goudie et al., 1959). It has been known for some time that the tendency for thyrotoxic patients to become myxoedematous after thyroidectomy can be correlated with the degree of lymphocytic infiltration in the operation specimen (Whitesell and Black, 1949). Thyroid antibodies are therefore of some value as a prognostic
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guide to the risk of hypothyroidism in thyrotoxic patients in whom surgery is contemplated (Irvine et al., 1962; Hjort et al., 1963).

A third use of thyroid antibodies as far as the clinician is concerned is in the diagnosis of early primary hypothyroidism. In an equivocal case the presence of high titres of thyroid antibodies would favour a diagnosis of thyroid insufficiency.

THE SIGNIFICANCE OF THYROID ANTIBODIES IN PATHOGENESIS

The effect of thyroid antibodies in vitro has been studied by growing human thyroid cells in tissue culture. Under suitable conditions these cells thrive and form a monolayer. Neither antibody to thyroglobulin nor antibody to the second colloid antigen has any adverse effect on such cultures. If, however, antibody to thyroid cytoplasm, even in trace amounts, is added to the culture in the presence of complement, rapid lysis of the thyroid cells occurs (Irvine, 1962; Forbes et al., 1962). While it is likely that dispersed thyroid cells under the conditions of culture (that is to say, trypsinisation) are more susceptible to penetration by the cytoplasmic antibody, the fact that such a reaction can occur indicates the possible cytotoxic potential of this type of thyroid antibody in vivo.

With regard to thyroid stimulating globulin a remarkable correlation has been demonstrated by Carneiro et al. (1966b) between the level of thyroid stimulating globulin in the serum and the rate of iodine metabolism in the thyroid gland. The correlation is such that it is tempting to speculate that thyroid stimulating globulin is the driving force behind the thyroid gland; in other words, that the presence of
thyroid stimulating globulin is the primary abnormality underlying thyrotoxicosis.

When considering immunological reactions one must not devote all one’s attention to the gammaglobulin in the serum, but remember that lymphocytes may well be carriers of cell-bound antibody and are certainly involved in the reactions of delayed hypersensitivity that are characteristic of graft versus host disease, for example. Lymphocytes are much more difficult to study than are the serum gammaglobulins.

Under the electron microscope one can clearly see that in chronic thyroiditis lymphocytes penetrate through the basement membrane of the thyroid vesicle and come into close apposition with the thyroid cells (Irvine and Muir, 1963). If such a lymphocyte is carrying or synthesising thyroid antibody then it can discharge its immunological missile directly at the target cell. Under these circumstances the levels of the antibody in the serum may not be of primary importance but merely represent an overspill of excess antibody from the target tissue. Plasma cells may be found in similar situations.

Genetic Aspects

The tendency to develop thyroid autoimmunity is familial and appears to be genetically rather than environmentally determined. Part of the evidence for this statement is based on twin studies; in thyrotoxicosis and in Hashimoto thyroiditis there is a high degree of concordance among uniovular twins (Irvine et al., 1962; Hassan et al., 1966). From family studies it would appear that the development of thyroid autoimmunity is controlled by a dominant autosomal gene with incomplete penetrance.
THYROID

Association with Other Diseases

In recent years an association between different disease conditions with autoimmune characteristics has been recognised. One of the most striking of these associations is that between thyroid disease and pernicious anaemia (Tudhope and Wilson, 1962; McNicol, 1961). In a study of 157 patients with one or other of the thyroid autoimmune disorders—thyrotoxicosis, Hashimoto thyroiditis, or primary non-goitrous hypothyroidism—histamine-fast achlorhydria was found in over 30 per cent. and frank or latent pernicious anaemia in 6 per cent. (Irvine, 1965). The histology of the gastric mucosa in the patients with achlorhydria is characterised by atrophy and lymphocytic infiltration, often with the formation of large germinal centres. There are few or no parietal cells or chief cells to be seen and the mucosa is thinned.

The recognition of this association between thyroiditis and gastritis led to the detection of antibody to gastric parietal cells and consolidated previous work on antibody to gastric intrinsic factor. Table III summarises the incidence

| Table III |
| Gastric Antibodies in Thyroid Disease |

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Percentage with Serum Positive for Antibodies to</th>
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<td>Parietal Cell</td>
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<td>Thyrotoxicosis</td>
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<td>Hashimoto goitre</td>
<td>300</td>
</tr>
</tbody>
</table>

Blood donors, F, 40-60 years | 141 | 0 | 0 |
AUTOIMMUNITY

of gastric antibodies in patients with pernicious anaemia, patients with thyroid autoimmune disease and in control subjects. The majority of patients with pernicious anaemia have parietal cell antibody in the serum and about half have antibody to intrinsic factor. Between a quarter and a third of patients with thyroid autoimmune disease have parietal cell antibody in the serum and 3 per cent. have antibody to intrinsic factor. This 3 per cent. may seem low but is highly significant, the incidence in the control subjects being zero. It correlates well with the finding of pernicious anaemia in 6 per cent. of this thyroid population.

Another condition in which a high incidence of organ specific antibodies has been described is idiopathic adrenal insufficiency. Antibodies specific for the secretory cells of the adrenal cortex only occur in patients whose Addison's disease is of idiopathic type and not in those in whom it is tuberculous. When patients with Addison's disease were studied for evidence of overt disease in other organs a high incidence of thyroid disorder, pernicious anaemia, idiopathic hypoparathyroidism and of diabetes mellitus was found in patients with idiopathic or probable idiopathic Addison's disease but not in those with tuberculous destruction of the adrenal. Seven out of 12 of the idiopathic group and 11 out of 23 of the probable idiopathic group had clinical and objective evidence of thyroid disease, pernicious anaemia, idiopathic hypoparathyroidism or diabetes mellitus, while these diseases were not found in any of the tuberculous patients (Table IV). Likewise, there was a high incidence of thyroid and gastric antibodies in the idiopathic and probable idiopathic groups but not in the patients with tuberculous adrenal insufficiency (Irvine et al., 1967).

Similar observations in patients with idiopathic hypoparathyroidism have been made by Blizzard et al. (1966).
**THYROID**

**Table IV**

*Diseases associated with Primary Adrenal Insufficiency*

(Reprinted from Irvine, Stewart and Scarth (1967). *Clin. exper. Immunol.*, 2, 31, by kind permission of the Editor.)

<table>
<thead>
<tr>
<th>Type of Adrenal Insufficiency</th>
<th>Type</th>
<th>Probable</th>
<th>Tuberculous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disease:</td>
<td>Idiopathic</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthyroid goitre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Moniliasis</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Eczema</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Schilder’s disease</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Alopecia totalis</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

| Total affected | 8 | 13 | 1 |
| Total patients | 12 | 23 | 16 |

In idiopathic hypoparathyroidism a significantly high incidence of antibodies to parathyroid, thyroid, stomach and adrenal were found in comparison to the incidence of these antibodies in control subjects. Likewise, a clinical association between idiopathic hypoparathyroidism, thyroid disease and idiopathic Addison’s disease was noted (Blizzard *et al.*, 1967).

Thyrotoxicosis, Hashimoto goitre and primary atrophic hypothyroidism would seem to belong to a group of disorders...
AUTOIMMUNITY

which may involve tissues other than the thyroid such as the gastric mucosa, adrenal or parathyroid. This group of diseases is characterised by a genetic trait, a predominantly female incidence, by aggregation within the same group of patients, by the histological appearance of lymphocytic infiltration in one or more tissues and by the occurrence of organ specific autoantibodies.

MECHANISMS IN THYROID AUTOIMMUNITY

To complete this discussion on thyroid autoimmunity I would like to consider the mechanisms that might be responsible for the formation of autoantibodies. There are two main mechanisms that have been postulated (Fig. 5). The first is that there is a primary defect in the target organ; that is to say, the thyroid vesicle (Hijmans et al., 1961; Mackay and Burnet, 1963). It used to be believed that thyroglobulin was kept within the thyroid vesicle and never normally escaped. It was therefore hidden from the reticuloendothelial system, the antibody-forming tissue never had an opportunity to get acquainted with thyroglobulin, and therefore there had been no opportunity for tolerance to be developed. Following damage to the thyroid vesicle, either by trauma or virus infection, thyroglobulin might be released. As it would then be treated as a foreign substance, antibodies would be formed against it and these might lead to further thyroid damage and so set up a chain reaction with the formation of more thyroid autoantibody. Against this concept is the demonstration that in many patients it is very difficult to induce thyroid antibodies even following gross thyroid damage such as may be produced by $^{131}$I treatment. Those patients that do form antibodies in response to thyroid damage show a transitory rise in titre.
THYROID
POSSIBLE MECHANISMS IN AUTOIMMUNITY

I DISTURBED ANTIGEN

THYROID FOLLICLE

Abnormal release of normal constituents,
or
Normal release of abnormal constituents.

II DISTURBED TOLERANCE

THYROID FOLLICLE

Normal release of normal constituents.

THYMUS
Normal control

ANTIBODY-FORMING CELLS
Normal

THYMUS
Defective control

ANTIBODY-FORMING CELLS

? Primary defect

FIG. 5
Possible mechanisms in autoimmunity. (Reprinted from Irvine (1964). Quarterly Journal of Experimental Physiology, 49, 324, by kind permission of the Editor.)

and there is no evidence of a chain reaction (Irvine, 1964). Likewise an animal will only make antibodies to thyroid so long as injections of antigen are continued to be given and it will only make antibodies at all if Freund's adjuvant is given along with the antigen. Moreover, it has been recently demonstrated that in animals thyroglobulin appears to be normally discharged into the thyroid lymphatics and is by no means confined to the thyroid vesicle (Daniel et al., 1967).

Alternatively, one might postulate that there has been
AUTOIMMUNITY

some subtle change in the thyroglobulin molecule that has rendered it antigenic. However, to account for the facts that I have described one would have to postulate a multiplicity of such antigen-producing alterations; four in the thyroid gland, two in relation to the stomach, a further one in relation to the adrenal and another in relation to the parathyroid gland, and one would need to postulate that these alterations were genetically determined. This would seem too complex to be realistic.

The second of the hypotheses discussed above is to my mind more acceptable. It is thought that the primary abnormality may not be in the target tissue but in the antibody-producing system. That is to say, that there is a genetically determined defect in immunological homeostasis (Irvine, 1964). In order for immunological tolerance to be maintained, there must be an immunological memory which recognises substances that are constituents of the body’s own tissues and substances that do not belong to the body and are foreign. By virtue of this immunological memory, clones of lymphocytes that tend to form antibodies against the body’s own tissues are normally destroyed. If this mechanism of immunological recognition or the deletion of aberrant clones of lymphocytes is defective, then autoantibodies against a particular constituent or against a group of constituents of the body may develop. In relation to thyroid autoimmunity these antibodies are highly tissue specific.

In recent years it has been shown in experimental animals that the thymus gland is essential for the development and maintenance of the immunological system (Miller, 1963) and it is therefore tempting to speculate that some abnormality in the thymus may be responsible for the occurrence of certain patterns of autoimmunity. Irvine and Sumerling
XI : 30

THYROID

(1965) studied the thymus radiologically in patients with different types of thyroid disease. A correlation was observed between the size of the thymus shadow on X-ray in the sagittal plane and the presence or absence of auto-antibodies in the patient's serum. Enlargement of the thymic shadow was a common occurrence in patients showing autoimmunity and was not found in those in whom auto-antibodies were absent.

A second way of studying the thymus in patients with thyroid disease is to obtain a biopsy of the upper cornu of the thymus during a standard thyroidectomy with due respect to the parathyroids (Gunn et al., 1964). The finding of germinal centres in the medulla of the thymus is highly abnormal. The occurrence of thymic germinal centres again correlates with serological and histological evidence of autoimmunity. The pathology of the thymus is reminiscent of that seen in the strain of New Zealand mice that spontaneously develops autoimmune haemolytic anaemia and systemic lupus erythematosus (Burnet and Holmes, 1964).

The problem that I must leave with you is whether the changes in the thymus that I have just described are simply another manifestation of an autoimmune phenomenon or whether a defect in thymic function might be of fundamental importance in the genetically determined disorder of immunological tolerance that underlies the thyroid autoimmune diseases.

REFERENCES

AUTOIMMUNITY


IMMUNOLOGICAL ASPECTS OF PERNICIOUS ANAEMIA

W. J. Irvine

Antibodies against two constituents of the gastric mucosa commonly occur in the sera of patients with Addisonian pernicious anaemia. The constituents of the gastric mucosa involved are (1) the microsomal fraction of the parietal cell cytoplasm and (2) intrinsic factor. There is evidence that there may be more than one antibody to intrinsic factor.

THE INCIDENCE OF ANTIBODIES TO GASTRIC MUCOSA

The discovery of gastric antibodies was achieved by somewhat devious means. Schwartz (1958, 1960) and Taylor (1959), while studying why pernicious anaemia patients became resistant to oral treatment with hog intrinsic factor, found that the sera of a number of patients with pernicious anaemia who had never received any treatment had the property of inhibiting the effect of hog intrinsic factor. This was demonstrated by mixing the serum with the intrinsic factor and giving this to the patient to swallow along with radioactive vitamin B12 in a standard Schilling test. At that time there was little evidence to substantiate the suggestion that the inhibitory substance might be an antibody.

In 1962 Tudhope and Wilson reported their detailed study of the relationship that had previously been claimed to exist between pernicious anaemia and thyroid disease (Lerman and Means, 1932). They found 24 out of 52 patients with primary hypothyroidism had a histamine fast achlorhydria and that 12.3% of 72 hypothyroid patients had Addisonian pernicious anaemia. Once again it was
inferred that immunological mechanisms may provide the explanation for this clinical relationship, but on the other hand it did seem relevant that both the thyroid and the gastric mucosa are related embryologically and that both tissues have the property of concentrating iodide.

The immunological methods that had proved so rewarding in the investigation of thyroid disease (see Doniach earlier in this symposium) were therefore applied to the study of pernicious anaemia. It was found that 75 per cent of the sera of patients with Addisonian pernicious anaemia gave a positive complement fixation test specific for a saline extract of gastric body mucosa and that the fraction responsible for this reaction was a gamma globulin (Irvine et al. 1962). Taylor et al. (1962) and Irvine (1963) subsequently demonstrated, using the fluorescent antibody method (Coons and Kaplan, 1950), that the complement-fixing gastric antibody was specific for the cytoplasm of the gastric parietal cell. Absorption and inhibition tests excluded the possibility of a common antigen between thyroid and gastric tissue and it was demonstrated that the gastric antibody would react equally well with the patient's own gastric mucosa provided it still contained an adequate concentration of parietal cells (Irvine, 1963). An overlap was shown to occur in the incidence of gastric and thyroid antibodies in pernicious anaemia and thyroid disease (Irvine et al, 1962; Markson and Moore, 1962; Taylor et al. 1962).

Jeffries et al. (1962) developed an in vitro method for the detection of antibody to intrinsic factor. The basis of the method was to observe the alteration of the electrophoretic mobility of radioactive vitamin B_{12}-intrinsic factor complex in the presence of test serum as opposed to normal serum. In the presence of antibody to intrinsic factor a larger complex is formed and is retained at the origin on starch gel electrophoresis. The method is illustrated in Fig. 1. The figure also demonstrates that antibody to intrinsic factor is distinct from antibody to parietal cell cytoplasm as detected by complement fixation or fluorescent staining. Abels et al. (1963) and Ardeman and Chanarin (1963) described another method for detecting antibody to intrinsic factor which depends upon the property of intrinsic factor to bind vitamin B_{12} to serum. The method of Ardeman and
Fig. 1. The electrophoretic migration of intrinsic factor-58 Cobalt B12 complex after incubation with normal serum and with serum from patients with pernicious anaemia (from Irvine, 1963. Quart J. Exp. Physiol. 48, 427). C.F.T. = Complement fixation test.

Chanarin (1963) is illustrated in Fig. 2. It only differs from the method of Abels in so far that activated charcoal is used to absorb the unbound vitamin B12; Abels uses dialysis for this purpose. As Roitt et al (1964) have pointed out, the method of Ardeman and Chanarin (1963) detects antibody to the vitamin B12-binding site of
The detection of antibody to intrinsic factor according to the method of Ardeman and Chanarin (1963). A reduction in vitamin $B_{12}$ binding $\geq 3\mu$/mg/ml. test serum indicates the presence of intrinsic factor antibody in the test serum.

Both the antibody to parietal cell cytoplasm and the antibody to the vitamin $B_{12}$-binding site of intrinsic factor are $\gamma$ gamma (IgG)
globulins, but neither are species specific. The site of secretion of intrinsic factor in the human gastric mucosa is not yet established although this question should be capable of being answered using autoradiography and studying whether or not activity in the specialised cells of the gastric mucosa can be inhibited by previous treatment of the section with antibody to intrinsic factor.

The incidence of parietal cell, intrinsic factor (vitamin B₁₂-binding site) and thyroid antibodies and of antinuclear factors (ANF) in the serum of patients with pernicious anaemia, thyroid disease, iron deficiency anaemia, adrenal insufficiency and in control subjects is shown in Table 1. The incidence of gastric parietal cell antibody in the sera of blood donors according to age and sex is illustrated in Fig. 3.

**Table 1—Incidence of Gastric and Thyroid Antibodies and of A.N.F. in the Sera of Patients with Pernicious Anaemia, with Related Disorders and in Control Subjects (from Irvine, 1965. New Eng. J. Med.—in Press).**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Percentage Positive for Gastric</th>
<th>Thyroid</th>
<th>Gastric and/or ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pernicious Anaemia</td>
<td>130</td>
<td>73 55 41 90 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latent P.A.</td>
<td>6</td>
<td>100 50 50 100 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Hypochromic Anaemia</td>
<td>55</td>
<td>14 2 12 20 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>302</td>
<td>24 3 48 57 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Thyroiditis</td>
<td>238</td>
<td>30 3 78 85 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic Adrenal Insufficiency</td>
<td>25</td>
<td>32 28 56 64 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–65, M &amp; F</td>
<td>629</td>
<td>5 13 16 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–60, F</td>
<td>141</td>
<td>9 0 26 28 1</td>
<td></td>
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</tbody>
</table>

P.C. = antibody to gastric parietal cells (fluorescent antibody method).
I.F. = antibody to intrinsic factor (see text).
T.C.H. = tanned cell haemagglutination.
C.F.T. = complement-fixation using 2 MHD complement and 50% haemolysis as end-point.
A.N.F. = antinuclear factors.
Fig. 3. The incidence of gastric cell antibody in the sera of blood donors and of patients with Addisonian pernicious anaemia, with chronic thyroiditis and with thyrotoxicosis according to age and sex (from Irvine et al., 1965 Ann. N.Y. Acad. Sci.—in Press).
THE CLINICAL SIGNIFICANCE OF ANTIBODY TO GASTRIC MUCOSA

It will be noted in Table I that the incidence of gastric antibodies is not restricted to patients with pernicious anaemia. In collaboration with Dr. Howard Davies, Haematology Department, Royal Infirmary, Edinburgh, gastric analysis and gastric biopsy and studies of vitamin B₁₂ metabolism have been carried out on patients with thyroid disease, chronic iron deficiency anaemia and adrenal insufficiency (Irvine et al., 1965). The findings in 124 patients with thyroid disease are shown in Fig. 4. It is apparent that the presence of gastric parietal cell antibody in the serum is associated with some degree of hypochlorhydria. The hypochlorhydria may vary from complete histamine fast achlorhydria to only a slight reduction in the gastric acid secretion. The histological appearance of the gastric biopsy is in keeping with the reduction in acid secretion in respect of the content of parietal cells. Severe lymphocytic infiltration is a common finding in the biopsy specimens that show a moderate or gross reduction in parietal cell content. Advanced atrophic gastritis may occur without gastric parietal cell antibody in the serum. Mackay (1964) found an incidence of complement fixing gastric antibody in a titre of 16 or greater in 28% of 50 patients who had chronic atrophic gastritis but who did not have frank pernicious anaemia.

In Fig. 4 antibody to intrinsic factor (vitamin B₁₂-binding site) was only found in the sera of patients with a histamine-fast achlorhydria and all the patients with this antibody had malabsorption of vitamin B₁₂ in the Schilling test. The follow-up of a positive serological test for gastric antibodies led to the discovery of latent or frank pernicious anaemia in a number of patients in whom the diagnosis had not been suspected clinically.

THE IMMUNOASSAY OF GASTRIC INTRINSIC FACTOR

Apart from the possible role in pathogenesis, the finding of antibody to intrinsic factor has an important application to the study of
ASPECTS OF PERNICIOUS ANAEMIA

SECRETION OF GASTRIC HCl (in mEq) IN THE POST-HISTAMINE HOUR

ACHLORHYDRIA

<table>
<thead>
<tr>
<th>IC POS.</th>
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</table>

COMPLEMENT-FIXATION TITRE

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<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>512</td>
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<td></td>
</tr>
<tr>
<td>256</td>
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<td></td>
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<tr>
<td>128</td>
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<td></td>
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<tr>
<td>64</td>
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<td></td>
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<td>32</td>
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<td></td>
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<tr>
<td>16</td>
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<td></td>
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<tr>
<td>8</td>
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<td></td>
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<tr>
<td>4</td>
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<tr>
<td>2</td>
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</tbody>
</table>

ANTIBODY TO GASTRIC PAROTAL CELLS

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| 2 = SERUM POSITIVE FOR ANTIBODY TO INTRINSIC FACTOR (IF POS.)
| 0 = SERUM NEGATIVE FOR ANTIBODY TO INTRINSIC FACTOR (IF NEG.)
| 3 PATIENTS WITH UNTREATED LATENT PERNICIOUS ANAEMIA (IF POS.)
| 1 PATIENT WITH UNTREATED LATENT PERNICIOUS ANAEMIA (IF NEG.)
| 6 PATIENTS WITH UNTREATED FRANK PERNICIOUS ANAEMIA (IF POS.)
| 4 PATIENTS WITH UNTREATED FRANK PERNICIOUS ANAEMIA (IF NEG.)

I.C. = Indirect Coombs fluorescent antibody method

Fig. 4. Gastric acid secretion in the post histamine hour correlated with the titre of gastric parietal cell antibody and the presence or absence of antibody to intrinsic factor in the sera of 124 patients with thyrotoxicosis, Hashimoto goitre or primary hypothyroidism (from Irvine, Davies and Sumerling, 1965). Xth Congress Internat. Soc. Blood Transf. Stockholm, 1964. Karger—in Press).

gastric secretion in health and disease. Ardeman and Chanarin (1963) have applied their method for detecting antibody to intrinsic factor to the immunoassay of the intrinsic factor content of human gastric juice. In Fig. 5 the secretion of intrinsic factor 10$^{-6}$ mg units is plotted against the secretion of HCl in the post histamine hour. The method of immunoassay of intrinsic factor is a modification of that of Ardeman and Chanarin (1963) and is shown in Table 2.
Patients with antibody to intrinsic factor in the serum and those with impaired absorption of oral vitamin \( B_{12} \) in the Schilling test are indicated. It will be seen that, as in Fig. 4, the antibody to intrinsic factor is only found in patients with achlorhydria or minimal acid
TABLE 2. Immunoassay of intrinsic factor in gastric juice; a modification of the method of Ardeman and Chanarin (1963).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Serum control</td>
<td>Radioactive standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (ml.)</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Test Gastric Juice (ml.)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Serum (ml.)</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Serum B/238 (ml.)</td>
<td></td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>400 μg. 60Co Vitamin B12 (ml.)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Activated Charcoal (mg.)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

Mix and stand 20 min. at room temperature

Shake 1-2 min. centrifuge count radioactivity in supernatant

secretion in the post histamine hour and that all the patients with antibody to intrinsic factor in the serum had impaired Schilling tests. There is a decline in the secretion of intrinsic factor with decline in acid secretion, but patients with histamine fast achlorhydria and advanced atrophic gastritis on gastric biopsy may still be capable of secreting sufficient amounts of intrinsic factor to maintain adequate absorption of vitamin B12. It would appear from Fig. 5, that if a patient is capable of secreting ≥80 μmg. units intrinsic factor in the post histamine hour, he is unlikely to have Addisonian pernicious anaemia. Routine gastric analysis using the standard augmented histamine test can now provide not only an index of the parietal cell mass, but also a direct measure of the ability of the stomach to secrete intrinsic factor. The immunoassay of intrinsic factor should add considerably to the value of gastric analysis in the differential diagnosis of anaemia and of vitamin deficiency states. Since the isolation of gastrin I and II (Gregory and Tracy, 1964) it has been shown that these substances are useful clinically in the
assessment of gastric acid secretion (Makhlouf et al., 1964) and that they have the property of stimulating the secretion of intrinsic factor (Irvine, 1965). The advantage of gastrin over histamine for routine gastric analysis is that it is entirely free of the unpleasant side effects of histamine. An example of the effect of gastrin (I and II) on the volume flow, secretion of HCl and intrinsic factor in a patient with a normal gastric biopsy and a normal response to histamine is shown in Fig. 6.

SIGNIFICANCE OF GASTRIC ANTIBODIES IN THE PATHOGENESIS OF ADDISONIAN PERNICIOUS ANAEMIA

The occurrence of autoantibodies in association with any disease process at once raises the question as to whether the antibodies are a primary cause of the disease or are a result of it. As yet it has not been convincingly demonstrated in a reproducible manner that atrophic gastritis can be induced in experimental animals by immunological means. The clinical and immunological association between pernicious anaemia and thyroid disease and possibly adrenal insufficiency suggests that these conditions may have the same basic pathogenic mechanism (Irvine, 1963).

The incidence of achlorhydria is greater among the relatives of pernicious anaemia patients than among control subjects (Callender and Denborough, 1957; McIntyre et al., 1959). Te Velde et al. (1964) studied the incidence of gastric antibodies in 220 relatives of 21 patients with pernicious anaemia whose serum gave positive tests for antibody to parietal cells. Antibodies were found in 42 out of the 220 relatives and biopsy studies in 20 of these positive subjects revealed histological evidence of gastric mucosal atrophy in 15, but only 6 of these 20 relatives had a defect of absorption of labelled vitamin B12 and only 11 had achlorhydria. An analysis of the pedigrees of the families included in their survey indicated that the development of parietal cell antibody is probably controlled by a dominant autosomal gene. Other genetic studies have been done in relation to pernicious anaemia (Doniach and Roitt, 1964; Irvine
ASPECTS OF PERNICIOUS ANAEMIA

Fig. 6. The effect of an intramuscular injection of (a) 2 µg/kg. body weight gastrin (I and II) and (b) 40 µg/kg. body weight histamine on the volume, acidity and intrinsic factor content of the gastric secretion in a female subject aged 56 yrs. with normal gastric histology and function (from Irvine, 1965.)
et al. 1965), thyroid disease (Hall et al., 1962; Doniach and Roitt, 1963) and adrenal insufficiency (Hung et al., 1963).

In order to explain the development of gastric antibodies in terms of tissue damage one must either postulate a genetically determined defect in the various tissues concerned (stomach, thyroid and adrenal) or that there is an environmental factor, such as a virus infection, that has a predilection for these tissues with resultant antibody formation against the tissues. There is no direct evidence that such a virus exists. Alternatively, the basic pathology may be immunological. As described elsewhere (Irvine, 1964) there is evidence that there may be a disorder of immunological tolerance in this group of patients and that the ability to recognise constituents of one's own body may be defective. One could imagine the central lymphoid tissue (including the thymus) as a computer with a programme unit that may be defective with regard to the recognition of a particular antigen or group of closely related antigens. The defect would then become manifest by the formation of autoantibodies and the establishment of delayed hypersensitivity reactions. The defect in self recognition may be limited to body constituents that are only found in certain organs or to equally specific constituents which are shared by all tissues. This would result in a spectrum of autoimmunity varying from that of the organ specific type (e.g. pernicious anaemia), through that of a mixed type (Sjögren's disease) to a generalised disorder such as systemic lupus erythematosus.

CONCLUSION

Immunological studies have advanced knowledge in relation to pernicious anaemia in so far as they have introduced another dimension in the study of the disease process. This is particularly so in the study of the early stages of the disease and in determining its genetic characteristics. Immunological studies have also led to the development of a simple and accurate in vitro method for the assay of intrinsic factor. Such a method has been long awaited and has already resulted in an advance in the understanding of the physiology of gastric secretion.
ASPECTS OF PERNICIOUS ANAEMIA

With regard to clinical practice, gastric antibodies are a useful means of screening a large number of patients for pernicious anaemia, although their limitations must also be recognised. The immunoassay of the intrinsic factor content of gastric juice in the augmented histamine or gastrin tests has a valuable application in the differential diagnosis of anaemia and of vitamin deficiency states.

ACKNOWLEDGEMENTS

I am indebted to the Editor of the Quarterly Journal of Experimental Physiology for permission to reproduce Fig. 1; to the Editor of the Annals of the New York Academy of Sciences for permission to reproduce Fig. 3; to the Editor of the Xth Congress of the International Society of Blood Transfusion, Stockholm, 1964, for permission to reproduce Fig. 4; to the Editor of the New England Journal of Medicine for permission to reproduce Fig. 5 and Table 1 and to the Editor of the Lancet for permission to reproduce Fig. 6.

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IMMUNOLOGICAL ASPECTS OF PERNICIOUS ANAEMIA

W. J. Irvine

MEDICAL PROGRESS
IMMUNOLOGIC ASPECTS OF PERNICIOUS ANEMIA

W. James Irvine
Edinburgh, Scotland

THERE were two facts that suggested that immunologic factors may be of importance in the study of Addisonian (pernicious) anemia. In the first place, this condition has a high incidence in patients with thyroid disease, in which autoimmune phenomena are a frequent occurrence. Secondly, the histology of the gastric mucosa in pernicious anemia is characterized by atrophy of the specialized cells of the body of the stomach, and this is accompanied by a variable but often marked degree of lymphocytic infiltration.

ANTIBODY TO GASTRIC PARIETAL-CELL CYTOPLASM

By the method of complement fixation, the sera of 75 per cent of patients with pernicious anemia were found to contain a gamma globulin that was specific for an extract of the mucosa from the body of the human stomach, and, by the technic of immunofluorescence, this antibody has been shown to be specific for the cytoplasm of the gastric parietal cell. Provided a sufficient number of parietal cells still remain, specific fluorescent staining of the gastric parietal-cell cytoplasm can be demonstrated equally well with the patient's own gastric-body mucosa obtained by Crosby capsule or with gastric mucosa obtained at partial gastrectomy from patients with duodenal ulcer. This establishes the autoimmune nature of the reaction. The factor in the serum is a 7S gamma globulin (IgG), it can be absorbed by an extract of gastric-body mucosa, and the immunofluorescence of the parietal-cell cytoplasm can be inhibited in the indirect Coons technic by a positive serum. Thus, the criteria for describing an antigen-antibody reaction as laid down in the original critical papers by Coons and Kaplan have been fulfilled, and the concept of autoimmunity in pernicious anemia put on a firmer basis.

†Member, Medical Research Council Clinical Endocrinology Unit and Department of Therapeutics, Royal Infirmary.

ANTI-INTRINSIC-FACTOR ACTIVITY

The first indication that immune phenomena may occur in association with pernicious anemia is to be found in the work of Schwartz.1 Faced with the problem of acquired resistance to intrinsic factor in such patients treated by mouth, he looked for inhibitors to intrinsic factor and found them in the serum of several of these patients. Taylor5 subsequently observed intrinsic-factor-inhibiting substance in 9 out of 19 patients either treated by parenteral administration of vitamin B₁₂ or else untreated. In a fuller account Schwartz10 described intrinsic-factor inhibitors in the serum of 36 out of 91 patients with pernicious anemia and in none out of 39 control subjects. The presence of the factor, which was localized in the globulin fraction of the serum, could not be correlated with treatment-induced blockade of intestinal absorption of vitamin B₁₂.

This method of studying intrinsic-factor inhibitor substance, although directly related to the problem of acquired resistance to oral treatment, is cumbersome and requires that the patient swallow 50 ml. of serum and have repeated doses of radioactive Co⁵⁹-labeled vitamin B₁₂, each followed by twenty-four-hour collections of urine. In vitro methods for the detection of antibodies to intrinsic factor have been developed by Jeffries, Hoskins and Sleisenger,11 Abels et al.,12 and Ardeman and Chanarin.13 The au-

**Table 1. Detection of Antibodies to Intrinsic Factor.**

<table>
<thead>
<tr>
<th>Period of Study</th>
<th>Saline Solution (or Buffer)</th>
<th>Normal Serum</th>
<th>Normal Gastric Juice</th>
<th>Test Serum</th>
<th>20 Min. Chroining of Co⁵⁹-Labeled Vitamin B₁₂</th>
<th>Activation Change$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>Tett:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>±50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>±50</td>
<td></td>
</tr>
<tr>
<td>Serum control:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>±50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>±50</td>
<td></td>
</tr>
<tr>
<td>Radioactive standard:</td>
<td>2.0</td>
<td>—</td>
<td></td>
<td>0.5</td>
<td>±50</td>
<td></td>
</tr>
</tbody>
</table>

$\dagger$ Modification of method of Ardeman and Chanarin.13
10 min. at room temperature.
5 Mix & stand for 20 min. at room temperature.
5 Shake for 1 or 2 min.; centrifuge; count radioactive label in supernatant.

**Table 2. Immunoassay of Intrinsic Factor in Human Gastric Juice.**

<table>
<thead>
<tr>
<th>Period of Study</th>
<th>Saline Solution (or Buffer)</th>
<th>Test Gastric Juice</th>
<th>Normal Serum</th>
<th>Positive Serum B₁₂ (200)</th>
<th>20 Min. Chroining of Co⁵⁹-Labeled Vitamin B₁₂</th>
<th>Activation Change$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>ml.</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>Tett:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Serum control:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Radioactive standard:</td>
<td>3.0</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
<td>±50</td>
<td></td>
</tr>
</tbody>
</table>

$\dagger$ Modification of method of Ardeman and Chanarin.13
10 min. at room temperature.
5 Mix & stand for 20 min. at room temperature.
5 Shake for 1 or 2 min.; centrifuge; count radioactive label in supernatant.

**Figure 1. Investigation of 20 Patients with Chronic Hypochromic Anemia.**

**Figure 1.** Investigation of 20 Patients with Chronic Hypochromic Anemia.
Author has modified the last of these methods to make it more suitable for the analysis of large numbers of sera. Co⁶⁷-labeled vitamin B₁₂ is used with a specific activity of 1 microcurie per microgram, and this is stored at −20°C, in a concentrated stock solution. Before use it is diluted in physiologic saline solution so that 20 microg of Co⁶⁷-labeled vitamin B₁₂ is contained in 0.5 ml. The procedure for screening serum for anti-intrinsic-factor activity is then as shown in Tables 1 and 2. With this technique the inhibition of vitamin B₁₂ binding by a test serum of more than 3 microg, per milliliter is considered as being significant. Serums with an anti-intrinsic-factor activity of more than 10 microg, per unit per milliliter require to be titrated.

**Immunologic Survey in Pernicious Anemia and Related Disorders**

The results of a survey to determine the incidence of gastric and thyroid antibodies in pernicious anemia and related disorders and in control subjects are given in Table 2; references for the technics used are indicated. The age and sex distribution of the patients with pernicious anemia and with thyroid disease and of the control subjects is given elsewhere.¹⁵

There is little difference in the incidence or titer of gastric parietal-cell antibody in newly diagnosed cases of pernicious anemia as compared to cases that have been treated for many years.¹¹ In this series 73 per cent of the patients with pernicious anemia have antibody to parietal cells, and 55 per cent have antibody to intrinsic factor, whereas 86 per cent have either or both of these antibodies. This indicates that the parietal-cell antibody and the antibody to intrinsic factor do not run in parallel and that they must therefore be separate antibodies, as previously noted by Taylor et al.¹³ and by Irvine.⁶ Few of the patients with pernicious anemia have antibodies of the type that are not tissue specific (for example, the autoimmune complement-fixation reaction or antinuclear factors). Similar findings in the small series of cases of latent pernicious anemia demonstrate that the antibodies are present before the process has reached its completion. In 1933 Heath¹⁶ discussed the interrelation between pernicious anemia and idiopathic hypochromic anemia. Dagg et al.¹⁷ found gastric parietal-cell antibody in the serum of 11 out of 33 anemic (iron-deficient) patients with a histamine-fast achlorhydria and in only 2 out of 31 patients with acid gastric juice. Many of the 55 patients with chronic hypochromic anemia included in Table 2 of the present paper were suffering from gross blood loss due to menorrhagia or intestinal hemorrhage, and, in general, these patients had negative serologic reactions. By contrast, antibodies to gastric parietal cells were common in the serum of patients in whom blood loss was occult or insufficient to explain the presence of a severe hypochromic anemia. Goldberg and his associates¹ have shown that in 2 well matched groups of female patients with iron-deficiency anemia, 1 having histamine-fast achlorhydria, and the other with acid in the gastric juice, the achlorhydric patients absorbed

---

**Figure 2. Investigation of 29 Patients with Thyrotoxicosis.**

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<table>
<thead>
<tr>
<th>SECREATION OF GASTRIC HC1 (p mEq/IN THE POST-</th>
<th>HISTAMINE HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACHLORHYDIA &gt;10</td>
<td>X</td>
</tr>
<tr>
<td>2.512</td>
<td>O</td>
</tr>
<tr>
<td>2.512</td>
<td>O</td>
</tr>
<tr>
<td>128</td>
<td>X</td>
</tr>
<tr>
<td>64</td>
<td>X</td>
</tr>
<tr>
<td>32 O</td>
<td>O</td>
</tr>
<tr>
<td>16</td>
<td>O</td>
</tr>
<tr>
<td>8</td>
<td>O</td>
</tr>
<tr>
<td>4</td>
<td>O</td>
</tr>
<tr>
<td>I.C. POS. ONLY</td>
<td>O</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>O</td>
</tr>
</tbody>
</table>

X • SERUM POSITIVE FOR ANTIBODY TO INTRINSIC FACTOR (IF POS.)
O • SERUM NEGATIVE FOR ANTIBODY TO INTRINSIC FACTOR (IF NEG.)
I PATIENT WITH UNTREATED LATENT PERNICIOUS ANAEMIA (IF POS.)
I PATIENT WITH UNTREATED FRANK PERNICIOUS ANAEMIA (IF POS.)
only a third as much iron from an Fe²⁺-labeled meal as the group with acid in the gastric juice. It therefore appears that at least some cases of idiopathic hypochromic anemia with achlorhydria are an early stage in the same pathologic process that culminates in frank pernicious anemia. One out of the total of 55 patients with chronic hypochromic anemia had a detectable titer of antibody to intrinsic factor in the serum, and this patient is being carefully followed to determine whether or not she will progress to malabsorption of vitamin B₁₂.

The clinical overlap between pernicious anemia and spontaneous hypothyroidism has its immunologic counterpart in that 30 per cent of patients with chronic thyroiditis (Hashimoto's thyroiditis, or primary hypothyroidism without goiter) have gastric parietal-cell antibody in the serum and 40 per cent of patients with pernicious anemia have thyroid cytoplasmic antibody or antibody to thyroglobulin, or both, in the serum. Gastric or thyroid antibodies are present in 90 per cent of the patients with pernicious anemia, in 100 per cent of patients with Hashimoto's goiter and in 80 per cent of patients with primary hypothyroidism without goiter. In thyrotoxicosis 18 per cent of patients have thyroid cytoplasmic antibody or antibody to thyroglobulin, or both, and 24 per cent have antibody to gastric parietal cells. Similar findings have previously been reported by Irvine et al., Markson and Moore,⁶ Doniachi and Roitt⁸ and Irvine.⁸⁻¹⁰

**Correlation between the Presence of Gastric Antibodies and the Secretion of Gastric Acid and of Intrinsic Factor**

By definition patients with frank or latent pernicious anemia of the Addisonian type have advanced atrophic gastritis. To study the earlier stages that must precede this terminal event, gastric analysis using the augmented histamine test was carried out in a number of patients suffering from chronic hypochromic anemia, in patients with thyroid disease and also in 13 hospital control patients who gave positive tests for gastric parietal-cell antibody. The secretion of free hydrochloric acid in the posthistamine hour has been correlated with the titer of gastric parietal-cell antibody in the patient's serum and with the presence or absence of antibody to intrinsic factor in the serum. These findings are shown in Figures 1-3. The selection of patients for this type of analysis is necessarily biased in favor of those with gastric antibody in the serum, but the degree of bias can be assessed from the incidence of this antibody in the unselected patients shown in Table 3.

Although the incidence of gastric antibodies differs in patients with different clinical disorders the pattern of gastric acid secretion in the presence of these antibodies is essentially the same. The presence of gastric parietal-cell antibody in the serum is almost invariably associated with a reduction in acid secretion, but the severity of this cannot be closely corre-
Table 3. Incidence of Gastric and Thyroid Antibodies and of Antinuclear Factor in the Serum of Patients with Pernicious Anemia and with Related Disorders and in Control Subjects.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Positive Gastric Parietal Cells</th>
<th>Positive Gastric Parietal Cell Hemagglutination or Complement Fixation Test</th>
<th>Positive Thyroid or Thyroiditis</th>
<th>Positive Gastric or Thyroid</th>
<th>Positive Antinuclear Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pernicious anemia</td>
<td>130</td>
<td>73</td>
<td>55</td>
<td>41</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>Latent pernicious anemia</td>
<td>6</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Chronic hypochromic anemia</td>
<td>35</td>
<td>14</td>
<td>2</td>
<td>12</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>302</td>
<td>24</td>
<td>3</td>
<td>48</td>
<td>57</td>
<td>3</td>
</tr>
<tr>
<td>Chronic thyroiditis</td>
<td>238</td>
<td>30</td>
<td>3</td>
<td>78</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Idiopathic adrenal insufficiency</td>
<td>25</td>
<td>32</td>
<td>28</td>
<td>56</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>Blood donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-45 yr. of age — male &amp; female</td>
<td>629</td>
<td>5</td>
<td></td>
<td>13</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>40-60 yr. of age — female</td>
<td>111</td>
<td>9</td>
<td>0</td>
<td>26</td>
<td>28</td>
<td>1</td>
</tr>
</tbody>
</table>

*Fluorescent-antibody method.*
†Method of Fulthorpe et al.*
‡Using 2 MHD complement and 50% hemolysis as end point.*
§Method of Irvine.*

Fluorescent-antibody method.
Method of Fulthorpe et al.
Using 2 MHD complement and 50% hemolysis as end point.
Method of Irvine.

Figure 4 demonstrates that the reduction in the gastric secretion of intrinsic factor parallels that in acid secretion with the titer of the antibody in the serum. The presence of this antibody is also associated with a reduction in the number of parietal cells and with an increase in the lymphocytic infiltration in the gastric mucosa as determined by biopsy using a Crosby capsule. The loss of chief cells parallels the loss of parietal cells although the mucus-secreting cells are unaltered; in terms of present knowledge of gastric immunology this is difficult to explain. Figures 1-3 show that antibody to intrinsic factor is correlated with an advanced degree of gastric atrophy and, with only one exception (Fig. 1), the presence of this antibody is associated with some abnormality of vitamin B₁₂ absorption in the Schilling test. Figure 4 demonstrates that the reduction in the gastric secretion of intrinsic factor parallels that in acid secre-

![Figure 4](image-url)
tion and that, in the presence of achlorhydria, the atrophic gastric mucosa may continue to form small amounts of intrinsic factor sufficient to maintain an adequate absorption of vitamin B_{12}. The method of assay of intrinsic factor is shown in Table 3: a normal serum and a serum that is known to contain a high titer of antibody to intrinsic factor are used. The variable is then the amount of intrinsic factor in the sample of gastric juice that is being tested.

These studies suggest that gastric atrophy and achlorhydria are a frequent occurrence in the presence of gastric parietal-cell antibody, but antibody to intrinsic factor may be required for this to progress to malabsorption of vitamin B_{12}.

**Genetic Aspects of Gastric Autoimmunity**

It is well known that there is a hereditary factor in the etiology of pernicious anemia. Figure 5 presents the clinical and serologic findings in a family in which both parents had gastric parietal-cell antibody in the serum and 1 of whom also had antibody to intrinsic factor.

Three out of 7 of the offspring have antibody to gastric parietal cells in the serum, and at least 2 of these have evidence of gastritis, 1 with hypochlorhydria and the other with achlorhydria, but both subjects have normal results on Schilling tests and normal levels of serum vitamin B_{12}. This family is included in Table 8 of Irving and his associates. Also reported in this paper is a pair of monozygotic twins, both of whom have parietal-cell antibody in the serum and both of whom were diagnosed, at the same time and in different hospitals, as having latent pernicious anemia. According to Tr Veille et al., the development of parietal-cell antibody is controlled by a dominant autosomal gene, which is also responsible for the processes leading to atrophic gastritis, gastric achlorhydria and impairment of vitamin B_{12} absorption. Complete expression of the pattern is the complete disease whereas incomplete expression provides the various situations found in the group of asymptomatic relatives. There can be little doubt that gastric serology is of value in detecting the early and subclinical stages of the process that culminates in frank pernicious anemia and which appears to have an increased incidence among the immediate relatives of patients with pernicious anemia.

**Disordered Immunologic Tolerance**

The similarities between gastric and thyroid autoimmunity are strong: both are characterized by a high incidence of tissue-specific antibodies, and in both there is an antibody directed against a constituent of the cell cytoplasm of the target organ and a second antibody against a secretion of the corresponding gland. The age and sex distribution of the two diseases is similar. It has been argued that thyroid autoimmunity is a manifestation of disordered immunologic tolerance and it is likely that the same basic mechanism underlies the occurrence of autoimmunity in the atrophic gastritis of pernicious anemia.

By means of pneumomediastinography, the size of the thymic shadow was found to be abnormally large (p less than 0.001) in thyroid patients with thyroid or gastric antibodies in the serum (34 patients, with a mean thymus size of 9.3 square centimeters; S.D., 4.9) when compared to the size of the thymic shadow in patients who had thyroid disease but no thyroid or gastric antibodies in the serum (10 patients, with a mean thymus size of 3.6 square centimeters; S.D., 1.3).

**Conclusions**

Tissue-specific autoimmunity is a prominent feature in patients with Addisonian (pernicious) anemia, and, provided this is what is meant by the term “autoimmune disease,” pernicious anemia must be classified among the most striking examples of this disorder. Whether autoimmunity is of basic importance in the pathogenesis of atrophic gastritis remains unproved, but the possibility that this is in fact the case is sup-

![Figure 5. Family Study of the Inheritance of Gastric Autoimmunity.](image-url)

ported by strong circumstantial evidence. Further work is required to demonstrate a cytotoxic effect of parietal-cell antibody on cells of the gastric mucosa, to induce atrophic gastritis in small laboratory animals by the injection of gastric extracts and Freund adjuvant and to study its adoptive transfer to an unaffected animal with the use of a suspension of sensitized lymphocytes.

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AUTOIMMUNE AND HYPERSENSITIVITY PHENOMENA
IN ALIMENTARY DISEASES

W. J. Irvine

(1968) in Recent Advances in Clin. Path. Ed. Dyke
Chapter 29

AUTO-IMMUNE AND HYPERSENSITIVITY PHENOMENA IN ALIMENTARY DISEASES

W. J. Irvine

Introduction

During the past decade immunological phenomena, with special reference to auto-immunity and hypersensitivity, have been the subject of much study in relation to diseases of the alimentary system. My object has been to review the present state of knowledge in this area, indicating where the existence of immunological phenomena has been clearly established and where its existence is less certain. In so doing, reference is made to the various immunological techniques that have been applied to the study of alimentary diseases. The diagnostic value of certain auto-antibody tests is stated and the possible role of immunological mechanisms in the pathogenesis of various diseases of the alimentary system is discussed.

Aphthous Ulceration

Recurrent aphthous ulceration of the mouth is a chronic disorder of unknown etiology which occurs more frequently in females. The histology is characterized by lymphocytic and plasma cell infiltration and the condition is said to be associated with idiopathic steatorrhea, Crohn's disease and ulcerative colitis, although the evidence for this is far from satisfactory. Aphthous ulceration also occurs as part of Behcet's syndrome (recurrent oral and genital ulceration with associated eye lesions, especially iritis). Using tanned cell haemagglutination, complement fixation and precipitin techniques with a saline extract of fætal oral mucosa, Lehner (1964, 1967) has described a higher incidence of positive results and titres with the sera of patients suffering from aphthous ulceration in its major form and also with the sera of patients with Behcet's syndrome than with the sera of control subjects. The antigen is said to reside in the prickle cells. Preliminary studies indicate that the active constituent in the serum is in the IgG and IgM globulin fractions.

A second immunological approach has been the demonstration of an increased incidence and titres of antibodies to milk proteins in the sera of patients with aphthous ulceration (Taylor, Truelove and Wright, 1964). As will be discussed later, this has also been described in patients with ulcerative colitis.

Sjögren's Syndrome

Sjögren's syndrome is a chronic benign disorder which occurs in middle-aged females and which is characterized by the triad of keratoconjunctivitis
sicca, xerostomia and in one-half to two-thirds of patients, some form of connective tissue disease, usually rheumatoid arthritis and less commonly systemic lupus erythematosus, progressive systemic sclerosis, polymyositis or periarteritis nodosa. The term “sicca” syndrome is used to describe the association of keratoconjunctivitis sicca and xerostomia in the absence of connective tissue diseases. The lacrimal and salivary glands show extensive chronic inflammatory cell infiltration and acinar atrophy with failure of secretion. Similar pathological changes may be seen in other secreting glands and lead to dryness of the nose, pharynx, larynx, trachea, bronchi and vagina. In the salivary glands the intercalated ducts frequently show epithelial and myo-epithelial proliferation (Morgan and Castleman, 1953; Bloch et al., 1965).

**Fig. 1.** Staining of direct epithelial cytoplasm in the indirect immunofluorescence test using an unfixed section of human parotid tissue, serum of a patient with Sjögren’s syndrome and anti IgG conjugated with fluorescein (UV × 225).

(From Feltkamp and van Rossum (1968), *Clin. exp. Immunol*).

Bertram and Halberg (1964) using the indirect fluorescent antibody technique with unfixed sections of normal parotid, submaxillary and sublingual glands, have demonstrated antibodies to the salivary duct cell cytoplasm in 11 out of 19 patients. Feltkamp and van Rossum (1968) have confirmed these findings, obtaining an incidence of 16 sera positive for antibody to salivary duct cells out of a series of 30 patients (Fig. 1). The antibodies were always present in the IgG serum fraction, but some also occurred in the IgM fractions and a few in the IgA serum fractions. These observations are of importance since previous serological studies using complement fixation or precipitin test had revealed only non-organ-specific antibodies (Anderson et al., 1961; Bloch and Bunim, 1963). Some sera react in the immunofluorescent tests with epithelium of sweat glands, breast, prostate and bronchus. Feltkamp and van Rossum (1968) found no salivary
duct antibodies in normal controls matched for age and sex. Bertram and Halberg (1965) describe a frequency of 27 per cent in controls above the age of 65 years.

Rheumatoid factor has been described in 48–100 per cent of sera from patients with Sjögren's disease. The prevalence and titre of rheumatoid factor is just as high in those patients with the sicca syndrome alone as in those with Sjögren's syndrome associated with a connective tissue disease, including rheumatoid arthritis.

Antinuclear antibodies (ANF) have been found in a high proportion of patients with Sjögren's syndrome. Positive L.E. cell tests appear to be related to associated connective tissue disease rather than to the sicca syndrome. By contrast, antinuclear antibodies have been detected by immunofluorescence techniques in approximately two-thirds of patients with Sjögren's syndrome. The increased prevalence of ANF in patients with the sicca syndrome is due to an increased incidence of "speckled" and "nuclear" antibodies (Beck et al., 1965).

In Feltkamp and van Rossum's series, the incidence of antibodies to skeletal muscle, gastric parietal cell, thyroid antigens, adrenal cortex, and mitochondria did not differ significantly from those in matched controls. Anderson et al. (1965) found an increased incidence of gastric parietal cell antibody in British subjects with Sjögren's but not in patients with this disease who were studied at the National Institute of Health, U.S.A. He maintains that antibody to thyroglobulin is more common in Sjögren's than in controls but the titres were generally low. There is no convincing evidence for an increased prevalence of clinical chronic thyroiditis or atrophic gastritis in Sjögren's syndrome.

In a preliminary report it has been stated that family studies in Sjögren's syndrome suggest an increased prevalence of rheumatoid arthritis, decreased tear formation (as detected by the Shirmer test), thyroglobulin antibodies, auto-immune complement fixation tests and elevated IgG levels in relatives of patients with the disease (Burch, Bunim and Bloch, 1963).

Although attempts have been made to produce Sjögren's syndrome by immunological means in experimental animals, the results do not closely resemble the lesions in man. There was no epithelial or myo-epithelial hyperplasia, but these features might be regarded as a form of repair and not specific for any particular injury (Chan, 1964).

In conclusion, the immunological features of Sjögren's syndrome resemble the non-organ-specific group of auto-immune diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis) more strongly than they do the organ-specific group (thyroiditis, pernicious anaemia and idiopathic Addison's disease). The role of the antibodies in the pathogenesis of the disease remains undetermined.

**Atrophic Gastritis**

A number of facts suggested that immunological phenomena may be important in the study of the atrophic gastritis of Addisonian pernicious anaemia. The histology of the gastric mucosa in pernicious anaemia is characterized by atrophy of the specialized cells of the body of the stomach accompanied by a variable but often marked degree of lymphocytic infiltration. Pernicious anaemia occurs predominantly in middle-aged females and has an increased...
incidence in auto-immune thyroid disease (thyrotoxicosis, Hashimoto goitre and primary atrophic hypothyroidism). It is known that corticosteroids can induce a reticulocyte response (Doig et al., 1957) and improve absorption of radioactive vitamin B₁₂ (Frost and Goldwein, 1958; Gordin, 1959; Kristensen and Friis, 1960) in Addisonian pernicious anaemia patients. During the past ten years auto-immune phenomena in relation to the gastritis of pernicious anæmia and in some cases of atrophic gastritis without pernicious anæmia have been clearly established.

**Antibody to Gastric Parietal Cells.** In a careful study of the relationship between pernicious anæmia and thyroid disease, Tudhope and Wilson (1962)

![Fig. 2. Positive indirect immunofluorescence test for gastric parietal cell antibody using an unfixed section of body of human stomach, serum from a patient with Addisonian pernicious anæmia and human polyvariant gammaglobulin conjugated with fluorescein (UV x 50).](image)

(From Irvine et al. (1965a), Annals, N.Y. Acad. Sci.).

found that 24 out of 52 patients with primary hypothyroidism had a histamine fast achlorhydria and that 12 per cent of 72 hypothyroid patients had Addisonian pernicious anæmia; immunological methods that had proved so rewarding in the investigation of thyroid disease were therefore applied to the study of pernicious anæmia. It was found that 75 per cent of the sera of patients with Addisonian pernicious anæmia gave a positive complement fixation test specific for a saline extract of gastric body mucosa and that the serum component responsible for this reaction was a gammaglobulin (Irvine et al., 1962). Using the immunofluorescence technique Taylor et al. (1962) and Irvine (1963) demonstrated that the complement fixation test specific for parietal cell cytoplasm (Fig. 2), and it was shown that the gastric antibody would react equally well with the patient's
own gastric mucosa provided it still contained an adequate concentration of parietal cells (Irvine, 1963). An overlap was shown to occur in the incidence of gastric and thyroid antibodies in pernicious anaemia and thyroid disease (Irvine, 1965a; Roitt, Doniach and Shapland, 1965). Approximately 30 per cent of patients with auto-immune thyroid disease (thyrotoxicosis, Hashimoto thyroiditis and primary atrophic hypothyroidism) have gastric parietal cell antibody and between 30–55 per cent of patients with pernicious anaemia have thyroid complement fixing antibody in the serum.

The parietal cytoplasmic antigen is associated with an insoluble lipoprotein microsomal element (Baur, Roitt and Doniach, 1965). Electron microscopic and ultraviolet absorption evidence suggests that the antigen component(s) of the microsomal fraction is associated with smooth membranes and not with the ribosomes (Ward and Nairn, 1967). The gastric parietal cell antigen is not species-specific; rat gastric mucosa (de Boer, Nairn and Maxwell, 1965) and pig gastric mucosa (Irvine, 1966a) reacting equally well. It is necessary to distinguish the gastric specific cytoplasmic fluorescence from that obtained with the mitochondrial antibodies in primary biliary cirrhosis (Doniach et al., 1966; Goudie, MacSween and Goldberg, 1966) and from lysosomal (Wiedermann and Miescher, 1965) and ribosomal (Sturgill and Carpenter, 1965) antibodies described in connective tissue disorders. To exclude this possibility human kidney sections are included as a control in the fluorescent tests and fresh rat kidney homogenate is used for a control in the complement fixation reaction. The gastric complement fixation reaction, although usually carried out by the Takasi microtitre method, can be automated (Irvine, 1966a).

Jeffries and Sleisenger (1965) and Fisher, Rees and Taylor (1965) have shown the presence of antibodies to gastric parietal cells in gastric juice of subjects who have circulating parietal cell antibodies. Whether these have any special function has not yet been elucidated.

Antibodies to Intrinsic Factor. The first direct demonstration that immune phenomena may occur in association with pernicious anaemia is to be found in the work of Schwartz (1958, 1960). In patients given oral intrinsic factor, he looked for inhibitors to intrinsic factor using an in vitro assay and found them in the serum of the majority of pernicious anaemia patients so treated but also in some who had not. Likewise, Taylor (1959) observed intrinsic-factor-inhibiting substance in nine out of 19 patients either treated by parenteral administration of vitamin B₁₂ or untreated.

In vitro methods for the detection of antibodies to intrinsic factor have been developed. The basis of the method of Jeffries, Hoskins and Sleisenger (1962) is to observe the alteration of the electrophoretic mobility of radio-active vitamin B₁₂ intrinsic factor complex in the presence of test as opposed to normal serum. In the presence of antibody to intrinsic factor a larger complex is formed and is retained at the origin on starch gel electrophoresis. Other methods depend upon the property of intrinsic factor to bind vitamin B₁₂ and that this can be inhibited by antibody to intrinsic factor (Abels et al., 1963). The method of Ardeman and Chanarin (1963) has been modified by Gottlieb et al. (1965) and by Irvine (1966b), and is dependent upon the separation of bound from unbound radio-active vitamin B₁₂ by protein coated charcoal. Abels et al. (1963) used dialysis to separate the unbound vitamin B₁₂.
Schade et al. (1967) have shown that there are two separate antibodies to intrinsic factor. Antibody I blocks the binding of B₁₂ by intrinsic factor and antibody II unites with intrinsic factor-B₁₂ or with intrinsic factor alone. Antibody I exchanges readily with the B₁₂ of intrinsic factor-B₁₂ complex. The complex of intrinsic factor and antibody I will absorb antibody II. Intrinsic factor would therefore appear to have at least two antigenic sites. The intrinsic factor antibodies are IgG immunoglobulins.

In man there is evidence that the gastric parietal cell is the site of secretion of intrinsic factor (Hoedemarker et al., 1964). Fisher, Rees and Taylor (1966) have produced circumstantial evidence that antibody to intrinsic factor may be present in the gastric juice of some patients with pernicious anemia. Schade et al. (1966) studied one patient who did not absorb normal amounts of vitamin B₁₂ from a standard dose of normal human gastric juice and vitamin B₁₂ given by mouth. The patient's gastric juice was shown by in vitro and in vivo techniques to contain antibody to a complex of human intrinsic factor and vitamin B₁₂. The inhibition of vitamin B₁₂ absorption by the presence of antibody to intrinsic factor in gastric juice would provide an interesting demonstration that an antibody can inhibit the physiological function of an endogenous antigen. Further studies are required to establish this point.

Incidence of Gastric Antibodies in Pernicious Anemia and Related Disorders. Seventy-three to eighty-nine per cent of patients with Addisonian pernicious anæmia have gastric parietal cell antibody detectable in the serum irrespective of the duration of treatment that the patient may have had with vitamin B₁₂ (Taylor et al., 1962; Irvine, 1965a; Jeffries and Sleisinger, 1965). There is a rising incidence with age, particularly in females, in control populations (Irvine et al., 1965a). As already mentioned, the incidence of parietal cell antibodies in the thyroid auto-immune diseases is 25-33 per cent. There is an increased incidence in patients with chronic iron deficiency anæmia (Dagg et al., 1964; Irvine et al., 1965a), in patients with idiopathic adrenal insufficiency (Irvine, Stewart and Scarth, 1967) and in the young diabetic subjects (Moore and Neilson, 1965; Irvine and Davies, 1963; Irvine, 1968). In all these groups the presence of parietal cell antibody in the serum correlates with some degree of atrophic gastritis. The incidence and titre of gastric parietal cell antibody shows a general correlation with the severity of the atrophic gastritis as indicated by a reduction in gastric acid secretion following stimulation by histamine and a reduction in the number of parietal cells and also chief cells in the gastric biopsy (Adams et al., 1964; Dagg et al., 1964; Irvine et al., 1965a; Irvine, 1965a). The incidence of parietal cell antibodies is increased in patients with simple atrophic gastritis without associated disease (Mackay, 1964; Coghill et al., 1965) but the incidence in gastric carcinoma, gastric ulcer, and in post-gastrectomy and post-gastro-enterostomy cases is low.

Intrinsic factor antibody I occurs in about 50-60 per cent of patients with Addisonian pernicious anæmia (Ardeman and Chanarin, 1963; Roitt, Doniach and Shapland, 1965; Irvine, 1965a) and intrinsic factor antibody II occurs in approximately 30 per cent (Jeffries, Hoskins and Sleisinger, 1962). Antibody to intrinsic factor shows a very high degree of correlation with malabsorption of vitamin B₁₂ due to deficiency in secretion of intrinsic factor (Irvine, 1966b). A small number of patients have been described in whom intrinsic factor antibodies are present in the serum although no abnormality could be detected in the patient's vitamin B₁₂ metabolism. These patients have occurred among those with thyroid auto-immune diseases (Ardeman and Chanarin, 1966), idiopathic adrenal insufficiency (Irvine, Stewart and Scarth, 1967) and in relatives of pernicious anæmia.
patients (te Velde et al., 1964). The majority of these subjects had a histamine fast achlorhydria, although one patient described by Ardeman and Chanarin had a normal secretion of both acid and intrinsic factor; in this patient it was shown that the antibody to intrinsic factor was a true auto-antibody. In general, patients with simple atrophic gastritis, although they may have the same gastric histology as patients with pernicious anemia, do not have intrinsic factor antibody in the serum (Coghill et al., 1965; Fisher, Rees and Taylor, 1965). Very few control subjects have yet been found with antibody to intrinsic factor in the serum.

When pernicious anemia of the Addisonian type occurs in children with gastric atrophy and loss of both acid and intrinsic factor secretion, there is a high incidence of both parietal cell and intrinsic factor antibodies in the serum (Doniach and Roitt, 1964). However, in infantile pernicious anemia (in which there is normal gastric histology and acid secretion but an impairment of intrinsic factor synthesis) no gastric antibodies have been detected (Doniach, Roitt and Taylor, 1965). It would appear that infantile pernicious anemia is a distinct and separate condition from Addisonian pernicious anemia of the adult. The adult type of pernicious anemia occasionally affects juveniles. Infantile pernicious anemia is due to a highly selective deficiency in intrinsic factor synthesis and is genetically unrelated to adult pernicious anemia and is not associated with auto-immunity (McIntyre et al., 1965).

Immunooassay of Intrinsic Factor. Possibly the most direct application of the study of gastric auto-immunity has been the development of a sensitive and accurately quantitative immunooassay for gastric intrinsic factor using intrinsic factor antibody I. The most satisfactory methods (Gottlieb et al., 1965; Irvine, 1966b) are modifications of the method of Ardeman and Chanarin (1963). The secretion of intrinsic factor in the post-histamine hour is less than 100-200 nanogram units in patients with pernicious anemia (Ardeman and Chanarin, 1965a; Irvine et al., 1965b; Irvine et al. 1968), and, as shown in Fig. 3, there is a good correlation between the direct immunooassay of intrinsic factor in gastric juice following histamine or pentagastrin in patients with achlorhydria and the absorption of vitamin B\textsubscript{12} in the Schilling test correctable with exogenous intrinsic factor.

The immunooassay of intrinsic factor has also enabled the description of the pattern of intrinsic factor secretion following gastric stimulation with histamine or gastrin or pentagastrin. Immediately following stimulation intrinsic factor would appear to be washed out of the gastric secretory cells or from the gastric crypts. This is followed by a much lower but sustained intrinsic factor secretion at approximately twice the basal level, if stimulation is continued (Irvine, 1965b; Wangel and Callender, 1965; Irvine, 1966b; Lawrie and Anderson, 1967).

According to Ardeman and Chanarin (1965a) 500 ng. units of human intrinsic factor in a single dose usually restores the absorption of vitamin B\textsubscript{12} in pernicious anemia. Since intrinsic factor antibody is not strictly species-specific, it can also be used for the immunoassay of preparations of hog intrinsic factor and thereby provide a ready means of standardization. Three hundred to six hundred nanogram hog intrinsic factor is sufficient to correct vitamin B\textsubscript{12} absorption in patients with pernicious anemia (Irvine, 1966b).
Relation of Gastric Antibodies to Pathogenesis of Atrophic Gastritis.

Achlorhydria and indeed frank Addisonian pernicious anaemia can occur without gastric parietal cell or intrinsic factor antibody being detectable in the serum. Electron microscopic studies (Irvine et al., 1965a) have shown that lymphocytes may lie within the gastric tubules, invaginating the specialized cells of the gastric mucosa. Crabbe, Carbonara and Heremans (1965) have demonstrated the production of IgA, IgM and IgG immunoglobulins in the lamina propria of the small gut in man but the antigenic specificities of these immunoglobulins is not known. A comparable study of the stomach in atrophic gastritis would be of interest. As already mentioned, parietal cell and intrinsic factor antibodies have been described in the gastric juice. The presence of intrinsic factor antibody in gastric juice may be independent of the level of the antibody in the serum. This suggests that gastric antibodies may be synthesised within the gastric mucosa.

Antibody to intrinsic factor may be effective at the site of vitamin B₁₂ absorption in the distal ileum. The fact that vitamin B₁₂ absorption can be corrected in Addisonian pernicious anaemia by the administration of oral intrinsic factor can be explained by assuming that the dose of intrinsic factor given is in excess of what can be inhibited in vivo by intrinsic factor antibody.

![Graph](image-url)
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It has been argued that the presence of gastric parietal cell antibody in the serum of patients with chronic hypochromic anaemia may be a consequence of atrophy of the gastric mucosa as a result of the iron deficiency (McFadyen et al., 1967). To others (Irvine, 1967) it would appear more probable that the chronic hypochromic anaemia may be due to atrophic gastritis as it has been shown that such subjects have some impairment of iron absorption (Goldberg, Lochhead and Dagg, 1963). The latter view is supported by the observations of Wright et al. (1965) that patients with certain diseases (e.g. auto-immune thyroid disease) are much more liable to produce gastric parietal cell antibodies than patients with other diseases (e.g. ulcerative colitis, idiopathic steatorrhoea), although the histological appearance of gastric atrophy is identical. Patients with achlorhydria may continue to form small amounts of intrinsic factor sufficient to maintain an adequate absorption of vitamin B12. In only a relatively small proportion of patients with gastric atrophy is the impairment of vitamin B12 absorption of such a degree as to lead to pernicious anaemia. The transition from a state of achlorhydria with adequate absorption of vitamin B12 to that of pernicious anaemia with malabsorption of the vitamin has only infrequently been observed.

Although the histological appearance of the atrophic gastric mucosa of pernicious anaemia would suggest a static end stage of an antecedent destructive process, Croft, Pollock and Coghill (1966) have shown, from the measurement of DNA in gastric perfusates, that in pernicious anaemia the rate of cell loss (and therefore presumably the rate of cell turnover) in the gastric mucosa was not reduced below normal. The capacity of the gastric mucosa to regenerate is shown by the response of some patients with pernicious anaemia to prolonged treatment with therapeutic doses of corticosteroids (15-40 mg. prednisolone/day). In some there is an increase in the secretion of intrinsic factor to levels sufficient to restore the Schilling test to normal and small amounts of acid may be secreted. In serial biopsies histological evidence for some regeneration of parietal cells and chief cells has been observed. The improvement in the histology and function is not maintained after the steroids are withdrawn (Jeffries, 1965; Ardeman and Chanarin, 1965b; Jeffries, Todd and Sleisenger, 1966; Rodbro et al., 1967; Wall et al., 1968). In two patients with megaloblastic anaemia following partial gastrectomy there was no improvement in the absorption of vitamin B12 with steroid therapy; it is possible that ischaemic factors are important in producing atrophy of the gastric remnant (Ardeman and Chanarin, 1965b). It would therefore appear that corticosteroids may counteract an immuno-destructive process in patients with pernicious anaemia permitting mucosal regeneration to occur.

Convincing experiments have yet to be done to show that a state of chronic atrophic gastritis can be produced in rats, guinea-pigs or dogs by immunological techniques, but in monkeys atrophic gastritis can be produced; for example, by the repeated intradermal injection of gastric antigen emulsified with Freund's adjuvant. (Andrada and Rose, 1969). It has also not yet been possible to demonstrate a cytotoxic effect of humoral antibody or of lymphocytes on parietal cells in tissue culture, the difficulty being to get parietal cells to grow in tissue culture.

Familial Tendency to Pernicious Anaemia and Gastric Auto-immunity. The relatives of patients with pernicious anaemia show an increased incidence of
overt pernicious anemia, latent pernicious anemia and of histamine fast achlorhydria (Callendar and Denborough, 1957; McInyre et al., 1959), te Velde et al. (1964) found gastric parietal cell antibodies in 20 per cent of 220 relatives of 21 patients with pernicious anemia. They concluded from an analysis of the pedigrees that the development of this antibody is controlled by a autosomal gene with incomplete penetrance showing a sex controlled bias. Roitt and Doniach (1967) in a careful re-assessment of the aggregation of auto-immunity in families of thyroiditis patients showed that the increased incidence of gastric antibodies which had been previously reported in the relatives was no longer evident.

It is often stated that the geographic distribution of pernicious anemia shows considerable variation. It is said to be rare in the coloured races. The incidence of gastric parietal cell antibodies and intrinsic factor antibodies in a population of Hong Kong Chinese thyrotoxic patients was the same as in a British population of similar patients (Irvine, McFadzean, Todd, Tso and Young; in preparation). A less complete reduction in intrinsic factor together with a high dietary intake of vitamin B$_12$ probably explains the lower incidence of PA in the Hong Kong Chinese.

Clinical Value of Gastric Antibodies. It is suggested that patients at risk of developing pernicious anemia (those with thyroid auto-immune disease, idiopathic Addison's disease, early onset diabetes, chronic hypochromic anaemia and relatives of patients with pernicious anemia) should be screened routinely for antibody to gastric parietal cells and for antibody to intrinsic factor. While such a procedure may indicate considerable subclinical disease it will also indicate a proportion of patients with latent or overt pernicious anemia that had previously escaped diagnosis. The immunoassay of gastric intrinsic factor is recommended as a simple and direct diagnostic aid and can be done on the samples obtained during routine gastric analysis for acid secretion.

Subclinical Atrophic Gastritis. The study of gastric auto-immunity has revealed that advanced atrophic gastritis without symptoms and without vitamin B$_12$ deficiency is a common occurrence. This observation, together with the development of an accurate and quantitative technique for the assay of intrinsic factor, should allow a better understanding of the natural history of atrophic gastritis and its mode of inheritance.

Liver Disease

There is evidence that auto-immunity is concerned with three types of chronic liver disease—active chronic hepatitis, cryptogenic cirrhosis and primary biliary cirrhosis.

Active Chronic Hepatitis ("Juvenile" Cirrhosis; "Lupoid" Hepatitis). Active chronic hepatitis is associated with the markers indicative of an auto-immune disease—hypergammaglobulinemia, circulating auto-antibodies in a proportion of cases to antigens derived from cell nuclei and cytoplasm, lymphoid and plasma cell infiltration and "piecemeal" necrosis in the liver, response to cortisone and an occasional association with other auto-immune diseases (Mackay, Weiden and Hasker, 1965). Lupoid hepatitis may be regarded as those cases of active chronic hepatitis that have a positive LE cell test and who may occasionally have manifestations, usually of a minor nature, of systemic lupus erythematosus. Some cases of active chronic hepatitis have an initial illness resembling infective hepatitis, but in
many the disease has an insidious onset. Some cases of active chronic hepatitis become quiescent, but others progress to post-necrotic cirrhosis.

Cryptogenic Cirrhosis. In this group of patients there is no history of alcohol abuse and the histology shows a post-necrotic cirrhosis.

Primary biliary cirrhosis describes a clinical picture of prolonged obstructive jaundice or cholestasis, but with demonstrable patency of the extrahepatic ducts. The disease begins in mid to late adult life and is relentlessly progressive; the majority of cases occur in females. The onset is insidious and the course prolonged. The early histological appearances on liver biopsy show destruction of bile ducts with lymphocytic infiltration, initially with minimal fibrosis and a well-preserved hepatic architecture. Subsequently, the development of fibrous tissue in the portal areas and regeneration of liver tissue produces a finely or coarsely nodular cirrhosis.

Immunological Features of Liver Disease

ANF. According to Doniach et al. (1966) by far the highest incidence of ANF is found in active chronic hepatitis (77 per cent compared to 2 per cent in matched controls for this young age group). Fifty-six per cent had moderate or high titres although only five of the total of 43 subjects gave a positive LE cell test. Seventy-nine per cent of patients’ sera gave a diffuse nuclear staining pattern and 21 per cent were of the speckled variety.

In primary biliary cirrhosis 46 per cent of 41 cases were positive for ANF, the titres being moderate or high in 31 per cent. Speckled and diffuse staining reactions were found in equal proportions and LE cell tests were consistently negative. Thirty-eight per cent of patients with cryptogenic cirrhosis were positive for ANF.

Mitochondrial Antibodies. Antibodies to mitochondria ("M" antibodies) have been described by Doniach et al. (1966) in the sera of virtually all patients with primary biliary cirrhosis (98 per cent). They were also found in 31 per cent of patients with cryptogenic cirrhosis (particularly those with obstructive features) and 28 per cent of cases of active chronic hepatitis. In contrast only two patients out of 28 with extrahepatic biliary obstruction and one patient out of 25 with infective hepatitis gave reactions and these in very low titre. Uniformly negative results for "M" antibodies were obtained in alcoholic cirrhosis, cholestatic drug jaundice and cholestasis associated with ulcerative colitis.

These antibodies are clearly of value to the clinician in the differential diagnosis of surgical from non-surgical jaundice, although care must be taken in interpretation of the results in cases with associated connective tissue disorders where a significant incidence of positive reactions has been observed. In confirmation of the findings of Walker et al. (1965), Goudie, MacSween and Goldberg (1966) noted that out of 30 cases diagnosed as primary biliary cirrhosis 26 had anti-mitochondrial antibody whereas none of 77 cases with jaundice due to extrahepatic bile obstruction showed this serological abnormality. The incidence of the "M" antibodies in the different types of liver disease suggest that they do not arise merely in response to liver damage.

As implied by the name, antibodies to mitochondria are not specific to
any tissue. In the indirect immunofluorescence procedure “M” antibodies give a bright uniform cytoplasmic fluorescence with renal distal tubules, gastric parietal cell, and thyroid Askanazy cells, and a characteristically coarse granular duller staining of renal proximal tubule, thyroid epithelial and gastric chief cells (Fig. 4). Human renal distal tubule is probably the best tissue to use for the detection of “M” antibodies so as to avoid confusion with other tissue specific antibodies that may also be present in the patient’s serum.

![Image of tissue staining](image-url)

**Fig. 4.** Positive test for mitrochondrial (“M”) antibodies using unfixed section of rat kidney, serum from a patient with primary biliary cirrhosis and anti-δ, C conjugated with fluorescein. Note the bright uniform cytoplasmic fluorescence in the renal tubules. A glomerulus is seen to be unstained (UV × 350).

**Complement Fixation Tests.** Many authors have applied the complement fixation reaction to the study of antibodies in liver diseases, but in no instance was evidence for organ-specificity obtained. Doubt has been cast on the truly immunological nature of the so-called “auto-immune complement fixation test” of Gajdusek (Beall, 1963). Complement fixation tests with liver sera will detect antibodies to mitochondria and also to other cell constituents. They have been described in acute infective hepatitis (Holborow, *et al.*, 1963).
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Pinckard and Weir (1966) have demonstrated a transient appearance of complement fixing antibodies which react mainly with mitochondrial fractions in rats developing liver cell necrosis after injections of carbon tetrachloride and other poisons. These reach a maximum 3–4 days after injury and disappear on recovery. Similar antibodies were obtained in rats by injections of homologous liver extracts, clearly showing that the rat responds immunologically to the products of liver cell breakdown. The finding of Doniach et al. (1966) of “M” antibodies in three out of five patients with drug related hepatic necrosis may be relevant in this context. These observations are difficult to reconcile, however, with the definite predilection of “M” antibodies for certain liver diseases and not for others as described above.

Smooth Muscle Antibodies. Smooth muscle antibodies as detected by the immunofluorescence test occur in about two-thirds of patients with active chronic hepatitis, about half of the patients with primary biliary cirrhosis and about a quarter of patients with cryptogenic cirrhosis (Johnson, Holborow and Glynn, 1965; Whittingham, 1966; Doniach et al., 1966). These antibodies were not found, or only in low incidence, in other forms of liver disease or in control subjects.

Rheumatoid Factor. The Latex fixation reaction is positive in 17–53 per cent of patients with viral hepatitis, primary biliary cirrhosis, and alcoholic, juvenile and cryptogenic cirrhosis. The incidence of a positive Rose-Waaler reaction is about half this figure except in primary biliary cirrhosis, when the incidence is comparable to that of the Latex fixation reaction (Bouchier, Rhodes and Sherlock, 1964).

Antibody to “Bile Canaliculi”. Antibody staining bile canaliculi of the liver parenchymal cells has been claimed to occur using the immunofluorescence test by Johnson et al. (1966) in a proportion of patients with active chronic hepatitis. However, the staining pattern is not convincing with regard to bile canaliculi but may represent liver cell membranes. This would appear to be the first observation in liver disease of an antibody reacting with a component specific to the liver parenchyma. Antibody to bile ductual cells has been described in the immunofluorescence test by Paronetto, Schaffner and Popper (1964). Its existence requires further confirmation.

The demonstration that serum from some patients with putative auto-immune disease of the liver gives rise to staining of various tissue components by immunofluorescence raises the question whether some at least of these reactions are attributable to common elements in the tissues studied. The pattern of incidence of positive reactions with the different tissues and the observation that absorption of primary biliary cirrhosis serum with liver mitochondria leaves the smooth muscle staining intact without removing all the “M” fluorescence indicates that these are separate antigen antibody reactions (Johnson et al., 1966). On the other hand, Ironside, de Boer and Nairn (1966) have reported that the reaction with smooth muscle can be inhibited by absorption with homogenates of liver, kidney or stomach.

Thyroid and Gastric Antibodies. Thyroid and gastric antibodies have been observed more commonly in active chronic hepatitis and in primary biliary cirrhosis than in normal controls while this difference was not observed in alcoholic cirrhosis, extrahepatic obstruction or infective hepatitis (Doniach et al., 1966).

Immunosuppressive Therapy. A positive role of immune processes is suggested by the clinical improvement following steroids (Mackay and Wood, 1961) or other immunosuppressive drugs such as 6-mercaptopurine and azathioprine (Mackay, Weiden and Unger, 1964) in patients with active chronic hepatitis. However, Page, Condie and Good (1964) reported similar clinical and biochemical improvement in four patients with plasma cell hepatitis treated with 6-mercaptopurine in dosage insufficient to inhibit antibody production and expressed the opinion that the agent’s beneficial effect could be related to suppression of a chronic virus infection.
Conclusion. In active chronic hepatitis, cryptogenic cirrhosis and primary biliary cirrhosis, there is evidence of a widespread immunological hyper-reactivity. The possibility of a co-existence of organ and non-organ specific antibodies in the sera of these patients requires further investigation, particularly in view of the demonstration of several liver-specific antigens (Milgrom, Tuggae and Witebsky, 1965). However, with the possible exception of antibody to “bile canaliculi” or to liver cell membranes there is no established evidence for the existence of antibodies that are specific for liver. Any causal relationship between the immune processes described so far and the pathogenesis of the disease remains unknown.

CHRONIC PANCREATITIS

Positive precipitin reactions with saline extracts of human pancreas have been reported in cases of chronic relapsing pancreatitis and pancreatic carcinoma (Thal, Murray and Enger, 1959; Fonkalsrud and Longmire, 1961). The serum from 14 of 16 patients with cystic fibrosis demonstrated clear and distinct precipitin lines to an extract of cystic fibrosis lung but only ill-defined precipitin lines were observed when the same sera were tested against an extract of cystic fibrosis pancreas (Stein et al., 1964). Rhesus monkeys immunised with pooled Rhesus monkey pancreas extract and Freund’s complete adjuvant developed pancreas-specific iso-antibodies, but auto-immunisation of rabbits with their now pancreas incorporated in Freund’s complete adjuvant failed to elicit auto or iso-antibodies and the pancreas of the immunised animals were normal grossly and histologically. Further studies are required before the phenomenon of auto-immunity can be defined in relation to chronic pancreatitis in man.

MALABSORPTIVE DISEASE

In coeliac disease in children and its counterpart in adults, malabsorption is due to an idiosyncrasy to gluten, a complex heterogeneous protein in wheat and certain other cereals. The harmful factor resides in gliadin, the alcohol soluble portion of gluten. In gluten-induced enteropathy there is atrophy of the villi of the small intestine and heavy infiltration of the lamina propria with lymphocytes and plasma cells. (See also chap. 21.) Direct instillation of flour or gluten on to the histologically normal distal small intestine of patients with adult coeliac disease produces similar acute mucosal inflammation, whereas the intestinal mucosa of a normal subject remains unchanged (Rubin et al., 1962). The malabsorption is usually correctable by a strict gluten-free diet, and corticosteroids are also effective.

In gluten-induced enteropathy of children and adults a significantly higher incidence and titre of antibodies to various fractions of wheat gliadin compared to controls has been shown (Heiner et al., 1962; Taylor, Truelove and Wright, 1964). A high incidence of antibodies to other dietary proteins, particularly to milk proteins, has also been described in coeliac disease, but not all published results are in agreement. Sewell et al. (1963), for example, have confirmed the finding of high antibodies to milk proteins in coeliac disease but have failed to find antibodies to gluten in the same sera. There is no apparent
correlation between the clinical severity of the disease and the presence of the antibodies. The factors involved in determining such antibody responses could include proteolytic activities at all stages in the pathway of absorption, the activity of immunocytes in the gut wall, the lymphatic drainage, and the predetermined immune responsiveness to specific antigens. There have been descriptions of acquired agammaglobulinaemia in adult celiac disease (Huizenga et al., 1961). The tests to gluten and its fractions have been negative in celiac patients, and peripheral eosinophilia and a personal or family history of allergy are unusual (Alvey, Anderson and Freeman, 1957).

Using the immunofluorescence method, Malik et al. (1964), claimed that the sera from patients with untreated celiac disease reacts with the cytoplasm of human and monkey jejunal mucosa. This suggested that both the healthy and celiac intestinal epithelium absorb antigen derivatives of gluten or possibly that some celiac sera contain a humoral autoantibody to small bowel epithelial cells. Rubin et al., (1965) were unable to confirm the presence of circulating autoantibodies to intestinal epithelium in celiac disease. Nor could they demonstrate synthesis of gliadin specific immunoglobulins in small bowel immunocytes. Clearly there is much to be done on the relationship between humoral antibodies against dietary proteins, hypersensitivity states to such proteins and gastrointestinal disease.

In contrast to the concept of immunological hypersensitivity in celiac disease, there is some evidence to suggest that immunological insufficiency may be of importance. Splenic atrophy, hypogammaglobulinemia and lymphoma of the small intestine may occur in association with idiopathic steatorrhoea (McCarthy et al., 1966). It has also been shown that patients with idiopathic steatorrhoea may be deficient in the synthesis of gamma A immunoglobulin in the alimentary tract and that gamma A immunoglobulins may be of importance in maintaining the integrity of the intestinal mucosa (Crabbé and Heremans, 1966).

CROHN’S DISEASE

While no circulating antibodies to intestinal mucosa have been found in Crohn’s disease, this condition shares with ulcerative colitis a number of extra-intestinal manifestations which are of considerable interest in an immunological context.

The granulomas of Crohn’s disease are similar to those found in sarcoidosis, but the gastro-intestinal tract is rarely affected in sarcoidosis and the two conditions would appear to be quite separate. They do resemble one another immunologically, however, in that both have a high incidence of negative Mantoux reactions, but the Kveim test is negative in Crohn’s disease and attempts to produce a similar reaction with Crohn’s tissue have not been successful (Williams, 1965). The analogy with sarcoidosis is not very close and immunology has added little to the understanding of this condition.

ULCERATIVE COLITIS

The clinical and pathological features of ulcerative colitis provide some circumstantial evidence of the possible importance of immunological factors in its characterization. The serum gammaglobulin level may be raised, there is a high frequency of extracolonic manifestations (many suspected of originating from hypersensitivity, such as arthritis, conjunctivitis, and erythema nodosum), the histology shows extensive lymphocytic infiltration in the colonic mucosa, and the response to corticosteroid therapy is well known (Broberger, 1964). There may be a slight but significant prevalence of ulcerative colitis in females (Evans and Acheson, 1965).

In ulcerative colitis abnormal immunological responses have been sought
to antigens derived from three sources—the patient’s own tissues, bacteria and food constituents.

**Auto-antibodies.** Broberger and Perlmann (1959) suggested that autoimmune reactions might be relevant to the study of ulcerative colitis and showed that haemagglutinating antibodies to a hot phenol-water extract of human foetal colon are present in the sera of a high proportion of children with ulcerative colitis, with a lesser incidence in adults with the disease. Some positive reactions in this test have been obtained using sera from patients with rheumatoid arthritis, systemic lupus erythematosis, some liver diseases and the nephrotic syndrome, but in a lower incidence than in ulcerative colitis (Asherson and Broberger, 1961). Immunofluorescence studies using unfixed sections of human colon have shown that the circulating antibodies react with an antigen in colonic epithelial-cell cytoplasm (Fig. 5).

There is a positive correlation between the results of the haemagglutination and the immunofluorescence tests, the former being more sensitive (Lagercrantz et al., 1966). Absorption of sera with foetal colon inhibited positive results with both techniques. 56 per cent of a group of 101 ulcerative colitis patients were found to have a haemagglutination titre $\geq 1:16$ compared to 13 per cent of age and sex matched healthy controls or surgical cases. Of 109 patients with other gastrointestinal disorders (chronic diarrhoeas of unknown aetiology, bacillary dysentery, Salmonella infection, cancer of colon and rectum, celiac disease) only 8 had a titre $\geq 1:16$. Fluorescent antibody staining of rat colon sections confirmed these results (Lagercrantz et al., 1966). In this series no clear difference was noted in the incidence or titre of control of antibodies in children compared to adults.

The haemagglutination procedure has shown that the colon antigen is also present in extracts from other parts of the gastro-intestinal tract of germ-free rats and is independent of blood group A and H antigens (Hammarström et al., 1965; Perlmann et al., 1965). Background reactivity somewhat decreases the sensitivity of the haemagglutination test as a diagnostic tool for the comparison of different groups of sera, since only titres elevated over the low and arbitrary chosen level of the controls can be counted as positive (e.g. $\geq 1:16$).

The antigen is probably a lipopolysaccharide, but it is difficult to prepare and many preparations are inactive. The antibody will react with the patient’s own colon tissue indicating that it is a true auto-antibody. The presence of colonic antibodies cannot be correlated with any clinical features, and may even persist after colectomy (Harrison, 1965a; Wright and Truelove, 1966; Lagercrantz et al., 1966). Using the fluorescent method Harrison (1965a) detected antibody reacting with mucosal cells of the small and large intestines in 27 out of 200 cases. All the control sera were negative. The sera of a few ulcerative colitis patients reacted with the superficial mucosal cells of the stomach. Lagercrantz et al. (1966) also noted a few patients with ulcerative colitis who had antibodies against the superficial mucous cells of the stomach which were distinct from those reacting with colon.

Positive sera have not been shown to be cytotoxic to colon cells, although such cells grown in culture will fix antibody (Broberger and Perlmann, 1963). The most powerful evidence so far implicating auto-immune mechanisms in ulcerative colitis is the finding that leucocytes from patients
with ulcerative colitis are cytotoxic for fetal colon cells in tissue culture (Perlmann and Broberger, 1963). Freshly obtained fetal colon cells were labelled with $^{32}$P-orthophosphate or $^{14}$C-amino acids and exposed to white cells from children with ulcerative colitis or from healthy controls. Exposure of the colon cells to patients' white cells led to a rapid isotope release, significantly higher than that obtained with normal white cells. The cytotoxic action of the patients' white cells was immunologically specific, since no difference from the controls was found in the isotope release when cells from other organs or animals were similarly treated. Complement was required in the system for a cytotoxic effect to be manifest. This observation has recently been confirmed by Watson, Quigley and Bolt (1966). It would seem that cellular antibody mediated by lymphocytes, rather than the antibody titre in the serum, may be responsible. In support of this, Watson, Styler and Bolt (1966) have shown that leucocytes from about one-third of patients with ulcerative colitis may produce autologous skin reactions. Taylor (1965) and Wright and Truelove (1966) report an increased incidence of gastric parietal cell antibody in ulcerative colitis, while Harrison (1965b)
found no increase in incidence compared to controls. The presence of anti-nuclear factor in the sera of ulcerative colitis patients has been reported, but again the results are conflicting, due to lack of standardization in the detection of antinuclear factor and lack of adequate controls matched for age and sex.

**Antibody to Bacteria.**—The clinical observation that an intercurrent infection may provoke a relapse of ulcerative colitis raises the possibility that an immunological response against normal or non-pathogenic bacteria might trigger off an auto-immune response against related antigens in the colon. Perlmann et al. (1965) suggested a cross-reactivity between bacterial lipopolysaccharides and colon antigen, since absorption of the sera of colitis patients with lipopolysaccharide derived from E. coli 014 reduced the reaction with colon antigen. A positive correlation was also found between the degree of reaction of sera with colonic and bacterial antigens. The bacterial antigen involved may be the heterogenous antigen of Kunin (1963). Rabbits injected with various dead enteric organisms in complete Freund’s adjuvant produce antibodies which react with their own colon and in some cases with ileum and stomach (Asherson and Holborow, 1966). There is yet no evidence that the production of these antibodies is associated with any pathology of the colon or elsewhere.

In testing for antibody to colon it is essential to use germ-free colon. If normal adult colon is used then 100 per cent of sera, whether from patients with ulcerative colitis or from normal controls, give positive reactions due to the bacterial contamination.

These facts are compatible with the view that ulcerative colitis is due to an auto-immune reaction against the colon mucosa perhaps caused by an antigenic similarity between certain bowel organisms and the mucosal cells of the colon. This would relate the immunological findings in ulcerative colitis to those of rheumatic heart disease and glomerulonephritis where cross-reactivity with streptococcal antigen is implicated. Alternatively, ulcerative colitis may be due to an immune response against bacteria or bacterial residues lodged in the wall of the colon, and the auto-antibodies may be an irrelevant concomitant of a strong immune response to bacteria.

**Food Hypersensitivity.** Taylor and Truelove (1961) reported higher serum antibody titres to the three main milk proteins (casein, lactoglobulin and lactalbumin) in patients with ulcerative colitis than in controls. Acheson and Truelove (1961) claimed that patients with ulcerative colitis had been weaned at an earlier age than control subjects. A beneficial effect of a milk-free diet in patients with a frank attack of uncomplicated ulcerative colitis has been suggested by Wright and Truelove (1965). However, the significance of antibodies to milk proteins in ulcerative colitis lacks conviction; the selective incidence and titres of milk antibodies in ulcerative colitis and the differences in the weaning habits compared to controls have not been confirmed (Dudek, Spiro and Thayer, 1965). A “milk free” diet would seem to be almost an impossibility; indeed, butter (which contains about 0.5 per cent milk protein) was allowed. Presumably this would be sufficient to perpetuate a state of hypersensitivity (Taylor, 1966). Some forms of milk intolerance may be non-immunological and due to intolerance to lactose (Jeejeebhoy, Desai and Verghese, 1964; Struthers, Singleton and Kern, 1965).

**Extracolonic Manifestations.** In both ulcerative colitis and Crohn’s
disease extra-intestinal manifestations may be prominent. Holdsworth et al. (1965) have presented strong clinical evidence of an association between ulcerative colitis and lupoid hepatitis or inactive cryptogenic cirrhosis. The relationship between ulcerative colitis and arthritis has been reviewed by McEwen et al. (1962) who state that there is no significant association with rheumatoid arthritis or rheumatoid factor, but that ulcerative colitis is clearly associated with a peripheral form of arthritis distinct from rheumatoid. In the study of 114 patients with ulcerative colitis Wright et al. (1965) reported that 12 per cent had evidence of past or present anterior uveitis and 17 per cent had evidence of sacroiliac involvement, whereas the incidence among controls was negligible. Wright and Watkinson (1965) described a colitic type of arthritis which usually begins as an acute arthritis, affecting a single joint of the lower limb, and then spreading predominantly in the legs in an asymmetrical manner. The improvement in the peripheral or colitic arthritis with a spontaneous or therapeutically induced remission of the ulcerative colitis would suggest that it at least is a secondary complication of the intestinal disease. The evidence that colitic arthritis, spondylitis and uveitis are related to auto-immune disease is nebulous. However, it is conceivable that these extracolonic manifestations of ulcerative colitis might result from a hypersensitivity reaction to antigens, for example, bacterial antigens released from the diseased colon, and represent true complications of the ulcerative colitis.

CONCLUSION

The alimentary diseases illustrate a wide range of immunological disorders or different forms of immunological aberration. At one end of the spectrum is the atrophic gastritis of pernicious anæmia which is one of the group of diseases (pernicious anæmia, auto-immune thyroid disease, idiopathic adrenal insufficiency and idiopathic hypoparathyroidism) that is characterised by the occurrence of organ-specific auto-antibodies and in which there is negligible incidence of antibodies reacting simultaneously with constituents of multiple tissues. In Sjögren's disease and the triad of liver diseases (primary biliary cirrhosis, active chronic hepatitis and cryptogenic cirrhosis) the majority of auto-antibodies described are non-organ-specific (e.g. antinuclear factors, antibodies to mitochondria and to smooth muscle, rheumatoid factor), but in both these groups of diseases there may be some overlap with organ-specific antibodies (e.g. thyroid and gastric) and in liver disease there may be a specific antibody to bile canaliculi or to liver cell membranes. In gluten enteropathy there is clearly an intolerance to a food constituent and this may be due to immunological hypersensitivity. The significance of food allergy in relation to ulcerative colitis is unknown; indeed, the alleged increased incidence and titre of antibodies to milk proteins in ulcerative colitis has been questioned.

Ulcerative colitis illustrates the interesting concept of immunological cross-reactivity between a bacterial antigen and an auto-antigen and places ulcerative colitis along with rheumatic fever and glomerulonephritis in this respect. The immunological aberration in relation to ulcerative colitis would therefore appear to be distinct from that occurring in pernicious
anemia and distinct again from that occurring in liver diseases although there is some overlap between hepatitis and ulcerative colitis. Ulcerative colitis is unique in the alimentary diseases in that a cytotoxic effect of lymphocytes from a patient with the disease has been demonstrated against colon cells in tissue culture. Attempts at producing lesions of the alimentary tract by immunological techniques in animals have met with little success, except for atrophic gastritis in the monkey.

With regard to auto-antibodies as an aid to clinical or subclinical diagnosis, the presence of parietal cell antibodies in the serum may be taken to indicate some degree of atrophic gastritis while the presence of intrinsic factor antibodies in the serum shows a strong correlation with malabsorption of vitamin B12 due to lack of intrinsic factor secretion with advanced atrophic gastritis. The application of serological tests to the screening of subjects at risk with regard to pernicious anemia leads to an earlier detection of the disease and should prove helpful in the elucidation of its natural history. While the detection of antibody to salivary duct tissue may prove valuable in the investigation of a suspected case of Sjögren's disease, the clinical value of mitochondrial ("M") antibodies in the differentiation between surgical and non-surgical jaundice has already been established.

Much further work requires to be done in relation to the immunological aspects of alimentary disease. In some disorders the occurrence of auto-antibodies and of hypersensitivity to exogenous antigens requires to be more clearly defined. In other disorders affecting the alimentary system where the occurrence of auto-antibodies has been defined, the reason for their development and their role in the pathogenesis of that disease remains an enigma.

References

PHENOMENA IN ALIMENTARY DISEASES

THE THYMUS AND AUTOIMMUNITY

W. J. Irvine

Leader in Lancet (1967), 1, 713-715.
The Thymus and Autoimmunity

The thymus is essential to the development and maintenance of immunological mechanisms in some animals: that much is certain. In man, the rare aplasia of the thymus (essential lymphocytophthisis or hereditary lymphohypoplastic dysgenesis) is the counterpart of neonatal thymectomy in the laboratory animal. Histological abnormalities of the thymus have long been recognized as an association of myasthenia gravis, hyperthyroidism, and adrenal insufficiency. The thymus of patients with systemic lupus erythematosus and with rheumatoid arthritis may also show histological peculiarities; and hemolytic anemia may accompany thymoma. Most diseases coupled with abnormality of the thymus are also linked with disordered immunological reactions and the formation of autoantibodies. Thus, antibodies reacting with skeletal muscle and thymic "epithelial" cells (probably more correctly called myoid cells) have been detected in patients with myasthenia gravis, and these patients also have an increase in antinuclear factors and in organ-specific antibodies to thyroid and gastric parietal cells. Four autoantibodies have been described in patients with thyroid autoimmune diseases (thyrotoxicosis, Hashimoto goitre, and primary atrophic hypothyroidism). The thymus in thyroid disease has been studied radiologically by Irvine and Sumerling and by a combination of radiology and biopsy, as described by Mr. Michie and his colleagues in their article on p. 691. Enlargement of the thymus shadow was shown to correlate with the occurrence of autoantibodies in the serum. Antinuclear factors are a feature of systemic lupus erythematosus; and antibody to γ-globulin is the basis of a factor characteristic of rheumatoid arthritis. Certainly, these associations between thymus abnormalities and diseases of immunological aberration are too common to be explained by chance alone.

Different patterns of histological abnormality may be seen in the thymus in the autoimmune diseases. In myasthenia gravis the predominant lesion is the formation of germinal centres in the medulla. While these appearances have often been called hyperplasia, the total size and weight of the thymus may be within normal limits in the absence of a thymoma; and the histological picture is probably better described as thymic dysplasia. Formation of germinal centres in the thymus is also characteristic of the changes associated with the thyroid autoimmune diseases; and it is seen in the NZB strain of mice in which autoimmune haemolytic anaemia develops spontaneously. The other elements in the thymus—spindle epithelial cells, Hassall's corpuscles, and plasma cells—are not significantly different from normal in myasthenia gravis. It has been postulated that thymomas tend to cause autoimmunisation to thymus: over 90% of thymomatous patients with myasthenia gravis have thymic-myoid and skeletal antibodies, and germinal-centre formation is common in thymic tissue lying next to a thymoma in patients with myasthenia gravis. In systemic lupus erythematosus the main lesion is loss of cortico-medullary differentiation and an increase in the number of spindle epithelial cells, cystic Hassall's corpuscles, and plasma cells. Germinal-centre formation is not so prominent as in myasthenia gravis.

To explain the relation between abnormality of the thymus and the autoimmune diseases two main hypotheses have been advanced. Burnet and Holmes regarded the germinal centres in the thymus as sites of production of forbidden clones of immunologically competent cells capable of reacting against the body's own tissues. While this hypothesis fits the morphological evidence in myasthenia gravis, it does not accord with the observations of Howie and Helyer that neonatal thymectomy does not prevent or delay the onset of autoimmune haemolytic anaemia in NZB mice but indeed accelerates it. Similarly, this hypothesis is not in keeping with the observation that thymectomy in rabbits is followed by an abnormally high incidence of positive Coombs tests. The alternative hypothesis is that germinal-centre formation in the thymus represents an antigen-antibody reaction within the gland and that the situation in the thymus is analogous to that in other tissues affected by an autoimmune disorder, such as the thyroid gland in Hashimoto thyroiditis. The germinal-centre formation and the increase in plasma cells in the thymus of myasthenia gravis may be regarded as the result of a chronic thymitis.

is particularly apt for myasthenia gravis, since auto-
 antibodies reacting against thymic myoid cells are often
 found in the sera of these patients. Whether or not the
 germinal centres in the thymus of patients with thyroid
 autoimmune diseases can be explained in the same way
 is less clear, because the frequency of antibody to
 thymic myoid cells is low in this group of diseases.
 As a consequence of thymitis, it is postulated that thymus
 function is impaired. \textsc{Goldstein and Whittingham} have
demonstrated that thymic lesions comparable with
 those in myasthenia gravis can be produced in labora-
tory animals by the injection of extracts of skeletal
 muscle or calf thymus together with Freund's adjuvant.
 Antibodies to skeletal muscle and thymic myoid cells
developed in the serum. These observations lend
 strong support to the view that thymic lesions in
 myasthenia gravis represent a chronic thymitis and not
 a primary hyperplasia of the thymic lymphoid tissue.

It is postulated that the thymus has an important
 physiological function in deleting aberrant clones of
 lymphocytes which, as a consequence of somatic
 mutation, react against constituents of the body's own
 tissues.\textsuperscript{1} If thymic function is impaired by an auto-
 immune process going on in the thymus itself, such
 aberrant clones of lymphocytes reacting against other
 constituents of the body may fail to be deleted. \textsc{de Vries}
 and \textsc{Hijmans} made a detailed study of the thymus
 gland in 5 autoimmune strains and in 5 normal strains of
 mice. In addition to observing lymphoid-follicle forma-
 tion in the autoimmune strains, they noted a gross
 depletion of thymic epithelial cells in the NZB strain
 of mice shortly after birth; and in other autoimmune
 strains (NZW and BWF) there was a striking though
 transitory neonatal hyperplasia of the large epithelial
 cells. At the same time many abnormally large Hassall's
 bodies appeared, most of which showed a central
 area of necrotic cells. These workers also observed
 invasion of the thymic epithelial cells by lymphoid cells,
 and this phenomenon was much more prominent in the
 autoimmune strains of mice than in the normal strains.
 Many of the lymphocytes seen near the epithelial cells
 were necrotic—a finding which seems to support the
 notion that "forbidden clones" of lymphoid cells are
 eliminated in the thymus.

The two hypotheses about the thymic disorder in
 autoimmune disease—proliferation of forbidden clones
 within the thymus and deficient thymic function
 in the deletion of autoreactive lymphocytes passing
 through the thymus—may not be mutually exclusive.
 The variation in the histological appearance of the
 thymus in the autoimmune diseases suggests that
 different mechanisms may operate. Thymectomy has
 been successful in autoimmune hemolytic anemia in
 infancy \textsuperscript{24} and in certain patients with myasthenia
 gravis,\textsuperscript{25} but not in systemic lupus erythematosus.\textsuperscript{27} On

\textsuperscript{23} Goldstein, \textsc{O.}, \textsc{Whittingham, S.} \textit{Lancet}, 1966, ii, 315; \textit{Clin. exp.
 Immun.}, 1967, 2, 278.

\textsuperscript{24} \textsc{de Vries, M. J.}, \textsc{Hijmans, W.} \textit{Immunology}, 1967, 12, 179.

\textsuperscript{25} \textsc{Walters, M. J.}, \textsc{Russell, P. A.} \textit{Lancet}, 1963, ii, 915.

\textsuperscript{26} \textsc{Br. med. J.} 1962, ii, 1291.

\textsuperscript{27} \textsc{Mackay, I. R.}, \textsc{Smiley, M.} \textit{Clin. exp. Immun.} 1966, 1, 129.
the other hand, systemic lupus erythematosus and Hashimoto thyroiditis have developed after thymectomy for myasthenia gravis. Why should thymectomy benefit some patients with myasthenia gravis—notably, young women who have had symptoms for not longer than five years and who do not have a thymoma? Thymectomy in myasthenia gravis leads to a reduction in titre of skeletal and thymic-myoid antibodies in patients who do not have a thymoma, but these antibodies per se are not the agents of the characteristic neuromuscular block. Immunofluorescence studies have shown that the skeletal antigen is attached to the A-bands of the myofibrils and not to the neuromuscular endplate; the skeletal antibodies are transmitted across the placenta, but the frequency of neonatal myasthenia cannot be related to the presence of these antibodies in the fetal circulation; patients with thymoma may have high titres of skeletal antibodies but no subjective or objective evidence of myasthenia. The observation that some of the mice in which an experimental thymitis was induced developed clinical and objective evidence of myasthenia is therefore of considerable interest. Mice that had previously been thymectomised did not develop myasthenia, although other experimental autoimmune diseases developed when they were appropriately stimulated (for example, allergic encephalomyelitis). These findings suggest that the disordered thymus in myasthenia gravis may be the site of production of a humoral neuromuscular blocking substance—evidence of which was detected by Parkes and McKinna. Whether or not the neuromuscular inhibitory substance released by the deranged thymus is an antibody has not been established, though it apparently exists in a globulin fraction of the plasma. The observation that patients with severe myasthenia gravis can be temporarily improved by haemodialysis suggests that the humoral blocking substance is not an antibody but a substance of smaller molecular size.

Autoimmune disease has been shown to be genetically determined both in man and in mice: this could mean either that the tendency for forbidden clones of immunologically competent cells to develop may be under direct genetic control or that the susceptibility of part of the reticuloendothelial system (the thymus, for example) to injury by exogenous agents (such as viruses) is genetically controlled. In any event, the connection between the thymus and autoimmune disease is likely to be complex. There is probably no single function or mode of action of the thymus in normal development or in normal adult life. Similarly, the nature of the thymic disorder—and the reason for it—are likely to differ between the autoimmune diseases.

IMMUNOBIOLOGY OF THE THYMUS
AND ITS RELATION TO AUTOIMMUNE DISEASE

W. J. Irvine

CHAPTER 9
IMMUNOBIOLOGY OF THE THYMUS AND ITS RELATION TO AUTOIMMUNE DISEASE
W. J. IRVINE

INTRODUCTION
In the attempt to approach an understanding of the significance of thymic changes in autoimmunity, it is necessary to consider what is known about the physiology of the thymus and what information has been gained from clinical studies. It is hoped that these two approaches will lead to a comprehensive understanding of the thymus in both health and disease. The purpose of the present review is to describe the state of current knowledge in the course towards this eventual goal.

EMBRYOLOGY AND MORPHOLOGY OF THE THYMUS
The immunological system can be broadly subdivided into the antibody-producing system, characterized histologically by plasma cells, and the system that has small lymphocytes as its main cellular component. An alternative subdivision is into the 'central' and 'peripheral' lymphoid tissue. The prototype of the central lymphoid tissue is the thymus. The cloacal lymphoid organ in birds, known as the bursa of Fabricius, and the appendix of the rabbit (Archer, Sutherland and Good, 1963) are other tissues of this type. In mammals, the tonsils may well represent the analogue of the bursa in birds (Miller and Davies, 1964). The major peripheral lymphoid tissues are the spleen and the lymph nodes.

Phylogenetic studies suggest that the development of the immune mechanism depends on the evolution of the thymus and organized lymphoid structures. The time at which a developing vertebrate can first react immunologically by rejecting skin grafts and by antibody production coincides with the period when lymphocytes can first be identified in the circulation (Good and Papermaster, 1964). The thymus in mammals arises as paired structures from the endoderm.
EMBRYOLOGY AND MORPHOLOGY OF THE THYMUS

of the third and fourth branchial clefts. Differentiation of the thymus is essentially autonomous during embryogenesis and the thymus lymphoid cells undergo histogenesis during the period when the embryo is most susceptible to the induction of immunological tolerance. The epithelial character of the thymus is plainly evident until the end of the second month of intra-uterine life in the human. At that time the small thymic cells begin to appear. Auerbach (1961) separated the thymic rudiments by trypsin into epithelial and mesenchymal components which were then studied in tissue culture. He demonstrated that the epithelial component of the thymic rudiment was capable of forming the lymphocytes of the thymus and that the mesenchyme provided the initial inductive stimulus and was responsible for the stromal elements of the gland. Cells migrating into the thymus did not appear to contribute significantly to the initial lymphoid population of the thymus. For immunological maturation to occur it appears from tissue culture studies that interaction of bone marrow, thymus and spleen are all essential (Auerbach, 1966).

The size of the thymus in man has been correlated with age in a careful analysis by Hammar (1929), in which he studied the thymus gland of persons who had died suddenly from trauma. The thymus gland increases in size until puberty and then slowly atrophies, but is still of considerable size in the adult. The large thymus glands of children dying suddenly from unexplained cause were shown to be physiological and the diagnosis of 'status thymolymphaticus' has gone into disrepute. The size of the thymus increases in proportion to body weight until about puberty, and then gradually decreases in relation to the body weight. Its phase of most active growth both in man and experimental animals is in early life.

The fully developed thymus is incompletely divided into lobules composed of a central core or medulla and an external part or cortex. The cells in the thymus are of two main types: lymphocytes (thymocytes) and reticular cells. The lymphocytes, which look the same as those in the circulation or in lymph nodes, are heavily concentrated in the cortex. Most of the cortical lymphocytes are small round cells with a relatively great nuclear/cytoplasmic ratio. The reticular cells are prominent in the medulla. They are large cells with ill-defined outlines and a large vesicular nucleus with little chromatin content. Most of the reticular cells are epithelial and some of them are typical reticulo-endothelial cells or macrophages. Certain of the reticular cells in the cortex and also in the medulla stain with periodic acid-Schiff reagent (PAS), indicative of a carbohydrate content. The PAS positive cells in the cortex tend to
be elongated structures, whereas the PAS cells in the thymic medulla are larger and more rounded. Using the electron microscope Clark (1963) has shown that thymus medullary epithelial cells have the nuclear and cytoplasmic fine structure of cells that secrete a proteinaceous or mucoid material. He demonstrated three types of cytoplasmic inclusions that might represent stored secretory material. Radioactive sulphate and $^{14}$C-glucosamine, detected by autoradiography, were rapidly and preferentially incorporated into the cytoplasm of medullary epithelial cells and into vacuoles and large inclusions. Clark (1966) has suggested that the medullary epithelial cells may contain a stored secretion which may be a sulphated acid mucopolysaccharide.

The rate of small lymphocyte production is very high in the thymus. Thymic lymphopoiesis, as measured by direct mitotic counts and by tritiated thymidine uptake studies (Metcalf and Wiadrowski, 1966), is comparable to the rate of division in lymph nodes under conditions of maximal antigenic stimulation. However, the unique feature of the lymphopoiesis in the thymus is that it is not dependent on antigenic stimulation, nor is it affected by resection of other lymphoid organs, partial thymectomy or the presence of one or numerous thymus implants (Metcalf, 1964). This suggests that the primary proliferative stimulus for thymic lymphoid cells must arise from within the thymus itself. The PAS positive reticular cells in the cortex may play some special role in stimulating mitosis in neighbouring lymphocytes, but the exact nature of the relationship between the proliferating lymphocytes and the PAS positive cells is not known.

It has recently been shown that the large cells in the thymic medulla contain an antigen that is shared with antigen in A bands of skeletal muscle (Van der Geld and his colleagues, 1963; Van der Geld and Strauss, 1966). Striated muscle fibres resembling those of skeletal muscle are present in the thymus of different species (Hammar, 1905), including man (Pappenheimer, 1910; Feltkamp-Vroom, 1966). It may be incorrect to describe the large cells in the thymic medulla which contain muscle antigen as 'epithelial cells'; they would appear to be of muscular origin and are probably derivatives of the myoid cells of the medulla.

Hassall's corpuscles are a feature peculiar to the thymic medulla. They may consist of single hypertrophic reticular cells or groups of reticular cells which may appear active, irregularly polygonal or flattened, hyalinized and concentrically arranged around a central core of nuclear debris. They have been variously interpreted as structures derived from the endothelium of blood vessels, or as
structures developed from reticular cells. While modern opinion favours the latter view (Sainte-Marie and Leblond, 1958), it is interesting to recall the evidence of Smith and Parkhurst (1949) that Hassall's corpuscles are composed of cells with the characteristics of keratinizing squamous epithelium.

While lymphopoiesis in the thymus is independent of antigenic stimulation, Marshall and White (1961) have shown that if bacterial antigen is injected directly into the thymus of an adult mouse, germinal centre formation and plasma cell infiltration result. They interpreted this evidence to indicate that a functional blood–thymic barrier exists which, under normal circumstances, prevents antigen from coming into contact with thymic lymphocytes. It has been demonstrated by electron microscopy that the thymus lymphocytes are separated from connective tissue and blood vessels by a basement membrane and a virtually continuous layer of epithelial reticular cells (Clark, 1963; Weiss, 1963). This epithelial cell layer does not, however, offer an absolute barrier to the passage of some antigens, notably ferritin and albumin (Clark, 1964).

Nossal and Mitchell (1966) compared the effectiveness of the blood–thymic barrier in adult and newborn rats and for various types of antigens. They used bovine serum albumen and flagella, polymerized flagellin and soluble flagellin from Salmonella adelaide as antigens. These antigens were labelled with $^{125}$I and then injected intravenously into adult and intraperitoneally into newborn rats. Autoradiographic studies were done to determine the distribution of the antigens. In the adult, retention of the antigen was mainly in the lymphoid follicles with little in the thymus. In newborn animals the distribution of radioactivity was much wider: with bovine serum albumen the thymus contained more isotope than the other lymphoid organs, and with the particulate antigens the spleen was only slightly more radioactive than the thymus. In the newborn not only did the antigen have a wider distribution but it persisted for longer than in the adult and, in contrast to the adult, the antigen was generally not engulfed by phagocytes. The partial blood–thymus barrier for antigen in adult animals cannot be regarded as an anatomical entity but rather as a functional concept depending, amongst other things, on the rapidity of phagocytosis of antigens. Whether the lack of a blood–thymus barrier in the newborn is due to an anatomical or functional difference has not been established. The inert nature of thymic tissue during immunization may be due to the paucity or absence of cells in the thymus which can initiate immunological reactions. Suspensions of thymic cells (i.e. with any structural blood–thymic barrier completely broken down) are less
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effective at producing runting or tolerance than other lymphoid organs (Billingham and Silvers, 1964).

It would seem likely that the different distribution and handling of antigen in the newborn, compared with the adult animal, is relevant to the problem of tolerance. Induction of tolerance appears to be favoured by the continued presence of antigen on or in lymphocytes; once tolerance has been induced it can be maintained for some time without the physical presence of antigen in the lymphoid cells. Tolerance would appear to depend on the antigen reaching all lymphoid areas including the thymus and on the antigen remaining there while lymphoneogenesis under thymic influence continues.

Traffic of Lymphoid Cells to and from the Thymus

There is unequivocal evidence that the thymus receives cells from the circulation which subsequently behave in the thymus as typical thymus lymphocytes. In studies on regenerating thymus tissue transplanted to a subcutaneous site, Harris and Ford (1964) demonstrated that most, if not all, of the lymphoid cells in the implant are eventually derived from invading host cells. Ford and his colleagues (1956) have shown that in lethally irradiated animals the thymus (as well as other lymphoid tissues and the myeloid tissue) is repopulated following an injection of cells obtained from marrow, spleen or liver. The irradiated thymus is not repopulated by cells obtained from lymph nodes or thymus (Ford and Micklem, 1963). Experiments with parabiotic mice, one member of which had a marker chromosome, have shown that an exchange of cells occurs between the two partners; cells from one parabiont were observed to be dividing in the thymus of the other (Harris and his colleagues, 1964). There is, at the present time, no direct experimental evidence to indicate the identity of the cell or cell types which migrate into the thymus. The thymus in adult animals appears to be excluded from the circuit of long-lived immunologically competent small lymphocytes that pass from the blood through the lymph nodes into the efferent lymphatics and back via the thoracic duct to the blood (Gowans and Knight, 1964).

Studies using tritiated thymidine indicate that the thymic small lymphocytes have a life-span in the thymus of only 3-4 days (Metcalf and Wiadrowski, 1966). Since the weight of the adult thymus remains constant over relatively long periods, cell death in the organ or migration from the organ must equal new cell production. Direct intrathymic injection of tritium-labelled thymidine has been followed by the appearance of some thymus-derived labelled
small lymphocytes in the mesenteric lymph nodes, spleen and intestinal lymphoid tissues (Nossal, 1964). The number of such 'migrant' cells was small in relation to the production rate of thymic cells. Likewise experiments with thymus grafts using chromosome marker techniques indicate that some cells dividing in spleen and lymph nodes had originated in the thymus graft.

Figure 1. Diagram illustrating the traffic of lymphoid cells to and from the thymus. It is possible that the proportion of immunologically competent lymphocytes leaving the thymus and entering the peripheral lymphoid tissue (shown here as 5 per cent) may be subject to control (after Miller, 1965).
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(Miller, Osoba and Dukor, 1965) although their numbers were not great. On this evidence most small lymphoid cells produced in the thymus do not appear to leave the organ but die in the thymus after 3-4 days. However, calculations based on the cell numbers, mitotic indices, generation time and pyknotic indices suggest that cells dying within the thymus can account for only a small percentage of cells produced by mitosis. The fate of the majority of thymic lymphocytes remains obscure. One might speculate that there is a mechanism which regulates the number of lymphocytes that can migrate to or enter peripheral lymphoid tissue and that the 'excess' of thymic lymphocytes may in some manner be lost from the body, but there is no direct evidence to confirm or refute such a hypothesis. A small proportion of the thymic small lymphocytes appear to be long-lived and to be exported from the thymus. These cells may eventually mature and become immunologically competent and join the circulating pool of lymphocytes (Figure 1).

Effects of Thymectomy in Normal Strains of Experimental Animals

The thymus plays a central role in the development of immunological capacity. Thymectomy, when performed at an early age, interferes with the normal and complete development of the lymphoid system and impairs various immunological functions, namely: delayed sensitivity, homograft rejection and antibody formation to certain antigens.

For example, thymectomy in the newborn is associated 1-2 months later with a striking decrease in the lymphocyte population (Miller, 1962a). In contrast to the depletion of lymphocytes in the circulating pool, plasma cells are not deficient in thymectomized animals. In some strains of mice neonatal thymectomy is followed 1-3 months later by the development of a wasting disease (Miller, 1962a; Parrott, 1962) very similar to the wasting syndrome that occurs in graft-versus-host conditions. Wasting disease does not occur, however, if the neonatally thymectomized mice are bred under germ-free conditions (McIntire, Sell and Miller, 1964). As might be deduced from the fact that circulating small lymphocytes are immunologically competent cells, thymectomy in newborn animals impairs transplantation immune reactions (Miller, 1962b) and delayed hypersensitivity reactions (Arnason and his colleagues, 1962). The majority of neonatally thymectomized mice of many strains fail to reject allogeneic skin homografts when grafted three days to five weeks after thymectomy. In some cases, skin grafts from distantly related donors and even from rats failed to be rejected...
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(Miller, 1962b). Some germ-free mice thymectomized at birth permanently accepted skin grafts from distantly related donors (McIntire, Sell and Miller, 1964). Cell suspensions from the spleens of adult mice thymectomized in the neonatal period are less capable of producing graft-versus-host disease in appropriate recipients than are similar dosages of spleen cells from normal mice (Dalmasso, Martinez and Good, 1962). The humoral antibody responses to most antigens (with the exception of a few antigens such as sheep red cells in the mouse) are not affected and immunoglobulin production is not impaired (Fahey, Barth and Law, 1965).

In contrast, thymectomy in adult life leads to no immediate significant impairment of immune capacity, although there may be some reduction in the population of lymphocytes in the peripheral blood, thoracic duct lymph, lymph nodes and spleen (Metcalf, 1960). For example, no significant defects in the capacity for rejecting allogeneic skin homografts or for producing serum antibodies to a variety of antigens have been reported in mice thymectomized in adult life and challenged within 1-2 months after thymectomy. If, however, the lymphoid system in the adult mouse is damaged or destroyed by ionizing radiation, then the thymus is important for the anatomical and functional regeneration of this system (Miller, Doak and Cross, 1963). Furthermore, when animals thymectomized in adult life are challenged with a new antigen six or more months later, there is clear evidence of immunological deficiency (Taylor, 1965). Presumably the adult thymus influences the development of an adequate population of long-lived immunologically competent cells. Only when this pool has become depleted, as a result of the limited life-span of its cells, do defects in immune capacity become apparent.

The thymus is not only essential in the development of the immunological system but it is also important for the maintenance of immunological function in the adult animal.

Reconstitution of Thymectomized Animals

Implanting with syngeneic or allogeneic thymus tissue subcutaneously or under the renal capsule enabled thymectomized, irradiated mice to recover practically normal immune functions (Miller and his colleagues, 1964). The majority of cells multiplying in the lymphoid tissues and in the thymus implant were shown, by means of the chromosome marker technique, to be of host origin and therefore could not have been the direct descendants of cells of the thymus donor strain (Miller, Osoba and Dukor, 1965). Discriminant spleen assays showed that host cells were largely
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responsible for immunological reactivity in restored mice (Dalmasso and his colleagues, 1963).

In order to test whether a direct cellular transfer was necessary for the restoration of immune capacity in the thymectomized host, Osoba and Miller (1964) placed thymus tissue in millipore diffusion chambers in the peritoneal cavity of young mice thymectomized at birth. Although the walls of the chambers were impermeable to cells, the mice were fully restored in their capacity to produce immune reactions. Cells surviving in the chambers were PAS positive. These experiments suggest that some of the action of the thymus may be mediated by means of a humoral factor. Thymus extracts have not been effective in restoring the immunological status of neonatally thymectomized mice. However, this could be achieved by thymus tissue that had been depleted of lymphocytes by irradiation in vitro with 500 r. Thymus tissue irradiated with 2,000 r failed to influence immunological capacities of neonatally thymectomized mice, even though the implants eventually became actively lymphopoietic. It is concluded that the thymus plays an essential role in inducing the differentiation of immunologically competent cells from non-competent precursors, and that this function is dependent upon the integrity of the thymus epithelial-reticular cells (Miller, 1966).

Klein, Goldstein and White (1966) have devised an assay of lymphocytotoxic activity in thymic extracts. It is based on the observation that particle-free supernatants of calf, rat and mouse thymic homogenates when injected into CBA mice result in the enhanced incorporation of tritiated thymidine into the DNA of the axillary and inguinal lymph nodes of these mice. This technique should prove valuable in the attempt to isolate and characterize the lymphocytopoietic factor of the thymus.

POSSIBLE MECHANISMS OF THYMUS ACTION

At the present time it is not possible to describe definitively the role of the thymus in immunological mechanisms. Our understanding of the function of the thymus is necessarily limited by lack of fundamental knowledge concerning many aspects of the physiology of the lymphocytes and our rudimentary knowledge of the humoral factors synthesized in the thymus.

Productive Hypothesis

According to elective theories of immunity and tolerance, contact of antigen with a cell at a certain stage of its differentiation might
POSSIBLE MECHANISMS OF THYMUS ACTION

eliminate in some way its potential to react to the antigen. Contact of antigen with the mature cell, on the other hand, might selectively stimulate that cell so that it subdivides, with the result that a clone of cells is formed. As already described, the thymus is at least partly shielded from the entry of extrinsic antigens and yet it has the greatest lymphopoietic activity of all the lymphoid tissues. The thymus would therefore seem to provide a suitable site for the differentiation of cells with immunological potential but without these cells being subjected to clonal proliferation. Burnet (1962) has suggested that all new or ‘primary’ immune patterns originate in the thymus; cells carrying primary patterns seed out in the lymph nodes and spleen and proliferate there in response to appropriate antigenic stimulation. However, as discussed above, apart from the earliest stages in development, the thymus does not apparently play a major role in populating the second-level or peripheral lymphoid organs.

Humoral Hypothesis

It is clear from the thymus graft experiments, in which millipore chambers were used, that at least for some aspects of thymus function the effective component is not cellular but humoral and can act at a site remote from the thymus. There may well be more than one such thymic hormone. A thymic hormone appears to be important in making immature lymphocytes immunologically competent and this factor is therefore referred to as ‘immunological competence inducing factor’ (ICIF). A thymic hormone may stimulate lymphopoiesis as indicated by Metcalf’s original observation that injection of thymus extracts into baby mice produced a lymphocytosis (see Chapter 5). A separate humoral factor may be required to permit the transformation of lymphocytes to plasma cells so that immunoglobulins can be synthesized and secreted. The evidence that such a factor is required follows from the observations that as the result of thymectomy there is a failure to react with the formation of immunoglobulins to certain antigens, that in the Swiss type of hypogammaglobulinaemia the thymus is poorly developed, and that in congenital lymphocytopenia with thymic aplasia there is hypogammaglobulinaemia.

Finishing School Hypothesis

In the intact animals, as opposed to thymus graft experiments, it may be that the hormones do not require to act outwith the thymus gland but exert their effect on cells that pass through the thymus gland. Lymphoid precursor cells—from the bone marrow
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for example—may need to migrate to the thymus for further education or modification before they can function as immunologically competent cells in other tissues (Miller, Doak and Cross, 1963).

The Thymus as an Immunological Censor

While it is clear that the thymus has some role in establishing immunological reactivity, it would be equally important to know if the thymus is involved in the induction of immunological tolerance. A censorship function has been postulated for the thymus whereby it is concerned with the elimination or inhibition of self-reacting clones (Burnet, 1962). The fact that neonatally thymectomized mice bearing foreign thymus grafts are specifically tolerant of thymus-donor-type skin, but not of third-party skin (Miller, 1962a), suggests that some such function may be thymus-dependent.

The thymus may be important in tolerance mechanisms in at least two ways:

(1) As previously discussed, there would appear to be a small but significant export of immunologically competent cells from the thymus. For tolerance to occur, antigen would have to reach these cells in the thymus cortex to ensure their 'clonal deletion'. Mackay and Goldstein (1967) have suggested that for destruction of self-reactive cells within the thymus, a 'library' of significant 'accessible' autoantigens of the body would need to be present within the gland. At least in the young animal many autoantigens could conceivably reach the thymus by the blood (e.g. thyroglobulin) (Hjort and Pedersen, 1962; Daniel and his colleagues, 1967). Squamous epithelium (skin, etc.) and muscle, by reason of their bulk and susceptibility to repeated trauma, would be tissues for which a high degree of tolerance would be required. Because of their fibrillar structure and insolubility such tissues may need to be represented in the thymus so that immunological tolerance to these tissues can be induced.

(2) If all the lymphoid cells of a given 'clone' were to be deleted at one point in time, the rate of regeneration of the immunological potential represented by that clone might well depend on the rate of formation of new immunologically competent cells (lympho-neogenesis). Lymphoneogenesis may occur in the thymus and, as described above, certainly appears to require a thymic factor. Thus, even if a single pulse of antigen 'deleted' all the cells of a given clone, or in some other way rendered the population tolerant, immune reactivity might shortly reappear through differentiation of new lymphocytes under thymic influence.
AUTOIMMUNITY

Before considering the relationship of thymic abnormality to diseases associated with autoimmunity it would be appropriate to consider in general the mechanisms that have been postulated to underlie the phenomenon of autoimmunity.

Although the existence of autoantibodies is recognized in association with many disease conditions, the significance of autoimmunity in the pathogenesis of these diseases remains obscure. The diseases associated with autoimmunity, often loosely referred to as the 'autoimmune diseases', form a spectrum of disorders. At one end of the spectrum is the group of diseases associated with organ-specific autoantibodies; examples of this group are chronic thyroiditis, Addisonian pernicious anaemia, idiopathic adrenal insufficiency and idiopathic hypoparathyroidism. At the other end of the spectrum are diseases, such as systemic lupus erythematosus, which are associated with non-organ-specific antibodies (e.g. anti-nuclear factors, non-tissue-specific complement-fixation reactions, etc.). A more appropriate name for the autoimmune diseases would be 'diseases of immunological aberration' (Fudenberg, 1966).

For a clinical condition to be suspected as possibly belonging to this group of diseases there are a number of characteristics that have come to be recognized. Among these is the demonstration of an autoantibody against a body component, an increase in the level of the serum gammaglobulin, accumulations of lymphocytes and plasma cells and the deposition of denatured gammaglobulin in the affected tissue, a significant transient or lasting benefit in the course of the condition as a result of treatment with corticosteroid drugs, and the tendency for more than one type of autoimmune manifestation to occur in the same individual.

There are a number of possible ways that autoantibodies could arise and it is unlikely that the same mechanism operates in all forms of autoimmune disease. The following mechanisms may be considered:

1. Change in antigen.
2. Introduction of foreign antigen into the body with determinant groupings resembling those of host antigen.
3. Change in tolerance.

Alteration of the antigen may occur in a number of ways. The tissue may be damaged by chemical agents or by viruses, so that tissue constituents that may not normally come in contact with the antibody-forming system are able to do so. Alternatively, antibodies
may be produced as a result of the tissue constituents being altered by the damaging agent so that they become antigenic. Thus, mitochondrial antibodies have been described by Pinckard and Weir (1966), following damage to rat liver by carbon tetrachloride (CCl₄), and Ehrenfeld, Gery and Davies (1961) have described heart antibodies following myocardial infarction. In both circumstances the rise in antibody titre was transient. Alternatively, there may be a somatic mutation in the tissue so that a component of the tissue, to which the body had previously been immunologically tolerant, now becomes antigenic. There are clearly difficulties in detecting such a somatic mutation as the change in molecular structure may be small.

Antibodies reacting against constituents of the body's own tissues may also arise as a result of the invasion of the body by bacteria—such as *E. coli* O14—that have an antigenic component similar to a component of the body's tissues. The antibody against the bacteria may then cross-react with the body's tissues—for example, the colon in patients with ulcerative colitis (Perlmann and his colleagues, 1965).

There has been an interesting controversy with regard to the mechanism of autoimmunity in chronic thyroiditis (Hijmans and his colleagues, 1961; Irvine, 1964) and which has been recently resolved. It was held that thyroglobulin was hidden from the antibody-forming tissues as a consequence of its being strictly confined to the thyroid vesicles, and to the fact that thyroglobulin was broken down into its hormonally active fractions before release into the circulation. If, as a result of thyroid injury or disease, the acinar contents should escape into the adjacent tissues, antigens to which tolerance had not been achieved might reach immunologically competent cells, with the consequent production of an immune response. Further study has revealed that this is not the case in relation to thyroglobulin, for it now appears that thyroglobulin is physiologically secreted into the lymphatics (Daniel and his colleagues, 1967).

Again, it used to be thought that once antibodies had been formed, for example to thyroid tissue, then a chain reaction would be set up with further tissue damage and release of antigen. However, I am not aware of any experimental situation whereby a single injury to a tissue or repeated injury over a finite time has led to persistent levels of the corresponding antibody. We may assume that for persistent titres to be produced either there must be a continuing abnormality in the tissue (i.e. somatic mutation or persistent virus infection) or there must be an alteration in the antibody-forming
AUTOIMMUNITY

A constant level of autoantibody titres over a period of years is characteristic of chronic thyroiditis, atrophic gastritis and idiopathic adrenal failure, although in systemic lupus erythematosus the titres of antinuclear factors may fluctuate.

A genetically determined change in the antibody-forming system is the most simple and readily acceptable explanation for the occurrence of persistent autoimmunity (Irvine, 1964). In order to explain the persistence of the antibody titres and the overlapping of diseases associated with autoimmunity one can postulate a breakdown of immunological homeostasis. This breakdown of tolerance to self antigens may occur primarily in the antibody-forming tissues or, possibly, be mediated through the thymus, as illustrated in Figure 2.

Tolerance to tissue antigens may only exist with respect to production of certain classes of immunoglobulin. It has been suggested that tolerance may be absent with respect to IgM antibody production to particulate subcellular tissue antigens, as it was not found possible to produce tolerance to such antigens despite an intensive course of injections in neonatal rats (Weir and Pinckard, 1967).

DISTURBED ANTIGEN

DISTURBED TOLERANCE

(Reproduced from Irvine, 1964, by courtesy of the Editor of Quarterly Journal of Experimental Physiology)

Figure 2. Possible mechanisms in thyroid autoimmunity
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CLINICAL SYNDROMES ASSOCIATED WITH THYMIC PATHOLOGY

Thymic abnormalities in man, as revealed by x-ray, surgery or post-mortem examination, have been observed in association with several clinical disorders.

Myasthenia Gravis

The thymus gland in myasthenia gravis has one of three microscopic pictures (Castleman and Norris, 1949). Approximately 10 per cent of patients with myasthenia gravis have true neoplasms of the thymus or thymomas (Figure 3). Of the remaining patients, about 20 per cent have thymus glands that, so far as can be determined grossly and microscopically, are completely within normal limits. The other 80 per cent of the non-thymomatous patients have abnormal thymus glands microscopically, although the gross appearance and weight of the glands are usually within normal
CLINICAL SYNDROMES ASSOCIATED WITH THYMIC PATHOLOGY

limits. Microscopically, the striking feature of the thymus gland of this last group of patients is the presence of germinal centres within the thymic medulla. These germinal centres are similar to those characteristic of any lymph node. The thymus in some patients with myasthenia gravis may contain so many germinal centres in the medulla that the cortex is compressed into a rim round the expanding medulla. In other respects the medulla is normal and contains Hassall's corpuscles in usual numbers. Although Castleman and Norris (1949) described the germinal change in the medulla as 'thymic hyperplasia,' a more appropriate terminology would be thymic dysplasia. Burnet and Holmes (1964) have regarded the germinal centres in the thymus as sites of production of forbidden clones of immunologically competent cells that are capable of reacting against the body's own tissues. An alternative hypothesis, and one that is more satisfying to many, is that the germinal centre formation in the thymus represents an antigen-antibody reaction within the gland and that the situation in the thymus is analogous to that in other tissues that are the site of an autoimmune disorder, such as the thyroid in Hashimoto's thyroiditis. The germinal centre formation and the increased number of plasma cells in the thymus of patients with myasthenia gravis may be regarded as being due to a chronic thymitis (Goldstein, 1966). This hypothesis is particularly applicable to myasthenia gravis, as autoantibodies reacting against thymic myoid cells (see Figure 4) are a common finding in the sera of these patients (Van der Geld and his colleagues, 1963; Van der Geld and Strauss, 1966).

Myasthenia gravis is the disease most frequently associated with thymoma. Thus 15–30 per cent of all thymomas are complicated by myasthenia gravis. Thymomas are benign or only locally invasive tumours. The thymoma associated with myasthenia gravis is usually well encapsulated, very slowly growing, and only rarely leads to the death of the patient, even if it does spread beyond the confines of the thymus gland. Thymomas may be classified according to the predominant cell type or types: (a) epithelial, (b) spindle-celled, (c) lymphocytic, or (d) lympho-epithelial (Schmid and his colleagues, 1965). The classical form of thymoma that is associated with myasthenia gravis is represented by an admixture of lymphocytes and plump, ovoid epithelial cells often associated with small spaces containing a blood vessel surrounded by lymphocytes. Castleman (1966) states that when this picture is seen in a thymoma removed from a patient who apparently does not have symptoms of myasthenia gravis, the pathologist may reasonably predict that at some later date the patient may develop signs and symptoms of
IMMUNOBIOLOGY OF THE THYMUS

myasthenia gravis. Castleman has reported five cases in which myasthenic symptoms developed some months or years after a thymoma had been known to be present. On the other hand, myasthenia gravis is seldom found in association with spindle-celled thymomas.

The hypothesis was advanced independently by Nastuk, Plescia and Osserman (1960), on laboratory evidence, and by Simpson (1960), on clinical evidence, that myasthenia gravis may be a disorder with an autoimmune aetiology. It has been shown that the serum gamma-globulin of 30 per cent of patients with myasthenia gravis (99 out of 336) reacted concurrently in vitro against alternative skeletal muscle striations (A bands) and thymic epithelial cell cytoplasm as demonstrated by indirect fluorescent antibody techniques (Strauss and his colleagues, 1965). This reactivity with skeletal muscle and thymus, as illustrated in Figure 4, is always concurrent when present in the sera of patients with myasthenia gravis. Serum from 19 out of 20 patients with myasthenia gravis and associated thymomas (95 per cent) showed identical cross-reactivity with skeletal muscle and thymus. Such reactions did not occur within test limits in the sera of 128 out of 129 healthy control individuals and 673 out of 674 disease control individuals, including patients with other forms of autoimmunity.

The very high incidence of antibody to muscle A bands in patients with thymomas has led to the suggestion that the thymoma initiates an autoimmune response (Goldstein, 1966). Strauss and his colleagues (1965) demonstrated that some of the large pale epithelial cells of the type of thymoma most commonly found in myasthenia gravis contain the myoid antigen of the muscle A bands and thymic myoid cells. An autoimmune response to the thymoma might also explain the observation that germinal centre formation is often seen in the 'normal' thymic tissue adjacent to thymomas (Lattes, 1962). One might draw an analogy between skeletal and myoid antibodies in thymomatous patients with myasthenia gravis and the occurrence of thyroid antibodies in patients with carcinoma of the thyroid (Hirabayashi and Lindsay, 1965). In patients with myasthenia gravis but without thymoma, the cause of the autoimmunization is unknown and the problem of its aetiology is common to the other spontaneous diseases associated with autoimmunity.

Strauss and his colleagues (1966) noted that the sera from 12 of 51 patients with thymomas unassociated with myasthenia gravis reacted against skeletal muscle striations and also against thymic epithelial cell cytoplasm. Six of these twelve antibody positive patients had no subclinical evidence of myasthenia gravis by electromyography. In three of the patients no evidence of myasthenia
was obtained following provocation with d-tubocurarine (McFarlin, Barlow and Strauss, 1966). It can therefore be concluded that the symptom complex and pharmacological responses of myasthenia gravis are not immediate or necessary consequences of having such antibodies in the circulation.

Figure 4. Demonstration of antibody in the serum of a patient with myasthenia gravis reacting concurrently with: (a) thymic epithelial (myoid) cells; and (b) the A bands of skeletal muscle.
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Patients with myasthenia gravis have a higher incidence of other autoimmune diseases than control populations; also, there is a considerable increased incidence of other autoantibodies in the sera of patients with myasthenia gravis. For example, in a series of 325 cases of myasthenia gravis, Osserman (1958) noted 11 patients (3 per cent) with hyperthyroidism, 5 patients who had undergone thyroidectomy for non-toxic goitre and 1 patient with thyroid carcinoma. Simpson (1958) studied 404 cases of myasthenia and described a goitre in 16 per cent of the females and 7 per cent of the males. Sahay, Blendis and Greene (1965) reported the co-existence of thyroid disease in 8 out of 260 cases of myasthenia, 5 of the 8 patients being hypothyroid. Simpson (1964) and Daly and Jackson (1964) both described cases of Hashimoto's disease occurring with myasthenia gravis. Oosterhuis (1964) has described 8 patients with hyperthyroidism, 7 patients with non-toxic goitre and 2 post-mortem cases of Hashimoto's thyroiditis in a series of 164 patients with myasthenia gravis. Becker and his colleagues (1964) found morphological changes suggestive of thyroiditis at autopsy in 6 out of 32 cases (19 per cent) of myasthenia gravis, while in a control group of 13,672 adults the frequency was only 0.9 per cent. Rheumatoid arthritis has been observed in 4 per cent of 491 patients with myasthenia gravis (Simpson, 1964) and in 6 per cent of 75 women with myasthenia gravis (Oosterhuis, 1964). The association in some cases of myasthenia gravis with systemic lupus erythematosus has been reported and reviewed by Simpson (1964), who also described pernicious anaemia in 9 out of 491 cases of myasthenia gravis. The simultaneous presence of aplastic anaemia, myasthenia gravis and thymoma occurs more frequently than would be expected (Castalgne and his colleagues, 1961; Oosterhuis and his colleagues, 1965).

If the series of White and Marshall (1962), Van der Geld and his colleagues (1963), Feltkamp and his colleagues (1963), Simpson (1964), Sturgill and his colleagues (1964) and Adner and his colleagues (1964) are put together, thyroid antibodies have been detected in the sera of 64 of 213 patients with myasthenia gravis. Antinuclear factors have been described in 48 out of 250 patients, and rheumatoid factors have been described in 14 out of 216 patients with myasthenia gravis. Simpson (1964) also reported antibodies to human stomach in 3 out of 35 patients with myasthenia gravis. Despite this incidence of serological reactivity to thymus and muscle and to other tissues, Kornfeld (1964) found no gross disorder in the serum electrophoretic pattern of 61 cases of myasthenia gravis.

In keeping with the serological overlap of other types of antibody in patients with myasthenia gravis, there is also an increase in the...
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incidence of skeletal muscle antibodies in patients with other types of autoimmune disease. Feltkamp and his colleagues (1964) demonstrated antibodies to skeletal muscle fibres in 19 per cent of 123 patients with rheumatoid arthritis, and Feltkamp and Feltkamp-Vroom (1965) reported a frequency of 13 per cent in 94 cases of pernicious anaemia, 11 per cent in 76 patients with autoimmune thyroiditis, and 19 per cent in 21 patients with idiopathic adrenal-cortical insufficiency. An incidence of 1–2 per cent was recorded in their control subjects. This is in contrast to the negative findings of Strauss and his colleagues (1965) referred to above, but the titre of skeletal muscle antibodies are probably higher in myasthenia gravis than in the other diseases of immunological aberration. Antibody factor has also been described in the sera of patients who have thymoma but who do not have any evidence of myasthenia (Strauss and his colleagues, 1966).

Attempts have been made to induce experimental myasthenia gravis by immunological techniques. Strauss (1963) injected guinea-pigs with muscle, actin and topomyosin in Freund’s adjuvant. No precipitating antibodies or symptomatic or histopathological changes in the muscles were observed. More recently, Goldstein and Whittingham (1966, 1967) claimed to have produced an analogue of human myasthenia gravis in guinea-pigs immunized with thymus or muscle in Freund’s complete adjuvant; half the immunized guinea-pigs showed lymphocytic infiltrates in the thymic medulla (Figure 5). Six of 24 animals immunized with thymus or muscle had electromyographic evidence of myasthenia. Guinea-pigs immunized with muscle formed autoantibody to thymic myoid cells. This experimental model provides strong evidence for the autoimmune basis of thymitis in myasthenia gravis in man.

What causes the neuromuscular block in myasthenia gravis is unknown. At the present time there is no direct immunological evidence for an antibody reacting at the neuromuscular end-plate. That a humoral factor is operative is inferred from the observation that neonatal myasthenia gravis affects some infants born to mothers with myasthenia gravis. The weakness in the infant is observed shortly after birth, responds to neostigmine, lasts about three weeks, and then disappears. As already discussed in relation to adults with myasthenia gravis, this blocking substance is apparently distinct from the autoantibody to thymic myoid cells and A bands of skeletal muscle. Neonatal myasthenia gravis has occurred in the infants of mothers who do not have this antibody in the serum, and conversely, these antibodies may be present in the serum of the baby without that baby having myasthenia (Oosterhuis, Feltkamp}
Figure 5. (a) Thymus of guinea-pig showing thymitis two weeks after immunization with thymus in complete Freund adjuvant. There is an accumulation of lymphocytes in the central medulla. (b) Normal guinea-pig thymus to show the degree of cellularity and regular pattern of cells in the medulla.
and Van der Geld, 1966). The possibility that the neuromuscular blocking factor may not be an antibody at all is suggested by the fact that the humoral blocking substance is dialysable and therefore is a substance of smaller molecular size than an antibody (Stricker and his colleagues, 1960).

It has been suggested that the humoral neuromuscular blocking substance in myasthenia gravis is liberated from the diseased thymus (Strauss and his colleagues, 1966). In the experimental model described by Goldstein and Whittingham (1966) the onset of myasthenia could be prevented by thymectomizing the animals before immunizing them with skeletal muscle or thymus. Although these thymectomized animals did not develop myasthenia when appropriately challenged, they could develop other experimental autoimmune diseases, such as allergic encephalomyelitis, when immunized with extracts of bovine spinal cord. This hypothesis would also fit the clinical observation that thymectomy leads to an improvement in symptoms in some patients with myasthenia gravis.

**Thyroid Disease**

Between 1910 and 1920 great interest was shown in the relationship between thymic enlargement and thyrotoxicosis (Crotti, 1938),

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![Graph of thymus weight over age](image-url)

*Figure 6. Weights of entire thymus, cortex and medulla, plotted by age in 'normal' subjects (cases of sudden death), in thyrotoxicosis and in myasthenia gravis. Each point represents a mean value of weights for all cases within successive five-year periods of life. The data for the 'normal' subjects were those of Hammar (1929), and for myasthenia gravis were derived from Castleman (1955), who cited only the weight of the entire thymus.*
and thymectomy was even performed as a treatment for thyrotoxicosis. Thyrotoxicosis was amongst the diseases chosen by Hammar (1929) when he analysed the weight of the thymus and its components. Mackay (1966) arranged Hammar's 43 cases of thyrotoxicosis into five-year age groups corresponding to the five-year age periods into which Hammar placed his control cases, these being victims of sudden death (Figure 6). The range of thymic weights for each age group was fairly wide, but the mean weights for all thymic components were considerably greater in thyrotoxicosis than in the controls, particularly in the younger subjects. The number of Hassall's corpuscles in the thymus glands of the thyrotoxic patients was greatly increased compared with the controls, suggesting increased epithelial activity. Gunn, Michie and Irvine (1964) obtained biopsies of the human thymus in the course of surgical thyroidectomy for thyrotoxicosis and found germinal centres in the medulla in 16 out of 50 thymic biopsies. Thymic medullary germinal centres have also been noted in thymus biopsies of patients with Hashimoto's thyroiditis (Figure 7). Thymic

Figure 7. A germinal centre within a biopsy of an upper corn of the thymus in a patient with histologically proven Hashimoto's thyroiditis

(Reproduced from Irvine, 1964, by courtesy of the Editor of Quarterly Journal of Experimental Physiology)
biopsies can only be done, however, when there are other reasons for operating in the neck or chest, e.g. thyroidectomy, parathyroidectomy, and cardiac or lung surgery.

A further way of assessing the thymus without recourse to surgery is by radiography. While many, but not all, thymomas can be demonstrated by conventional radiography, pneumomediastinography is required to visualize the thymus and distinguish it from adjacent structures. By this technique (Figure 8) it was found that the thymus shadow was frequently enlarged in patients with thyroid disease, such as thyrotoxicosis controlled with antithyroid drugs or \(^{131}I\), primary hypothyroidism treated with thyroxine, or euthyroid patients with Hashimoto's thyroiditis (Irvine and Sumerling, 1965).

The enlargement of the thymus shadow could be correlated with the occurrence of autoantibodies in the patient's serum. The limitation
of pneumomediastinography is that it can make no distinction between the relative amounts of fat and lymphoid tissue that together make up the thymus gland. Michie and his colleagues (1967) have attempted to overcome this limitation by combining the techniques of biopsy and of pneumomediastinography. They

![Autoantibodies Graph](Reproduced from Irvine, Davies and Sumerling, 1965, by courtesy of Academic Press)

**Figure 9.** The thymus in thyroid disease. The cross-sectional area of the shadow in the central plane was determined by pneumomediastinography in 52 patients with thyroid disease. The size of the thymus is compared in patients with positive thyroid or gastric serology and in patients with no serological evidence of autoimmunity. All patients were euthyroid at the time of examination.
calculated what they refer to as the 'corrected thymic size' (radiological measurement multiplied by percentage of parenchyma in a biopsy specimen of thymus). Relatively large amounts of thyroxine were shown by Warner (1964) to increase the thymus cortex in fowls, and it is conceivable that excessive thyroxine may be responsible for thymic enlargement and for the accompanying peripheral lymphocytosis and generalized lymphoid hyperplasia that occurs in some patients with hyperthyroidism. This would not seem to be an adequate explanation for the presence of germinal centres in the thymus or for the radiological evidence of thymic enlargement in euthyroid patients with thyroid disorders. Thyroid disease has been extensively studied in the past decade with regard to its autoimmune aspects. Hashimoto's thyroiditis, primary hypothyroidism and thyrotoxicosis are all associated with the occurrence of thyroid autoantibodies in the circulation and these three conditions have come to be known as the thyroid autoimmune diseases. Long-acting thyroid stimulating substance (LATS) has recently been shown to be an immunoglobulin, and it is tempting to consider that LATS may be an autoantibody that has stimulating properties on the thyroid cells and which is basically important in the pathogenesis of thyrotoxicosis (Adams, 1965). As yet no correlation has been made between the presence of muscle antibodies in the sera of patients with thyroid disorders and the presence of germinal centres in the thymus glands of these subjects. In the attempt to get an explanation of the occurrence of germinal centres in the thymus of patients with thyroiditis it would be helpful to carry out an immunofluorescent study using the sera of patients with thyroid disease and thymus tissue. Irvine and Sumerling (1965) noted a correlation between radiological evidence of thymus enlargement and the presence of autoantibodies in the patient's serum (Figure 9). Gunn, Michie and Irvine (1964) could not detect a correlation between autoantibodies in the sera and germinal centres in the thymus but did detect a correlation between the occurrence of lymphoid follicles in the thyroid and germinal centre formation in the thymus.

**Adrenal Disorders**

Adrenocorticoids of exogenous origin, as well as increased levels of these hormones produced by the body itself as a result of administration of ACTH or conditions of stress, produce a marked depletion of thymic lymphocytes and a decrease in size of the gland. Sloan (1943) found lymph follicle formation comparable to that in myasthenia gravis in the thymus glands of three of seven patients.
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with Addison’s disease; one had a thymoma. This observation is of particular interest in view of the description of adrenal specific antibodies in the sera of the majority of patients with idiopathic Addison’s disease, in contrast to their absence in the sera of patients with tuberculous destruction of the adrenal (Irvine, Stewart and Scarth, 1967). Unfortunately, Sloan did not state whether his patients had idiopathic Addison’s disease or whether the primary adrenal failure was due to other causes.

Cushing’s syndrome of adrenal hyperplasia may rarely be associated with a thymoma. The thymic tumours associated with Cushing’s syndrome are frequently malignant and consist of epithelial elements only and these tumours are totally or almost completely devoid of lymphocytic elements. Such thymomas probably secrete ectopic ACTH or ACTH-like substance in the same manner as has been shown for certain carcinomas arising from ‘non-endocrine’ tissues (notably lung) (Meador and his colleagues, 1962).

Systemic Lupus Erythematosus

In two patients with systemic lupus erythematosus (SLE) Hare and Mackay (1963) found no radiological evidence of thymic enlargement using the technique of pneumomediastinography. In the nine cases of SLE studied histologically by Goldstein and Mackay (1965) the thymus gland was considered to be smaller than normal. The histological changes in the thymus included gross lymphoid depletion of the cortex and medulla, collapse of normal architecture as shown by reticulin staining, and accumulation of cells in the medulla which occasionally resembled epithelial cells but were in most cases spindle-shaped; they often resembled the cell type which constitutes spindle-cell thymomas and the cells of thymic grafts which have survived in diffusion chambers. The number of plasma cells and cystic Hassall’s corpuscles were considered to be increased. In two cases, typical germinal centres were present in the medulla. Essentially similar changes were noted in the thymus glands of three patients with acute SLE and who underwent thymectomy (Milne and his colleagues, 1967). These appearances are in contrast to myasthenia gravis in which the only significant change seen in the thymus (in the absence of thymoma) was germinal centre formation in the medulla. This striking difference in the histology of the thymus gland in patients with SLE, as opposed to patients with myasthenia gravis, with respect to spindle epithelial cell concentration is illustrated in Figure 10.
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Mackay (1966) has also reported a relatively increased area of spindle epithelial cells in the medulla in certain other conditions, including rheumatoid arthritis, Goodpasture's syndrome (nephritis and pulmonary haemorrhage), chronic hepatitis, and also in the thymus of two patients with chronic glomerular nephritis who had both received a renal homotransplant. The number of patients with these diseases who have been studied is small.

Figure 10. Histology of thymus in systemic lupus erythematosus (SLE), myasthenia gravis (MG) and controls. The percentage areas of spindle epithelial cells in medulla, and numbers of cystic Hassall's corpuscles and plasma cells per mm. of a thymic section are shown as mean values. The controls suffered from miscellaneous diseases and were age-matched. All values for SLE were significantly increased (* p < 0.05).

Hypogammaglobulinaemia and Other Haematological Disorders

Adult acquired hypogammaglobulinaemia has been reported as co-existing with thymoma in 14 cases—10 females of mean age 51 years and 4 males of mean age 62 years—and it has been stated that 5–10 per cent of cases of adult hypogammaglobulinaemia will be associated with a thymic tumour (Comings, 1965; Peterson, Cooper and Good, 1965). An associated bone marrow deficiency was present in 5 of the 14 cases. The tumour was spindle-celled in 8 cases, epithelial in 2, and lympho-epithelial in 2. Relatives of cases of acquired hypogammaglobulinaemia have a significant incidence of rheumatoid arthritis and other diseases of autoimmune character and autoimmune serological reactions (Fudenberg, German and Kunkel, 1962; Wolf, Gokcen and Good, 1963).

Essential lymphocytopenia is a rare condition with a familial incidence and affects infants of both sexes. Infants with this condition
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appear to be normal at birth but fail to thrive. The syndrome is usually recognized at about the age of three months, when the presenting clinical features are wasting, diarrhoea, morbilliform rashes, bacterial and fungal infections, agammaglobulinaemia, and marked lymphopenia. The condition is fatal despite antibiotic therapy, blood transfusions and gamma-globulin infusions. At necropsy there is a marked atrophy of the lymphatic tissue and moderate to marked reticulo-endothelial hyperplasia. Lymph follicles and plasma cells are absent. The thymus is markedly atrophic and has few or no small lymphocytes and no Hassall’s corpuscles. The primary cause of this condition is probably a genetically determined agenesis of the thymus. Essential lymphocytophthisis in man with thymic agenesis is the exact counterpart of the wasting syndrome in neonatally thymectomized mice.

Thirty-nine instances of a thymic abnormality and haemopoietic insufficiency that have been observed since 1928 are listed and reviewed by Fisher (1964). The haematological disorders include pure red cell anaemia, pancytopenia, autoimmune haemolytic anaemia, thrombocytopenia, thrombotic thrombocytopenic purpura, leucopenia and pernicious anaemia. The most common thymic abnormality in this group of conditions is a neoplasm usually of the spindle-cell or lympho-epithelial types. It is believed that the relationship between thymic tumour or abnormality and haemopoietic insufficiency is more than fortuitous. There is no known physiological relationship between the thymus and erythropoiesis, although it is of interest that autoimmune haemolytic anaemia and pernicious anaemia, and possibly also the other haemopoietic disorders listed, are associated with autoimmunity. In keeping with this line of thought is the observation that out of the 39 examples of thymic abnormality and haemopoietic insufficiency, 2 also had agammaglobulinaemia and 4 had myasthenia gravis.

With regard to myasthenia gravis it is clear that in some cases the presence of a thymoma may precede the onset of the myasthenia. The time relationship between the haemopoietic disorder and thymic abnormalities is less well understood.

In view of the close similarities between the immunological aspects of pernicious anaemia and thyroid disease, it might be expected that the pathology of the thymus in patients with pernicious anaemia might resemble that described in chronic thyroiditis. Thymic dysplasia or hyperplasia has rarely been described in patients with pernicious anaemia, but this may be due to the fact that these non-thymomatous changes are entirely subclinical, and that they have not been adequately searched for by radiographic
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techniques or by post-mortem study in patients who have had a sudden accidental death. The same limitation applies to autoimmune haemolytic anaemia; before the thymus is available for histological examination the patient has generally been on steroid therapy or has undergone a protracted or acute illness which in itself would lead to marked alteration of the thymus histology.

Autoimmunity in Experimental Animals

Two forms of autoimmune disease have been shown to develop spontaneously in laboratory animals. The NZB strain of mice all show an autoantibody type of haemolytic anaemia, which develops after three months of age and is characterized by circulating antibody to red blood cells as shown by direct and indirect Coombs tests (Helyer and Howie, 1963a; Holmes and Burnet, 1963). Certain types of F₁ hybrids derived from the NZB strain, notably NZB and NZW hybrids, all develop a disorder after six months of age which closely resembles lupus nephritis and which is characterized by a positive lupus erythematosus cell test (Helyer and Howie, 1963b). The haemolytic anaemia occurring in NZB mice is similar to the warm-antibody type of haemolytic anaemia occurring in man (Dacie, 1962).

The occurrence of lesions in the thymus in NZB mice has been well documented (Burnet and Holmes, 1964). The thymus lesions consist of areas of expanded medulla, often with undoubted germinal centres containing mitoses and pyknotic cells and surrounded by a segment of a cuff of small lymphocytes with a cleared area of medulla outside this. Germinal centres occur singly or in massive lesions where germinal centres of different ages can be found together. These centres, although not as well defined as the germinal centres in man, are very similar to those occurring in the spleen or lymph nodes in mice, and have an obvious resemblance to the germinal centres in myasthenia gravis in man. The thymus lesions are first recognized histologically at about the time the Coombs test converts to positive—that is, at about 150 days. Male mice are often Coombs positive when no lesions can be found in sections from the thymus, but the females generally show thymic lesions before they become Coombs positive.

Effects of Thymectomy on Clinical Syndromes Associated with Thymic Abnormalities

The only human autoimmune disease for which thymectomy is regularly performed is myasthenia gravis. The results of this have
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been difficult to evaluate, because of the tendency of the disease to undergo spontaneous remission, the effect of age and sex on the response to thymectomy and the alleged adverse influence of thymoma. There are three series in which a long-term comparison has been made between thymectomized patients and medically treated 'controls' not subjected to thymectomy. Simpson (1958) reported that 58 per cent of 182 non-thymomatous female patients with myasthenia gravis treated surgically obtained a definite improvement, compared with 36 per cent of 59 'controls', and 17 per cent died compared with 36 per cent of the 'controls'. The results for females were statistically significant in favour of thymectomy. Similarly, Schwab (1961) reported improvement following 61 of 101 thymectomies in female subjects, compared with 21 improvements in 100 controls not subjected to operation. Henson, Stern and Thompson (1965) reported a remission in 67 per cent of 30 adults (28 females and 2 males) following thymectomy, as compared with 31 per cent in non-operative cases. Thus, thymectomy has its best effects in patients, especially females, with non-thymomatous myasthenia gravis. In other cases, thymectomy may fail because irreversible structural changes have occurred in the motor end plates (Woolf, 1966) or in the muscle fibres (Keynes, 1954; Fenichel, 1966). In keeping with these suggestions is the repeated observation that the best ultimate prognosis after thymectomy is in patients with the shortest pre-operative duration of symptoms, irrespective of the severity of the disease (Simpson, 1958). A further explanation for failure of the myasthenia gravis to improve after thymectomy for thymoma—and which may account for the occasional occurrence of myasthenia gravis developing after thymectomy for thymoma—could be incomplete thymectomy, possibly due to the presence of ectopic thymic tissue (Harvey, 1948; Fisher, 1964).

There are two reported cases of autoimmune haemolytic anaemia in infancy treated successfully by thymectomy. The patient of Wilmers and Russell (1963) was an infant, aged 2½ months with severe acute haemolytic anaemia and presenting positive indirect and direct Coombs reactions, who did not have a remission after corticosteroid treatment and splenectomy. Thymectomy produced prompt remission, and prednisolone was discontinued without ill effect and the response to the Coombs test became negative. The resected thymus showed absence of cortex and conspicuous epithelial tissue in the medulla. Karaklis and his colleagues (1964) reported the case of a male infant, aged ten months, with severe autoimmune haemolytic anaemia and positive direct and indirect Coombs
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reactions; transfusion requirements remained high despite treatment with corticosteroids and splenectomy. Thymectomy induced full remission, prednisolone was discontinued without ill effect, and the Coombs test became negative after six months. The resected thymus showed cortical atrophy and striking accumulations of cells resembling plasmoblasts in the medulla.

On the other hand, there are reports that thymectomy, whether for tumour or for treatment of myasthenia gravis, has been followed by the occurrence of autoimmune diseases, including myasthenia gravis, lupus erythematosus and Hashimoto's disease, which were either not present or not recognized before the operation (Alarcón-Segovia and his colleagues, 1963; Fisher, 1964; Simpson, 1964; Kerr and his colleagues, 1965). Again in these circumstances it may be difficult to exclude the possibility that not all the thymus gland was removed, and that a significant amount of thymus tissue may have been left in situ.

Mackay and Smalley (1966) studied three female patients aged 20, 14 and 56 years before and after thymectomy for severe SLE. Two of the patients had not received corticosteroids pre-operatively. None showed any evidence of benefit in the immediate post-operative period and all three required full treatment with prednisolone within 2-4 weeks of thymectomy and have continued to require maintenance dosage of prednisolone since. There was no change in titre of antinuclear antibodies after thymectomy in any of the three patients, as judged by the persistence of positive LE cell phenomena and the high titres of ANF. It was of interest that after thymectomy there was a transient rise in titre of antithyroglobulin antibody in two of the cases and a positive Coombs test in one, suggesting that thymectomy could allow the expression of autoimmune reactivity. Milne and his colleagues (1967) did not detect any alteration in the immunological status of three patients with acute SLE following thymectomy, apart from a lymphopenia in one patient. Nor was there any evidence of unequivocal clinical improvement attributable to the thymectomy. The situation was similar in their two patients with rheumatoid arthritis, who underwent thymectomy. There is one other report on the influence of thymectomy on autoantibody reactions in man, this being by Osserman and Weiner (1965) on the incidence of anti-muscle autoantibody before and after thymectomy in cases of myasthenia gravis; after thymectomy the serological reactions tended to become negative in cases without thymoma, but persisted in cases in whom a thymoma had been present. In rabbits, thymectomy has been observed to result in an increased incidence of positive Coombs reactions and of amyloidosis, again suggesting
that thymectomy might allow the expression of autoimmune activity (Sutherland and his colleagues, 1965).

In autoimmune strains of mice neither neonatal thymectomy nor the neona\-tal replacement of the thymus by a normal gland is able to prevent the development of autoimmune disease. With a normal exchange-grafted thymus the disorder develops in the same manner as in intact animals and is not influenced by the graft being normal or autoimmune. Holmes and Burnet (1964) found that complete thymectomy in NZB mice, either neonatally or at four weeks of age, significantly delayed the onset of the positive Coombs reaction, but most of the surviving mice eventually developed it. Helyer and Howie (1963c) and Howie and Helyer (1966) maintain that neonatal thymectomy in NZB and NZB-NZW hybrid mice accelerates the onset and increases the incidence of autoimmune serological reactions and increases the severity of the renal lesions.

The success of the thymectomy in myasthenia gravis may be explained in terms of the hypothesis that the thymus in such patients produces a significant amount of neuromuscular blocking factor. The poor results of thymectomy in other conditions associated with autoimmunity may be interpreted in the light of the above discussion on the mode of action of the thymus. Thymectomy as a treatment for autoimmune diseases other than myasthenia gravis may be effective only in childhood when any proliferative process involving lymphoid cells would be predominantly active in the thymus, and thymectomy would be expected to remove a highly significant fraction of that activity. However, the failure of neonatal thymectomy to ameliorate significantly the course of the haemolytic anaemia in NZB mice suggests that the development of forbidden clones of immunologically competent cells, either within or under the influence of the thymus, must occur and must undergo extra-thymic dissemination during intra-uterine life in the mouse. During the post-natal period in the mouse these clones must continue to grow and proliferate independently and to be influenced by the thymus only in a regulatory or inhibitory manner. If, on the other hand, autoimmune disease is due to the development of clones of lymphoid cells resistant to thymic control, thymectomy would not be expected to improve the disorder.

**Immunological Deficiency, Autoimmunity and Malignant Disease**

One of the most important developments in the study of the immunological deficiencies is the link with autoimmunity on the one hand and malignant lymphomas on the other. The association of
diseases of immunological aberration ('autoimmune diseases') and lymphoma within the same family, and indeed in the same patient, has been noted with increasing frequency in the past decade. Similar associations have been noted between the diseases of immunological aberration and immunological deficiencies, and between immunological deficiencies and lymphomatous malignancies (reviewed by Fudenberg, 1966). Dameshek (1966) has emphasized the autoimmune manifestations of the lymphoproliferative disorders. Lewis, Schwartz and Dameshek (1966) have indicated that x-radiation and alkylating agents may act as possible 'trigger' mechanisms in the autoimmune complications of malignant lymphoproliferative disease. In the experimental animal, malignant lymphoma has been noted in ageing, Coombs positive, NZB mice (Mellors, 1966). East and her colleagues (1967) have shown that the lymphoma can be transferred by serial passage of the animal's lymphoid tissue in young intact or neonatally thymectomized syngeneic recipients. Helyer and Howie (1963c) have shown that the thymic grafts of NZB mice with autoimmune haemolytic anaemia are able to transfer the disease to healthy mice. The possibility that the so-called autoimmune lesions and malignant lymphomas, and also the proliferative lesion within the thymus seen in autoimmune disease, may be a direct manifestation of 'slow' viruses in a host of appropriate genetic constitution requires further consideration.

CONCLUSION

Although much knowledge has been gained both with respect to the physiological importance of the thymus and the disease states that are associated with its abnormalities, in many respects the thymus remains an enigma. It is known that the thymus is essential for the development and maintenance of the immune system, and recent work is giving some indications as to how the thymus may achieve this function. Nevertheless, there is much that is still highly speculative about the ways in which the thymus is believed to achieve these important physiological functions.

The association between thymic abnormalities and the diseases of immunological aberration ('autoimmune diseases') is too frequent to be explained by chance alone. However, in view of our incomplete understanding of the physiology of the thymus, it is hardly surprising that at the present time we are unable to determine accurately whether the defect in the thymus in the autoimmune diseases is of primary or of secondary importance.

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In either event a genetic factor would appear to operate. According to one hypothesis this may operate primarily in relation to the deletion or lack of deletion of undesirable autoreactive clones of immunologically competent cells. Alternatively, the genetic factor may determine the susceptibility of the individual to invasion of specific tissues or groups of tissues by 'slow' viruses or other pathogens.

With the exception of myasthenia gravis, the place of thymectomy in the management of patients suffering from autoimmune disease would appear to be limited to diseases of acute onset when standard methods of treatment have failed in the very early years of life.

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THE THYMUS IN AUTOIMMUNE DISEASE

by

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The thymus is essential for the development of cellular immune mechanisms and for the reconstitution of the immunological system if damaged, for example, by radiation. The thymus is also implicated in certain types of humoral immunity probably due to cooperation between thymus-derived and bursa-derived lymphocytes (see Roitt et al, 1969). In addition to the link with immunological deficiency, it would therefore be reasonable to ask if some abnormality of the thymus might not be associated with other disorders of the immunological system, such as occur in autoimmunity.

The association between morphological abnormalities of the thymus and conditions characterised by disordered immunological function is impressive (Table I). This is not a comprehensive review of all the various conditions that have been associated with thymic pathology, but it includes the most important ones. For a more detailed account see Irvine (1967) and Goldstein and Mackay (1969).

**CLINICAL CONDITIONS IN MAN**

**Myasthenia Gravis:**

Myasthenia gravis is the condition linked with autoimmunity that shows the clearest correlation with thymic pathology. Approximately 10% of patients with myasthenia gravis have thymomas. The classical form of thymoma that is associated with myasthenia gravis is the predominantly lymphoid or the predominantly epithelioid types. The spindle cell type is not associated with myasthenia gravis. When a thymoma consisting of abundant lymphocytes and plump ovoid epithelioid cells is removed from a patient without myasthenia gravis, it is likely that the patient will develop myasthenia gravis at a later date (Castleman, 1966). According to Lattes (1962), patients with thymoma invariably show germinal centres in the surrounding thymus tissue. An important clinical point is that the presence of a thymoma may not be shown by
### Table I

The Correlation Between Conditions Characterised by Disordered Immunological Function and Morphological Abnormalities of the Thymus

<table>
<thead>
<tr>
<th><strong>THYMIC PATHOLOGY</strong></th>
<th><strong>ASSOCIATED DISEASE STATE</strong></th>
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<tr>
<td>Aplasia</td>
<td>Immunological deficiency</td>
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<tr>
<td>Dysplasia or Thymitis</td>
<td>Myasthenia gravis</td>
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<td></td>
<td>Thyroid autoimmune disease</td>
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<td>Systemic lupus erythematosus</td>
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<td>Rheumatic carditis (NZB mice)</td>
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Thymic Tumours:

(a) Lympho-epitheliomas
   - Myasthenia gravis
   - Aplastic anaemia and other blood dyscrasias
   - Hypogammaglobulinaemia (Mastomys)

(b) Lymphosarcoma
   - Lymphocytic leukaemia
conventional radiography even with tomography. Pneumomediastinography may be required to differentiate the outline of the thymoma from other tissues in the anterior mediastinum (Hare and Mackay, 1963; Irvine and Sumerling, 1965; Kreeel and James, 1965; Sumerling and Irvine, 1966).

In the 90% of patients with myasthenia gravis without a thymoma, the thymus is of normal size, but the majority of these patients show a marked degree of germinal centre formation in the medulla of the thymus and an increase in the number of plasma cells. In fact only about 15% of patients is the thymus histologically normal. The histological appearance of germinal centre formation in the thymus in myasthenia gravis and its resemblance to that of the thyroid in Hashimoto thyroiditis first prompted the suggestion by Smithers (1959) that myasthenia gravis may be of autoimmune origin.

**Thyroid Autoimmune Diseases (thyrotoxicosis, Hashimoto thyroiditis and primary atrophic hypothyroidism):**

It has long been recognised from post mortem studies that enlargement of the thymus gland and germinal centre formation in the medulla may occur in thyroid disease, particularly in thyrotoxicosis. More recently, the thymus in thyroid disease has been studied radiologically and by thymus biopsy during the course of routine partial thyroidectomy for thyrotoxicosis or simple goitre (Gunn, Michie and Irvine, 1964; Irvine and Sumerling, 1965). All the patients were euthyroid at the time of study. The size of the thymus shadow in the sagittal plane was measured by planimetry. Those with evidence of autoimmunity in the form of thyroid or gastric antibodies in the serum were found to have considerably larger thymus shadows than in the non-autoimmune cases. The X-ray, of course, does not indicate how much of the thymus shadow is connective tissue (as a result of involution) or how much is thymic cortex or medulla. To overcome this, Michie et al (1967) calculated the "corrected thymus size", this being the product of the
size and the percentage of parenchyma in a biopsy of the thymus. Patients who were under treatment for thyrotoxicosis had an increased amount of thymus tissue which often contained germinal centres in the medulla. The occurrence of germinal centres in the thymus medulla showed a correlation with germinal centre formation in the thyroid. On the other hand, patients with Hashimoto thyroiditis tended to have large thymic shadows radiologically but relatively little thymic tissue and rather few germinal centres in the medulla. At the present time, these findings are difficult to interpret but are unlikely to be entirely due to the differences in thyroid function in previous years.

In Addison's disease, although increased lymphocytic infiltration has been described in the thymic medulla, no distinction has been made in post mortem studies between patients with idiopathic (i.e. autoimmune) Addison's disease and those in whom the adrenal destruction was tuberculous or due to some other cause.

In systemic lupus erythematosus, the thymus tends to be smaller than normal, even in patients who have not previously been given steroid therapy (see Goldstein and Mackay, 1969). Although germinal centre formation may occur, the most conspicuous feature is the occurrence of spindle-epithelial cells, plasma cells and numerous cystic Hassall's corpuscles.

Henry (1968) has compared the histology of the thymus in patients with rheumatic heart disease and congenital heart disease. The thymus biopsies were taken during cardiac surgery. Germinal centres in the thymic medulla were more frequent and more conspicuous in the patients with rheumatic heart disease. In the past it has been denied that germinal centres occur in normal subjects, but it does seem that they do occur but to a lesser extent than in patients with certain autoimmune diseases.
ANIMAL MODELS

(a) NZB Mice:

(b) Spontaneously occurring and experimentally induced animal models of myasthenia gravis:

(a) The NZB strain of mice that develop autoimmune haemolytic anaemia spontaneously and show evidence of glomerulonephritis have a high incidence of germinal centre formation in the thymic medulla developing in adult life (Burnet and Holmes, 1964). The time of onset of the thymic changes does not have a close correlation with the appearance of positive Coombs tests. The mice all develop positive Coombs tests and thymic changes with increasing age.

It may be that the germinal centres in the thymus in autoimmune disease are the site of production of forbidden clones of lymphocytes that seed out into the lymphoid system and which are responsible for the production of self-reactive immunity. Alternatively, the normal thymus may have a censorship function, whereby it somehow deletes any aberrant clones of lymphocytes that might arise, for example, by spontaneous mutation (Burnet, 1962; Miller & Osoba, 1969). It may be that the thymus in order to do this has a repertoire of the antigens that are present throughout the various tissues of the body.

There is evidence, however, that the thymus is not primarily involved in causing the autoimmune disease in NZB mice. For example, neonatal thymectomy in NZB mice does not prevent the onset of autoimmune haemolytic anaemia although germinal centre formation in the thymic medulla does not appear in these animals until they are much older. Grafting of a normal thymus into an autoimmune strain does not prevent the development of the haemolytic anaemia or of the renal disease (Howie and Helyer, 1966). Thymic grafts inserted into NZB mice develop lesions similar to those occurring in the animal's
own thymus. This suggests that the thymus is a target organ, being one further site in which pathological evidence of an autoimmune reaction can be found (Holmes and Burnet, 1966).

On the other hand there is evidence that the autoimmune disease in NZB mice is initiated by a vertically transmitted virus. Thus, autoimmune disease with positive Coombs and LE cell tests can be transmitted to other strains of mice by grafts of thymus from autoimmune strains, by inocula of spleen cells and most significantly by cell free extracts from spleen. Virus particles can be consistently demonstrated by electronmicroscopy in the tissues of NZB mice and the development of positive Coombs tests can be significantly delayed by vaccinating baby NZB mice with formaldehyde-inactivated cell-free filtrates of older NZB mouse spleens (Mellors, 1969).

The NZB mice, by nature of their inbreeding, may be particularly susceptible to a certain type of virus infection. It can be postulated that the virus induces autoimmunity by altering the cells' antigens sufficiently to render these antigenic, and the immune reaction thus stimulated cross-reacts with normal antigens. In this way, an autoimmune reaction may be set up and the thymus may be involved as a target organ.

(b) Animal Models for Myasthenia Gravis:

1. Spontaneously occurring - Mastomys

2. Experimentally Induced

A high incidence of thymomas has been described in the species of South African rodent called mastomys and some of these animals have myositis and atrophy of skeletal muscle. Nearly a third of the animals with thymomas have severe myocarditis. Strauss et al (1968) recently described the presence of circulating antibody in the serum of such an animal with a thymoma that was reactive with cardiac and skeletal muscle striations in a manner comparable to the antibody found in patients with myasthenia gravis. It would, therefore, appear that these animals
provide an experimental model approaching that of thymomatous myasthenia gravis in man.

**Experimentally Induced Myasthenia Gravis in Animals:**

If myasthenia gravis is an autoimmune disorder then it should perhaps be possible to induce something like it in experimental animals by immunological techniques. This should mimic:

1. The thymitis, i.e. germinal centres in the thymic medulla,
2. The muscle and thymus myoid cell antibodies, and
3. The characteristic type of neuromuscular block.

The literature on the experimental production of myasthenia gravis is conflicting, but a positive finding is likely to be more significant than a negative one, particularly when immunisation procedures are involved. Goldstein and Whittingham (1966, 1967) and Kalden et al (1969) have shown that it is possible to produce an experimental model of myasthenia gravis in guinea pigs or rats by immunising the animals with skeletal or thymic extracts together with Freund's adjuvant via the foot pads. In the thymus of the normal guinea pig there is an even distribution of lymphocytes throughout the medulla. The majority of animals immunised with skeletal muscle or thymus showed a marked infiltration of lymphocytes around the Hassall's corpuscles in the thymus. There was no change in the weight of the thymus or in the other histological components. This appearance in the thymus is referred to as a "thymitis" as it does seem to be due to an immune reaction against an antigenic component of the thymus.

As demonstrated by the indirect immunofluorescence technique, the immunised guinea pigs produced circulating antibody reactive with skeletal and cardiac muscle striations and with myoid cells in the thymus. The control animals injected with lymph node extract or with saline in
Freund's adjuvant did not produce these antibodies.

Evidence for a defect in neuromuscular transmission was looked for in these animals comparable to that found in myasthenia gravis using the techniques of electromyography. The behaviour of the first 10 muscle action potentials during tetanic supramaximal nerve stimulation was used to study the neuromuscular transmission in the control animals and in the animals immunised with thymus or skeletal muscle (Fig. 1). In the control animals, the height of the amplitudes of the first 10 action potentials normally showed a slight increase or remained constant. In a significant number of the immunised animals, there was a rapid decline in the successive muscle responses similar to that described in patients with myasthenia gravis. Five to 10 minutes after the injection of Tensilon, a significant improvement in the muscle tetanus pattern was recorded in all guinea pigs with a partial neuromuscular block. In the majority, the pattern returned to normal.

The development of this partial neuromuscular block was found to be thymus-dependent. Animals that had been thymectomised did not develop neuromuscular block on immunisation with thymus or skeletal muscle. Furthermore, in animals in which a partial neuromuscular block had been induced, the tetanus pattern returned to normal within 3 to 4 days of thymectomy, as opposed to 7 to 10 days without thymectomy (Kalden et al, 1970). All the animals showing partial neuromuscular block had histological evidence of thymitis. The production of thymitis does seem to be an essential step in inducing neuromuscular block by immunological means. Some recent publications (Vetters, Simpson and Folkarde, 1969; Kaufman, Rushworth and Wright, 1969) deny that a myasthenia-like syndrome can be induced in experimental animals by immunological techniques. However, these papers do not give a histological description of thymitis. According to the hypothesis discussed above, a partial neuromuscular block would not be expected in the absence of experimental
thymitis. A further positive finding was that of Goldstein and Hofman (1968) who showed that the miniature end-plate potentials were significantly reduced in appropriately immunised animals in contrast to the controls, again in keeping with the findings in human myasthenia gravis.

Several workers have shown that thymic extracts may have a blocking effect on neuromuscular transmission. Recently, Goldstein and Hofman (1969) have given further support to the concept that the thymus may secrete a substance, thymin, which affects neuromuscular transmission. This substance may be released in excess following severe histological lesions in the thymus, such as may occur in myasthenia gravis in man or be induced experimentally by immunisation in animals. There is no evidence that this neuromuscular blocking substance is an antibody.

THYMECTOMY IN PATIENTS WITH AUTOIMMUNE DISEASE

The only human autoimmune disease for which thymectomy is regularly performed is myasthenia gravis. It has its best effects in females with non-thymomatous myasthenia gravis, particularly when the pre-operative duration of the disease has been short (Keynes, 1954; Simpson, 1958). This would be compatible with the experimental studies described above.

Thymectomy has also been successful in two infants with intractable autoimmune haemolytic anaemia (Wilmers and Russell, 1963; Karaklis et al, 1964). Apart from these examples, thymectomy has not produced beneficial results in relation to autoimmunity in adults, when it has been tried for example in systemic lupus erythematosus (Mackay and Smalley, 1966). Thymectomy as a treatment for autoimmune disease, other than myasthenia gravis, may be effective only in childhood when the influence of the thymus on the immunological system is particularly pertinent.

In conclusion, we are clearly a long way from fully understanding the role of the thymus in relation to autoimmunity. I have tried to describe and to interpret the facts that have emerged so far.
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continued:


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ADRENALITIS, HYPOPARATHYROIDISM AND ASSOCIATED DISEASES

W. J. Irvine

(in press)
IDIOPATHIC ADRENAL INSUFFICIENCY

The main clinical features of chronic adrenal insufficiency in man are malaise, tiredness, loss of weight, pigmentation of the skin and sometimes of the buccal mucosa, and hypotension followed in the acute form by abdominal pain and vomiting as the patient goes into adrenal crisis prior to death. Objective confirmation of the diagnosis may be obtained by measuring the level of 11-hydroxycorticosteroids in the plasma by the fluorimetric method of Mattingly (34) and determining the response in this level to adequate stimulation of the adrenals with natural or synthetic corticotrophin. While tuberculous destruction of the adrenals used to be the commonest cause of adrenal failure, this is no longer so in Western countries. The majority of cases are now due to what was variously referred to as simple adrenal atrophy, idiopathic Addison's disease or what might best be called chronic autoimmune adrenalitis.

A diagnosis of simple adrenal atrophy had hitherto been made by the process of exclusion. If the patient has radiological calcification of the adrenal glands, it may be assumed that the aetiology is other than idiopathic. However, only a proportion of patients with tuberculous adrenal glands show adrenal calcification. In the absence of calcification, it would be reasonable to assume a tuberculous aetiology if the patient had evidence of gross tuberculous infection elsewhere in the body either preceding or coincident with the diagnosis of adrenal insufficiency. Other causes of primary adrenal failure include metastatic tumour, haemorrhage, infarction, mycotic infections and amyloidosis. The sex incidence of idiopathic Addison's disease is predominantly female, while in cases of tuberculous adrenal failure the sex ratio is approximately equal (Table I).

The histopathology of simple adrenal atrophy (idiopathic Addison's disease) is illustrated in Fig. 1. In place of the normal three-layered architecture of the adrenal cortex (zona glomerulosa, fasciculata and reticularis), the adrenal cortical cells are reduced to islets and the
Fig. 1. The histology of the adrenal gland of a female patient aged 74 who died with idiopathic adrenocortical insufficiency (Case No. 8 of Irvine et al., ref. 28). Note the disruption of the normal architecture of the adrenal cortex and the lymphocytic infiltration.

H. & E. × 360
Fig. 2. The titration of adrenal antibody in the sera of patients with primary adrenocortical insufficiency using the methods of indirect immunofluorescence and complement fixation. The serum of the patient in the tuberculous group that gave a positive complement fixation test with a saline extract of adrenal stained the capillary and sinusoidal endothelium in the indirect immunofluorescent test, but did not stain the adrenocortical secretory cells.
Fig. 3. Positive immunoflorescence reaction with the secretory cells of the zona glomerulosa and zona fasciculata using the serum of a patient with idiopathic adrenocortical insufficiency and anti IgG-F.I.T.C. conjugate. Dark ground U.V. - blue light x 420.
TABLE 1. AGE AND SEX DISTRIBUTION IN ADDISON'S DISEASE

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Age Mean</th>
<th>Age Range</th>
<th>Sex Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>105</td>
<td>40</td>
<td>5 - 77</td>
<td>2.1 : 1.0</td>
</tr>
<tr>
<td>Tuberculous</td>
<td>35</td>
<td>46</td>
<td>19 - 80</td>
<td>1.2 : 1.0</td>
</tr>
<tr>
<td>Metastatic</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

stroma is increased by fibrous tissue and lymphocytic infiltration. While the adrenal glands are generally greatly reduced in size, this may not necessarily be so on account of the intensity of the lymphocytic infiltration (28).

While adrenalitis and adrenal antibodies have been produced in experimental animals following injection of adrenal tissue homogenate plus Freund's adjuvant, the results have been variable. In 1958, Colover and Glynn (12) reported round cell invasion of the adrenal cortex and adrenal necrosis in guinea pigs following immunisation with homologous adrenal in Freund's complete adjuvant. The lesions were described as distinctive or "specific", affecting only the adrenal. Similar results with guinea pigs were reported by Steiner et al. (49) and by Barnett, Dumonde and Glynn (3). However, in rabbits immunised with homologous adrenal in adjuvant, no difference in the adrenal lesions was observed when compared to those induced by injection of adjuvant alone (49). Barnett et al. (3) observed adrenal lesions in rabbits injected with heterologous (guinea pig or rat) but not with homologous adrenal tissue. Antibody reacting specifically with guinea pig and rabbit adrenal tissue, including autologous adrenal, was demonstrated by Witebsky and Milgrom (55) in the complement fixation, tanned cell agglutination and precipitation reactions. These antibodies did not cross-react with ovary or testis. Barnett et al. (3) found that rabbit immunised with homologous and heterologous adrenal homogenates in complete adjuvant
TABLE 2. ADRENAL ANTIBODIES IN OTHER CONDITIONS

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. cases</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyrotoxicosis</td>
<td>89</td>
<td>-</td>
</tr>
<tr>
<td>Hashimoto goitre</td>
<td>79</td>
<td>3</td>
</tr>
<tr>
<td>Primary atrophic hypothyroidism</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>Steatorrhoea</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>432</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
responded with autoantibody primarily directed against adrenal by complement fixation. Immunofluorescence tests showed that the antibody reacted not only with the cytoplasm of adrenocortical cells but also with the interstitial cells of ovary and testis and sometimes with spermatozoa and their precursors. These workers observed that the adrenal lesions were more severe and the antibody titres higher if heterologous rather than homologous adrenals were used. The adrenal lesions observed in these experiments consisted of foci of lymphocytes and histiocytes, with smaller numbers of plasma cells and eosinophil leucocytes, and with degenerative changes of the adrenocortical cells in the foci. Usually the lesions were observed to be most numerous and extensive in the deeper layers of the cortex, but similar infiltrates have been described in the medulla and may be related to isolated adrenocortical cells which occur there (49).

Skin tests with homologous adrenal have revealed no evidence of delayed type hypersensitivity in either the guinea pig or the rabbit injected with adrenal homogenate plus adjuvant (3). Levine and Wenk (31) produced allergic adrenalitis in Lewis rats by a single injection of isologous or homologous adrenal tissue aided by adjuvants, the most effective of which was a combination of Freund's complete adjuvant and pertussis vaccine. They showed that adrenalitis could be transferred passively from actively immunised donors to normal recipients by means of living lymph node cells.

The antigenic adrenal constituents in the above experiments have been shown to be heat labile (55, 3) and to be protein in nature (42). Centeno et al. (10) demonstrated by Ouchterlony precipitation techniques that antisera prepared in rabbits by immunisation with rabbit adrenal homogenates contain as many as 3 or 4 distinct antibodies to adrenal extract.

Adrenal Antibodies:

In 1957, Anderson et al. (1) noted that the sera of 2 of 10 patients with Addison's disease gave positive complement fixation tests with saline extracts of human adrenal and of human thyroid. Cross-absorption studies
with the serum of a patient with Hashimoto's disease and clinical adrenocortical insufficiency indicated the separate identity of the complement fixing antibodies reacting with thyroid and adrenal, respectively (35). Adrenal antibodies have been sought using complement fixation and/or the immunofluorescence technique in four large series totalling 235 patients thought during life to have idiopathic Addison's disease (16, 28, 7, 40). A higher incidence of adrenal antibodies was found among the females than the males, which is contrary to what has been observed in most organ-specific autoimmune diseases, such as Hashimoto's disease or pernicious anaemia. When a comparison is made between patients with idiopathic and with tuberculous Addison's disease, it is clear that adrenal antibodies are only associated with the idiopathic type and not with the tuberculous (Fig. 2). The complement fixation titres of adrenal antibodies tend to be lower than those for thyroid and gastric antibodies in thyroid autoimmune disease and in pernicious anaemia, respectively, but as in these other conditions the antibody titres tend to persist for many years following medical replacement therapy. A positive immunofluorescence test for adrenocortical antibody is shown in Fig. 3.

There is a very low incidence of adrenocortical antibodies in the sera of control subjects, patients with other organ-specific autoimmune disorders and in other disease groups so far tested (Table 2).

Adrenals from patients undergoing adrenalectomy for metastatic carcinoma or for Cushing's disease due to adrenal hyperplasia or adrenal glands obtained at early autopsy are all suitable for antigen. The adrenals of small experimental animals are less suitable as they are relatively weak antigenically. The adrenocortical antigen or antigens are particulate components of the cytoplasm, and are found predominantly in the microsomal but also in the mitochondrial fractions of adrenal saline extracts (5, 16, 17).

Nerup et al. (38, 39) applied the leucocyte migration test in a series of patients with Addison's disease, using pooled foetal adrenal extracts
as antigen. The results indicated the existence of a state of organ-specific, antiadrenal hypersensitivity of the cellular type in 8:11 males and in 6:19 females with idiopathic Addison's disease. The reactivity was not seen in patients with Addison's disease of unquestionably tuberculous origin. A parallel study showed that adrenal extract did not induce blast transformation in lymphocyte culture, as estimated by examination of cell morphology and (14C)-thymidine incorporation. The occurrence of anti-adrenal cellular hypersensitivity in males more frequently than in females is in contrast to the sex distribution of circulating adrenal antibodies in patients with idiopathic Addison's disease (28).

Clinical and Serological Overlap Between Idiopathic Addison's Disease and Other Conditions Associated with Autoimmunity

Idiopathic Addison's disease is an uncommon condition and surveys of even fairly large numbers of cases for autoimmune lesions in tissues other than the adrenal are likely to be biased by case selection. It has been possible to overcome this bias to some extent by the use of antibody tests for the detection of subclinical autoimmune disease and, especially, by comparison with control cases of tuberculous Addison's disease. By this means, it is now evident that patients with idiopathic Addison's disease are remarkably prone to a variety of other autoimmune disorders (16, 28, 7).

Table 3 illustrates the association of clinical diseases in the author's series in patients with primary adrenal insufficiency, and Table 4 summarises the immunological findings in the sera of these patients. The difference in the clinical and immunological findings in patients with idiopathic Addison's disease compared to those with tuberculous adrenal failure is clear. There is no set sequence in which an individual patient may develop associated disorders such as thyroid disease, pernicious anaemia, idiopathic hypoparathyroidism, diabetes mellitus or gonadal failure in relation to his Addison's disease, and the development of these conditions is not dependent on underactivity of the adrenal gland. The various conditions associated with idiopathic Addison's disease are discussed in more detail under separate headings.
**TABLE 3. CLINICAL DISORDERS ASSOCIATED WITH ADDISON'S DISEASE**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Idiopathic</th>
<th>Tuberculous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Hashimoto</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Hypoparathyroidism (primary)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Moniliasis</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gonadal failure</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asthma &amp; Eczema</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>CNS disorder</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Total Affected</strong></td>
<td>54</td>
<td></td>
</tr>
<tr>
<td><strong>Total Patients</strong></td>
<td>105</td>
<td>35</td>
</tr>
</tbody>
</table>
### TABLE 4. CIRCULATING ANTIBODIES IN PATIENTS WITH ADDISON'S DISEASE

<table>
<thead>
<tr>
<th>No. with Antibodies to</th>
<th>Steroid-producing Cells</th>
<th>Stomach</th>
<th>Thyroid</th>
<th>Mitochondria</th>
<th>A.N.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenal</td>
<td>Parietal Cells</td>
<td>Intrinsic Factor</td>
<td>Cytoplasm</td>
<td>Tg 2</td>
</tr>
<tr>
<td>No. Sera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Addison's</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>105</td>
<td>57</td>
<td>13</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>Tuberculous</td>
<td>35</td>
<td>Nil</td>
<td>Nil</td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td>Controls</td>
<td>140</td>
<td>1</td>
<td>Nil</td>
<td>13</td>
<td>Nil</td>
</tr>
</tbody>
</table>

1. Intrinsic factor antibody I determined by radioimmunoassay using albumin coated charcoal.

2. Tanned cell titre ≥1:25.

Other antibodies detected by indirect immunofluorescence tests.
Thyroid Diseases

Table 3 illustrates that the whole spectrum of thyroid autoimmune disease (thyrotoxicosis, Hashimoto goitre and primary atrophic hypothyroidism) has a remarkably high incidence in patients with idiopathic as opposed to tuberculous Addison's disease, the diagnosis of thyroid function being established in the majority of cases by modern techniques including P.B.I. and radio-iodine studies.

In 1926, Schmidt (41) described two patients with non-tuberculous Addison's disease and chronic lymphocytic thyroiditis. Although neither of these two patients had clinical signs of hypothyroidism, the coexistence of adrenal and thyroid insufficiency has come to be known as Schmidt's syndrome. Since then, there have been a number of reports of this biglandular deficiency, reviewed by Carpenter et al. (9). Brenner (8) and Wells (53) drew attention to pathological changes in the thyroid gland in cases of idiopathic Addison's disease - namely diffuse lymphocytic infiltration, formation of lymphoid follicles, loss of many of the thyroid acini and eosinophilia of the epithelium of the surviving acini. These changes, which appear similar to but less pronounced than Hashimoto's disease, were infrequent and slight in tuberculous Addison's disease. Similar findings are reported by Sloper (44). It is clear that the incidence of subclinical chronic thyroiditis is much higher than that of overt thyroid disease, as one would expect in the case of such an organ as the thyroid with its normally large reserve of function. The incidence of subclinical thyroiditis in idiopathic Addison's disease is reflected in the occurrence of thyroid cytoplasmic antibodies in contrast to control subjects matched for age and sex and to patients with tuberculous destruction of the adrenal (Table 4).

Retrospective studies on the incidence of thyroid disorders in Addison's disease are likely to give misleadingly low figures particularly if the distinction is not made between idiopathic and tuberculous Addison's when the case series extends back to the days when tuberculosis was
common. Thus in a large retrospective series (14, 15) consisting of 538 cases diagnosed as having Addison's disease between 1913-1958, myxoedema was described in only 2%, thyrotoxicosis in only 3%, and 7% had thyroid glands that were abnormal on palpation with lymphocytic thyroiditis being established in the two cases in whom tissue was obtained. It is probable that much higher figures would have been obtained if the incidence of thyroid disease in patients with idiopathic Addison's disease had been studied and if it had been possible to apply modern diagnostic methods to such a series.

Pernicious Anaemia:

Pernicious anaemia occurring in a patient with Addison's disease has been only infrequently described in the literature to date. Meechan and Jones (36) refer to 6 cases in the literature and added a further case of their own. Another case has been described by Turkington and Lebovitz (50). However, the careful analysis of a large series of patients with Addison's disease makes it clear that the association of pernicious anaemia with idiopathic Addison's disease is not a rare one (Table 3). No fewer than 9 out of 105 patients with idiopathic Addison's disease also had pernicious anaemia with malabsorption of vitamin $B_12$ and lack of intrinsic factor secretion measured by direct immunoassay or by the correction of the Schilling test with oral intrinsic factor. There is also a substantial incidence of subclinical atrophic gastritis as revealed by gastric analysis and gastric biopsy obtained by Crosby capsule in patients with idiopathic Addison's disease (28) (Table 5). While it is known that corticosteroids in pharmacological doses may affect gastric acid secretion (29, 51), the incidence of achlorhydria and of pernicious anaemia in patients with Addison's disease can scarcely be accounted for by a transitory lack of cortisol as the achlorhydria may be present after many years of replacement therapy with cortisone and fludrocortisone. Moreover, the pernicious anaemia may precede or follow the adrenal insufficiency. Again pernicious anaemia has not been reported in unequivocal tuberculous Addison's disease.
The association between atrophic gastritis and idiopathic Addison's disease is further substantiated by the increased incidence of gastric parietal cell and intrinsic factor antibodies in the sera of patients with idiopathic Addison's disease (Table 4). As described by Jeffries (see Chapter **), the occurrence of parietal cell antibodies correlates with some degree of atrophic gastritis while intrinsic factor antibodies in the serum show in general a strong correlation with malabsorption of vitamin $B_{12}$ of the pernicious anaemia type. The sera of patients with idiopathic Addison's disease contain an unusually high incidence of intrinsic factor antibodies even in patients who have as yet not progressed to overt pernicious anaemia, although all those so far tested have severe gastric atrophy. The statement that achlorhydria in patients with Addison's disease is reversible after therapy (46) is often quoted, but its validity is highly doubtful.

**Idiopathic Hypoparathyroidism**

This condition is considered in greater detail at the end of this Chapter.

**Diabetes Mellitus**

Beavan et al. (4) reviewed 55 cases in the literature of diabetes mellitus and Addison's disease and added a further 8. They noted that the majority of these patients had idiopathic rather than tuberculous adrenal insufficiency. Sorkin (47) reported 14 autopsy cases of combined Addison's...
disease and diabetes, 12 of whom had idiopathic adrenal failure and in only 2 was the adrenal failure tuberculous. Of 10 patients with idiopathic Addison's disease and diabetes in Table 2, 9 had diabetes of the insulin-dependent type. This is of some interest since, although adrenal antibodies are rare in patients with diabetes mellitus, it has been shown that in a series of 883 diabetics of approximately even age and sex distribution there is an increased incidence (p <0.005) of thyroid cytoplasmic and gastric parietal cell antibodies in the diabetics compared to controls, particularly in young diabetics of the insulin-dependent type (26). Older diabetics had an increased incidence of antibody to intrinsic factor. As discussed by * * * * in Chapter ***, there does seem to be some disturbance of the immunological system in diabetes with a propensity towards organ-specific autoimmunity. Autoimmune mechanisms of a type that have not yet been clearly elucidated may be the link between diabetes of the insulin-dependent type and idiopathic adrenal insufficiency.

**Gonadal Failure:**

Turkington and Lebovitz (50) drew attention to the high incidence of premature ovarian failure in patients with Addison's disease. Of 32 patients with Addison's disease who had been adequately followed, 7 or nearly 23% had evidence of gonadal failure. That gonadal failure in relation to Addison's disease is characteristic of idiopathic Addison's is illustrated in Table 3. Other cases have been previously noted (11, 13, 14, 6).

In 1968 Anderson et al. (2) described antibodies reacting with theca interna and corpus luteum of ovary, interstitial cells of testis, placental trophoblasts and adrenal cortex in 2 male patients with idiopathic Addison's disease without overt evidence of gonadal insufficiency. Irvine et al. (24) found a strong correlation between the presence of antibodies in the serum reacting with the steroid-producing cells of the gonads and a clinical history of either complete failure of ovarian function or the onset of premature ovarian failure. Only one patient with idiopathic Addison's disease who did not have antibodies reactive with steroid-producing cells of the gonads was
<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Human Adrenal Cortex</th>
<th>Human Placenta</th>
<th>Human Testis</th>
<th>Human/Rabbit Testis</th>
<th>Human Corpus Luteum of Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticularis</td>
<td>++ + + + + + + + + +</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasciculata</td>
<td>+ + + + + + + + + +</td>
<td></td>
<td></td>
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<tr>
<td>Glomerulosa</td>
<td>+ + + + + + + + + +</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Trophoblasts</td>
<td>++ + + + + + + + + +</td>
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<td></td>
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<tr>
<td>Interstitial Cells</td>
<td>+ + + + + + + + + +</td>
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<td></td>
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<tr>
<td>Theca</td>
<td>++ + + + + + + + + +</td>
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<tr>
<td>Confluent</td>
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<tr>
<td>Clumpy</td>
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<td></td>
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</tr>
</tbody>
</table>
known to have ovarian failure. From immunofluorescence patterns and from absorption studies, it is clear that there are a variety of different antibodies reactive with different constituents of steroid producing cells in the gonads but that all these antibodies cross-react with antigens in the adrenal cortex (Table 6).

Fig. 4 shows positive staining in the indirect immunofluorescence test of human corpus luteum and the surrounding cells while staining of the cells of a human Graafian follicle is shown in Fig. 5 with serum from a patient with Addison's disease and gonadal failure. The sera of the majority of the adrenal antibody positive patients with idiopathic Addison's disease react with all three layers of the adrenal cortex in the immunofluorescence test. However, some sera of patients with gonadal failure and Addison's disease show adrenal reactivity only with the zona glomerulosa and fasciculata, while others show it only with respect to the deeper layers of the zona fasciculata and the zona reticularis, using contiguous sections of the same adrenal as antigen (25, 23). The correlation between the presence of antibodies in the serum reacting with steroid-producing cells in the gonads and the clinical history of gonadal function in patients with Addison's disease is shown in Table 7. The occurrence of other clinical conditions in those patients is shown in Table 8.

An ovarian biopsy from one patient with Addison's disease and premature ovarian failure showed the presence of numerous primordial follicles. As the follicles enlarged, there was a progressively severe lymphocytic and plasma cell in infiltration and destruction of the follicle (24, 23). Immunisation of experimental animals with adrenal extracts has not produced unequivocal lesions of the ovaries or testes, although Barnett et al. (3) described the formation of antibodies reactive with ovary and testis after such a procedure.

**Neurological Disorders**

In this series there was a remarkable incidence of neurological disorders among the patients with idiopathic adrenal atrophy (Table 3). These
Fig. 4. Positive immunofluorescence reaction with human corpus luteum given by the serum of a patient with idiopathic adrenocortical insufficiency who was also suffering from premature gonadal failure. The fluorescence is in a clumpy pattern diffusely throughout the corpus luteum. In addition there is strong immunofluorescent staining of the theca cells in the trabeculae. Dark ground U. V. - blue light x 250
(From Irvine et al., ref. 25 by kind permission of the editor of Clin. exper. Immunol.)
Fig. 5. Positive immunofluorescence reaction with a Graafian follicle of human ovary using the serum of a patient with idiopathic adrenocortical insufficiency and premature ovarian failure.

Dark ground U. V. - blue light x 260.

(From Irvine et al., ref. 24 by kind permission of the editor of the Lancet.)
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</tr>
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</tr>
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TABLE 8. ASSOCIATED CLINICAL DISORDERS IN PATIENTS WITH STEROID CELL ANTIBODIES

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<tr>
<th>Age &amp; Sex</th>
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<th>Pernicious anaemia</th>
<th>Hypoparathyroidism</th>
<th>Diabetes Mellitus</th>
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consisted of Schilder's disease, spastic paraplegia, two cases of idiopathic epilepsy, suspected disseminated sclerosis and mental retardation. Blizzard and Kyle (5) refer to a case of Schilder's disease associated with idiopathic adrenal insufficiency. It is of interest that autoimmune phenomena may be present in demyelinating conditions (30).

Idiopathic Hypoparathyroidism

Idiopathic Addison's disease, thyroid disease and pernicious anaemia frequently occur in patients with idiopathic hypoparathyroidism (37, 20, 6). Table 3 also shows that idiopathic hypoparathyroidism is a common occurrence among patients with idiopathic Addison's disease. The histology of idiopathic hypoparathyroidism is characterised by atrophy and lymphocytic infiltration as illustrated in Fig. 8. Lupulescu et al. (33) have demonstrated that repeated inoculation of homologous parathyroid tissue into dogs may induce isoimmune hypoparathyroidism with the characteristic biochemical and histopathological features and the presence of complement-fixing parathyroid antibodies in low titres in the serum. The isoimmune hypoparathyroidism in dogs was similar to that previously described in rats (32).

The demonstration of antibodies specific for the parathyroid in the sera of patients with idiopathic hypoparathyroidism has only recently been reported from two laboratories (6, 27), although a number of laboratories have been unable to convincingly demonstrate such antibodies using either the immunofluorescence or complement fixation method. Blizzard et al. (6) reported finding parathyroid antibodies by immunofluorescence in 38% of 74 patients with idiopathic hypoparathyroidism, 26% of 92 patients with idiopathic Addison's disease, 12% of 49 patients with Hashimoto thyroiditis, and in 6% of 245 control patients. They showed that antibody(s) against parathyroid tissue are not reactive with parathyroid hormone and do not cross-react in absorption studies with gastric, thyroid, adrenal, liver or kidney tissues. Normal parathyroids were suitable for detecting the anti-
Fig. 6. The histology of the parathyroid in a case of idiopathic hypoparathyroidism showing foci of lymphocytic infiltration.

H. E. x 180.

(This photograph was generously provided by Dr W. G. R. M. de Boer of Monash University, Melbourne.)
body but they found that not all parathyroid adenomas were suitable. Irvine et al. (27) noted that fresh postmortem human parathyroid tissue could be used, but out of a limited series they only found one serum from a patient with idiopathic hypoparathyroidism (who also had idiopathic Addison's disease and ovarian failure) that was unequivocally positive for parathyroid antibody. This serum stained both the oxyphil and the chief cells of the parathyroid. Control sera were negative. Blizzard et al. (6) found that patients with idiopathic hypoparathyroidism had a raised incidence of gastric, thyroid and adrenal antibodies even excluding patients known to have pernicious anaemia, thyroid disease or adrenal insufficiency. This is in keeping with idiopathic hypoparathyroidism belonging to the club of organ specific autoimmune diseases discussed below.

Mechanisms of Autoimmunity in Addison's Disease

Why thyroid disease, pernicious anaemia and diabetes mellitus of the insulin-dependent type should be so common among patients with idiopathic Addison's disease and yet idiopathic Addison's should be so rare among patients with these other diseases remains an enigma. It appears that if an individual is making autoantibodies to adrenal cortex, he stands a high chance of making antibodies to thyroid, gastric parietal cells, intrinsic factor, parathyroid and to steroid-producing cells in the gonads; whereas if an individual is making autoantibodies to thyroid cytoplasm, he stands a fair chance of making autoantibodies to gastric parietal cells but little chance of making autoantibodies to adrenal, parathyroid or to other steroid-producing cells. Incidentally, the 3 patients with Hashimoto (lymphadenoid) goitre shown in Table 2 who had adrenal antibodies in the serum had no clinical evidence of Addison's disease and all had a good reserve of adrenocortical function as determined by a satisfactory rise in the plasma 11-hydroxycorticosteroid levels following stimulation with synthetic ACTH. Blizzard et al. (6) noted that 4 of their patients with idiopathic hypoparathyroidism in whom antibodies to adrenal had been previously demonstrated went on to develop unequivocal Addison's disease. The patient with steatorrhea in
Table 2 who had adrenal antibodies in the serum was subsequently shown to have primary adrenal failure (18).

The difference in the incidence of clinically associated disease and of autoantibodies in patients with idiopathic as opposed to tuberculous Addison's is striking. The occurrence of autoimmunity in the idiopathic type cannot therefore be due to adrenal insufficiency per se. It is clear the destruction of the adrenal is by itself not an adequate stimulus to account for the continued production of adrenal autoantibodies. The remarkable clinical and immunological association between idiopathic Addison's disease and other diseases that are characterised by autoimmunity would indicate that idiopathic Addison's disease belongs to a group or club of disorders in which organ-specific autoimmunity is a distinctive feature. The basic abnormality would appear to be in relation to antigen recognition by the immunological system rather than some mutation or alteration in the tissue constituents themselves to make them antigenic. The immunological disorder could be described as a disorder of immunological tolerance which is characterised by the formation of autoantibodies that are predominately tissue-specific.

Circulating antibodies themselves would appear not to be detrimental to the adrenal insofar that, as already mentioned, 3 patients have been described with adrenal autoantibody in the serum and yet have a perfectly normal reserve of adrenocortical function. Secondly, adrenocortical antibodies, being IgG, can pass the placental barrier and enter the foetal circulation and yet overtly normal babies have been born of mothers who have idiopathic Addison's disease with high titres of adrenocortical antibody detectable in the mother's serum and in serum obtained from umbilical cord blood. There has been no evidence of any impaired function of the babies' adrenal glands. Presumably if immunological mechanisms are of importance in the aetiology of the idiopathic Addison's disease, they operate through the mechanisms of delayed hypersensitivity or of synergism between delayed
hypersensitivity and circulating antibody. This probably also holds true for the related conditions of idiopathic hypoparathyroidism and the autoimmune forms of premature gonadal failure.

Sloan (43) described germinal centre formation in the thymic medulla in 3 of 7 patients with Addison's disease; one had a thymoma. The significance of this in relation to the cause of disturbed immunological tolerance or autoantibody formation is obscure (21).

**Genetic Factors in Idiopathic Addison's Disease**

The combination of idiopathic Addison's disease with hypothyroidism (Schmidt's syndrome) is sometimes familial and is thought to be an autosomal recessive character (9, 4). Likewise, idiopathic Addison's disease alone, idiopathic hypoparathyroidism alone and the simultaneous occurrence of these two conditions have been described as familial and their genetic origin as autosomal recessive characters has been suggested (54). Idiopathic Addison's disease has been described in two pairs of identical twins (45, 19). It is not clear whether the association of various disorders in patients with idiopathic Addison's disease could be caused by mutant genes at a single locus or by genes at different loci, nor is there any indication as to how many alleles might exist in either case. Spinner et al. (48) made an analysis of the clinical history from 140 families containing patients with idiopathic Addison's disease without idiopathic hypoparathyroidism, Addison's disease with hypoparathyroidism, and hypoparathyroidism alone. They demonstrated a significantly greater similarity of clinical and other attributes among affected persons within the families than among unrelated persons. Some cases were apparently genetically determined and the distribution of affected persons within the family was compatible with an autosomal recessive pattern. Other cases did not fit any mendelian pattern even although their families contained more than one affected person. Clearer information is likely to be obtained when antibody studies are done on pedigrees of propositi with idiopathic Addison's disease preferably including
three generations and a goodly number of siblings.

**Cushing's Syndrome**

The finding of adrenocortical antibody in the serum of a patient with bilateral adrenal hyperplasia together with lymphocytic infiltration in the adrenal glands (52) suggests that adrenal specific antibody may be associated with hyper- as well as hypo-function of the adrenal cortex in a manner comparable to thyroid autoimmunity in thyrotoxicosis. The only other reported incidence of adrenocortical antibody in Cushing's syndrome is one out of a series of 24 patients studied by the author (24, 25, 23), but this patient was shown to have an adenoma of the adrenal cortex. The significance of the uncommon occurrence of adrenal antibodies in Cushing's syndrome is obscure, but the possibility exists that there might be in a minority of cases a factor comparable to the long acting thyroid stimulating immunoglobulin of thyrotoxicosis.
REFERENCES


AUTOIMMUNE OVARIAN FAILURE

W. J. Irvine

(1970) in 'Reproductive Endocrinology',
Symposium of Endocrine Section of
AUTOIMMUNE OVARIAN FAILURE

W. J. Irvine

Department of Endocrinology, The Royal Infirmary; M.R.C. Clinical Endocrinology Unit, Edinburgh

It is now well recognized that primary adrenal atrophy (Addison's disease) of idiopathic type is associated with the occurrence of autoantibodies specific for the adrenal cortex and that this condition belongs to a group of disorders that are characterized by the occurrence of organ-specific autoantibodies (Goudie et al., 1966; Nerup et al., 1966; Blizzard et al., 1967; Irvine et al., 1967). Anderson et al. (1968) first described the occurrence of an antibody in two patients with Addison's disease that reacted not only with adrenal cortex but also with steroid-producing cells in other tissues. Turkington and Lebowitz (1967) have emphasized the clinical association between Addison's disease and ovarian failure. In the present paper the results of a study of the clinical and immunological relationship between idiopathic adrenal failure and premature gonadal failure with particular reference to the ovary are briefly summarized (Irvine et al., 1968; Irvine et al., 1969).

With the help of many colleagues I have been able to study 140 patients with Addison's disease. One hundred and five of these were considered to have idiopathic Addison's disease while in 35 there was good evidence that the adrenal destruction was the result of tuberculosis (Table I). The patients with idiopathic Addison's disease had a remarkably high incidence of thyroid autoimmune disease (thyrotoxicosis, primary atrophic hypothyroidism, Hashimoto goitre), Addisonian pernicious anaemia, idiopathic hypoparathyroidism, insulin-dependent diabetes mellitus and idiopathic gonadal failure. Taken together, 56 of these disorders occurred in 44 of the 105 patients with idiopathic Addison's disease, while none of these conditions was recorded in the patients with tuberculous Addison's disease. The association of each of these conditions with organ-specific autoimmunity is well documented (Doniach and Roitt, 1968;
Fig. 1. Positive test for antibody to adrenocortical secretory cells in the indirect immunofluorescence technique using the serum of a patient with idiopathic Addison’s disease and anti-IgG-FITC conjugate. (from Irvine, 1968). Dark ground UV-blue light × 252

Fig. 2. Positive test for antibody to Graafian follicle of human ovary in the indirect immunofluorescence technique using the serum of a patient with idiopathic Addison’s disease and premature ovarian failure and anti-IgG-FITC conjugate (from Irvine et al., 1968). Dark ground UV-blue light × 144

To face page 106
Fig. 3. Positive immunofluorescence staining of human corpus luteum given by the serum of a patient with idiopathic Addison’s disease and premature ovarian failure, showing a plumpy pattern diffusely throughout the corpus luteum and brilliant staining of the theca cells in the trabeculae. (from Irvine et al., 1969) Dark ground UV-blue light x 150

Fig. 4. Positive staining in the indirect immunofluorescence technique of the interstitial cells and of the spermatids in rabbit-testis given by the serum of Dr. Kolb’s patient (p. 116). (from Irvine et al., 1969) Dark ground UV-blue light x 162

**Table I**

*Clinical disorders associated with Addison's disease*

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<th>Disorder</th>
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<tr>
<td>Gonadal failure</td>
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<td>Total patients</td>
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*Antibodies to adrenal and to other steroid-producing cells*

Table II summarizes the incidence of IgG antibodies in the sera of patients with Addison's disease that react with steroid-producing cells in the endocrine glands of reproduction (ovary, testis or placenta) and those that react with the cells of the adrenal cortex. Thirteen of the 105 patients with idiopathic Addison's disease had antibodies reacting with steroid-producing cells in tissues other than the adrenal and 57 had antibodies that reacted with adrenal cortex. These antibodies did not occur in the tuberculous group. One out of 24 patients with over-activity of the adrenal (Cushing's syndrome) had steroid cell antibody, and one out of 164 control subjects matched for age and sex had antibody specific for the adrenal cortex only.

Figure 1 shows the immunofluorescent staining of the adrenal cortex in the indirect method with the serum of a patient with idiopathic Addison's disease. The serum stained the glomerulosa, fasciculata and reticularis zones in the adrenal cortex and did not cross-react with steroid-producing cells in other tissues. This is the commonest immunofluorescence reaction with regard to steroid-producing cells in patients with idiopathic Addison's disease.
Table II

Steroid cell antibodies in Addison's disease and Cushing's syndrome

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<th>Number of patients</th>
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* Antibodies reactive with extra-adrenal steroid cells are referred to as steroid cell antibodies.

The 13 sera from patients with Addison's disease reacting with steroid-producing cells in tissues other than the adrenal gave a variety of immunofluorescence staining patterns described briefly below. For example, certain of these sera reacted with the theca cells of the Graafian follicles in human ovary (Fig. 2) while others of the same sera reacted with human corpus luteum (Fig. 3). The reaction with human corpus luteum showed four different patterns, summarized in Table III. The corpus luteal cells showed either a clumpy staining pattern throughout the corpus luteum or a confluent staining pattern so that the cell boundaries were poorly defined. Other sera gave patchy immunofluorescent staining of the corpus luteum, only certain cells in the corpus luteum combining with the antibody in the patient's serum. The same or other sera stained the theca interna cells surrounding the corpus luteum and in relation to the trabeculae (Fig. 3). Rarely, the sera of patients with idiopathic Addison's disease and idiopathic gonadal failure may give a positive immunofluorescence reaction with rabbit or human ova (Irvine et al., 1968, 1969). Such a patient is described by Kolb et al. (p. 116).

Two staining patterns were noted with testis sections, either with human or rabbit tissue. The commonest is staining of the interstitial cells, which is particularly easy to detect using rabbit-testis. Two of the three sera that reacted with ova also showed positive reactions...
with spermatids in the tissue sections of testis. Figure 4 shows immunofluorescence staining of the testicular interstitial cells and spermatids given by the serum of Dr Kolb’s patient. The sera from a proportion of patients with idiopathic Addison’s disease and idiopathic gonadal failure also reacted in the indirect immunofluorescence technique with the trophoblast cells of human placenta.

The positive tests with adrenal, ovary, testis and placenta obtained by the immunofluorescence method can be confirmed by complement fixation. There is no clear pattern of positive reactions with the different steroid-producing tissues. That is to say, some sera may give a positive reaction with corpus luteum or only with the theca cells of the Graafian follicles and the same serum may or may not give a positive reaction with the interstitial cells of testis or with the trophoblasts of placenta. However, sera that react with any of the steroid-producing cells in either the ovary, testis or placenta all react with the adrenal cortex. On this evidence alone it can be inferred that there must be a multiplicity of antibodies reacting with steroid-producing cells and varying in their specificity, but that all the antigens are represented in the adrenal cortex. The exception to this is the rare occurrence of antibodies to ova and to spermatids.

Table III contrasts the immunofluorescence staining patterns obtained using sections of human corpus luteum and sections of human adrenal cortex as antigen. The reactions with corpus luteum are subdivided according to whether the pattern of immunofluorescence was clumpy, confluent, patchy or involving the theca cells surrounding the corpus luteum. The reaction with the adrenal cortex is subdivided according to whether the glomerulosa, fasciculata or reticularis zones were stained. In keeping with the statement made above, all sera positive with corpus luteum were positive with adrenal cortex but some sera only reacted with certain zones of the adrenal cortex. Thus a serum may give confluent staining of the corpus luteum without staining the zona reticularis in the adrenal; the serum of another patient may give patchy luteal staining and theca staining in the ovary without any reaction with the zona glomerulosa in the adrenal. Invariably, however, the zona fasciculata was stained indicating that all antigens in the corpus luteum involved in clumpy, confluent, patchy or theca reactions are probably also represented in the zona fasciculata of the adrenal.

Absorption studies have also emphasized that the antigens of the
steroid-producing cells in the gonads and placenta are represented in the adrenal cortex. Thus, adrenal extracts are capable of absorbing the different antibodies reacting with the corpus luteum, with cells of the Graafian follicles, with placental trophoblasts or with interstitial

<table>
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<th>Corpus Luteum of Ovary</th>
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<td>Theca</td>
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Menstrual history and gonadal antibodies

The menstrual histories of the 14 patients with steroid cell antibody in the serum are summarized in Table IV. There are 13 patients with Addison’s disease and one patient with Cushing’s syndrome. The age of the patients is shown in chronological order. The menstrual history of the younger females up to age 43 is of particular interest. At age 12 it is too early to make any comment. Of the three girls aged 20, one had normal menstruation while two had never menstruated. The four women aged 28 to 43 at the time of study had all started menstruating at the normal time and two had had normal
### Table IV

**Gonadal function in patients with steroid cell antibodies (from Irvine, 1970)**

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</tr>
</tbody>
</table>
pregnancies, but in every case the periods had stopped spontaneously between the ages of 18 to 26 and had not recurred during the past 8 to 25 years. Such complete amenorrhoea from such an early age and lasting so long would be a very rare occurrence in the general female population and is highly significant in the present context.

In older women (aged 51 to 72) in whom steroid cell antibodies reacting with ovary were detected, menstruation had continued up until the age of at least 40.

The boy of 16 with Addison’s disease and antibodies reacting with

**Table V**

*Associated clinical disorders in patients with steroid cell antibodies (from Irvine, 1970)*

<table>
<thead>
<tr>
<th>Age &amp; sex</th>
<th>Thyroid disorder</th>
<th>Pernicious anaemia</th>
<th>Hypoparathyroidism</th>
<th>Diabetes mellitus</th>
</tr>
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<tbody>
<tr>
<td>12</td>
<td>hypo</td>
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<td>✓</td>
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</tr>
<tr>
<td>20</td>
<td>toxic</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
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<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

The testis had some clinical signs of hypogonadism but 16 is again rather young to make conclusions about his future development. The Cushing’s patient aged 43 was still menstruating. In summary, although it is not known for how long any patient had had steroid cell antibodies in the serum, the occurrence of these antibodies either before or during reproductive life in the female shows a strong correlation with either complete or premature ovarian failure.

Table V shows the other clinical disorders in addition to idiopathic Addison’s disease and Cushing’s syndrome that occurred in the patients with steroid cell antibodies in the serum. There is a remark-
able concentration of these associated clinical disorders in the younger patients; notably idiopathic hypoparathyroidism. These younger patients would appear to have a particularly strong propensity towards autoimmunity.

**Ovarian histology**

Histology of the ovary was available in two cases with Addison’s disease and gonadal failure.

The first case is the patient described by Dr Felix Kolb in this symposium (p. 116), who had idiopathic hypoparathyroidism and Addison’s disease and failure of menstruation with streak ovaries. No chromosomal abnormalities could be detected. The patient had antibodies to ova and to the theca cells of the Graafian follicles. The possibility exists that the total failure of her ovaries to develop may be explained on an immunological basis.

The second patient is a 26-year-old female, who was studied at the Royal Post-Graduate Hospital, London. She developed idiopathic Addison’s disease at 13 years, normal menarche at 14 years and her periods stopped permanently at 18 years. Steroid cell antibodies were detected at age 26. Since the patient was particularly anxious to know the prognosis with regard to her fertility, laparoscopy was done. The ovaries were noted to be of normal size on ovarian biopsy. Numerous primordial follicles were present but as the follicles attempted to develop, they failed to do so and were apparently destroyed, with evidence of plasma cell and lymphocytic infiltration. This patient’s serum did not react with her own primordial follicles but it did react with follicles that were beginning to develop. It is tempting to postulate that the reason for the failure of the follicles to develop was due to an immunological reaction mounted against them.

**Gonadal failure not associated with Addison’s disease**

The sera of 37 patients with cessation of menstruation persisting for more than one year and of unexplained origin in women younger than 35 years and of nine women older than 18 years with total failure of menstruation and no menarche have been studied. None of these patients had Addison’s disease and their sera were all negative for steroid cell antibodies. Likewise the sera of 50 normally menopausal women were found to be uniformly negative for steroid cell antibodies.
It would therefore appear that autoimmunity as a possible cause of ovarian failure is only likely to be relevant in the rather few cases where this occurs in conjunction with primary autoimmune adrenal insufficiency.

SUMMARY
There is a significant incidence of premature ovarian failure in patients with idiopathic Addison’s disease. Both conditions may have an autoimmune basis. The reason for their association seems to be that there is a sharing of antigens between the adrenal and the gonads. While the adrenal is likely to be affected if autoimmunity develops to any of the antigens in the steroid-producing cells, the ovary and the testis may or may not be implicated according to exactly what steroid cell antigens are involved.

REFERENCES

DISCUSSION
Hall: Did you study the sera of the parents of any of the patients with steroid cell antibodies?
Irvine: A few have been studied but not a sufficient number to make any valid comment. I would expect there to be more evidence of autoimmunity in these patients’ parents than in the general population.
DISCUSSION

Kolb: K. D. Wuepper, L. C. Wegienka and H. Fudenberg (Am. J. Med. 1969. 46, 206) have reported an increased incidence of antibodies in the relatives of patients with idiopathic Addison’s disease.

Irvine: As in our own studies, the family data described by Wuepper et al. are too limited for statistical appraisal.

Klopper: I wonder if Dr Govan would comment on the ovarian pathology of the last patient you mentioned.

Govan: I think the critical point is whether plasma cells were present in fairly large numbers around the ovarian follicles. Plasma cell reaction does not occur around normal follicles at any time. Occasionally a small granuloma may occur in the cortex of the ovary but this is usually in patients near the menopause. I would like to ask if there was a plasma cell reaction around these follicles. If so, then I think I would accept that this was an auto-immune reaction or likely to be.

Irvine: There were plasma cells in relation to the follicles in this patient. This is an abnormal finding.

Lathe: A number of these tissues have in common the fact that they respond to pituitary trophic hormones. Have you eliminated the possibility that the antibodies interact with the pituitary hormones in the end organs?

Irvine: I agree that when multiple end organ deficiencies occur it is necessary to consider that the primary pathology might be in the pituitary or in the action of the pituitary hormones. However, we have not been able to find any antibodies to growth hormone in these patients, for example. I don’t think your suggestion would explain the variety of antibody patterns that I described to you or the results of the absorption studies. Also, although I have looked for antibodies to pituitary in these patients I have not been able to detect any.

Kolb: The second most common tissue involved is the parathyroid and there is no evidence that there is any parathyrotrophic hormone. This made us think that we are dealing with multiple end organ insufficiency rather than primary pituitary insufficiency.
AUTOIMMUNITY AND ENDOCRINE DISORDERS

W. J. Irvine

(1967) in 'Symposium on Autoimmune Disease'
Practitioner, 199, 180-191
AUTO-IMMUNITY AND ENDOCRINE DISORDERS

By W. J. IRVINE, M.B., M.R.C.P.Ed.
Endocrine Clinic and Department of Therapeutics, Royal Infirmary; Medical Research Council Clinical Endocrinology Research Unit, Edinburgh

Endocrine disease has been the subject of much research with regard to the concept of auto-immunity. Along with auto-immune haemolytic anaemia, myasthenia gravis and pernicious anaemia, certain of the endocrine disorders constitute some of the clearest examples of what might be called auto-immune disease or, more correctly, disorders of immunological aberration. The latter term does not imply that the disease in question is necessarily caused by immunological mechanisms, but indicates that these conditions are closely associated with immunological abnormality.

THYROID DISEASE

Four cases of chronic thyroiditis with goitre were described by Hashimoto in 1912, and it was from his pathological description that chronic goitrous thyroiditis became known as Hashimoto’s disease. Clinically the patient is typically a middle-aged woman with a firm, rubbery, diffuse goitre which may give rise to an aching discomfort in the neck and mild obstructive symptoms. The patient may be euthyroid but is often hypothyroid. Hashimoto thyroiditis is by far the commonest cause of goitre in myxedematous middle-aged women. The histology of Hashimoto thyroiditis (otherwise known as lymphadenoid goitre) is characterized by:

1. Reduction in size of the thyroid vesicles, which contain little or no colloid but which may contain macrophages.
2. Askanazy cell change in the epithelial cytoplasm.
3. Lymphocytic infiltration.
4. Fibrosis.

The relative severity of these features varies in different goitres and often in the same goitre. Primary hypothyroidism without goitre is regarded as an atrophic variant of the same condition.

It is known from clinical observation that thyrotoxicosis is in some way related to Hashimoto goitre and to primary hypothyroidism, as if all three conditions were different manifestations of what was fundamentally the same disease process. Some patients with Hashimoto goitre give a previous history of thyrotoxicosis, whilst in others Hashimoto thyroiditis and thyrotoxicosis coexist. In the older reports, before effective methods of treatment for thyrotoxicosis became available, a proportion of thyrotoxic patients who survived their illness subsequently went on to develop euthyroid goitre and eventually, often over a period of decades, became
hypothyroid. Furthermore, thyrotoxicosis not infrequently features in the family history of a patient with Hashimoto goitre or with primary hypothyroidism. Recent studies on the immunology of thyrotoxicosis go a long way to providing a unitary concept for this group of thyroid disorders. It is now held that there are three thyroid auto-immune diseases: thyrotoxicosis, Hashimoto goitre and primary atrophic hypothyroidism.

THYROID ANTIBODIES

Antibody to thyroglobulin.—Antibody to thyroglobulin was first detected by the agar diffusion or precipitin method (Roitt et al., 1956).

This is a crude method for the detection of large amounts of antibody to thyroglobulin, but it remains a useful test in the differential diagnosis of thyroid disease. A more sensitive and semi-quantitative method for detecting antibody to thyroglobulin is provided by the tanned-cell haemagglutination (TCH) test (Fulthorpe et al., 1961).

A third method of detecting antibody to thyroglobulin, and one which is also highly sensitive, is by the immunofluorescence technique.

Antibody to second component of colloid.—The antigen involved in this reaction is distinct from thyroglobulin in so far as it does not contain iodine. In the fluorescent technique, using fixed sections of thyroid tissue, this antigen-antibody reaction gives a diffuse staining of the colloid.

Thyroid cytoplasmic antibody.—The immunofluorescent method using unfixed sections of human thyrotoxic gland have shown that this antibody is directed against a particulate insoluble component of the cytoplasm of the thyroid secretory cells. Ultracentrifugation studies have shown that the antigen is predominantly in the microsomal fraction.

Thyroid cytoplasmic (microsomal) antibody shows a high degree of species and organ specificity. It can also be detected by the classical immunological technique of complement fixation.

Thyroid-stimulating globulin.—Thyroid-stimulating globulin is a more appropriate name for the substance otherwise known as long-acting thyroid stimulator (L.A.T.S.). The long-acting thyroid stimulator was first recognized by Adams (1961) in New Zealand when he was using a bioassay technique for thyroid stimulating hormone (T.S.H.).

As currently used, this technique depends upon the thyroidal uptake of $^{131}$I given intraperitoneally to mice that have been fed a diet deficient in iodine. The animal’s own T.S.H. secretion is then suppressed by giving thyroxine. Finally, the animal is injected with test or control sera. If the serum contains T.S.H. the $^{131}$I is released from the animal’s thyroid into the blood stream.

With T.S.H. the rise in the level of radioactivity in the blood reaches a maximum at about three hours and the level of radioactivity then falls. The serum of a patient with primary hypothyroidism would give an identical response. The sera of certain patients with thyrotoxicosis, however, give a different type of response, being much more prolonged. The maximum level of blood radioactivity occurs at about twelve hours with thyrotoxic sera in
contrast to the three hours characteristic of T.S.H. (fig. 1). Hence the name: long-acting thyroid stimulator (L.A.T.S.). L.A.T.S. is an immuno-globulin of type IgG (Dorrington and Munro, 1965) as are the other thyroid antibodies already described. L.A.T.S. is not produced in the pituitary and there is some direct evidence to suggest that it is synthesized by the reticuloendothelial system (McKenzie and Gordon, 1965). The levels of L.A.T.S. in the blood fall in response to treatment with steroids.

**INCIDENCE OF THYROID ANTIBODIES IN THYROID DISEASE**

Table I summarizes the incidence of three of the thyroid antibodies in patients with thyroid disease and in the control subjects. The actual figures obtained for the percentage of positive patients depend on the sensitivity of the methods used. With the sensitive technique of immunofluorescence the incidence of thyroid microsomal antibody is about 70 per cent. in thyro-
Auto-immunity and Endocrine Disorders

Auto-immunity and Endocrine Disorders

Toxicosis. The main point is that antibodies to thyroglobulin and to thyroid cytoplasm are a common occurrence in lymphadenoid goitre, primary atrophic hypothyroidism and also in thyrotoxicosis. The incidence of these antibodies in simple goitre and malignant goitre is only slightly higher than in the controls. Thyroid-stimulating globulin (L.A.T.S.) is found exclusively, or virtually exclusively, in thyrotoxicosis. It has been detected in the sera of 80 per cent of patients with thyrotoxicosis when methods of concentrating the sera have been used (Carneiro et al., 1966a).

Thyroid-stimulating globulin (L.A.T.S.) is found exclusively, or virtually exclusively, in thyrotoxicosis. It has been detected in 80 per cent of patients with thyrotoxicosis when methods of concentrating the sera have been used (Carneiro et al., 1966a).

The highest incidence and titres of thyroid-stimulating globulin among patients with thyrotoxicosis occur in those who have large goitres and particularly those who have pretibial myxoedema. The association between exophthalmos and thyroid-stimulating globulin is not clear.

Value of Thyroid Auto-antibodies in Differential Diagnosis

A euthyroid patient with goitre may have a Hashimoto thyroiditis, simple goitre, or malignant goitre. In many cases the diagnosis will be apparent from the clinical features but in others, a definitive diagnosis cannot be reached without further investigation. In these circumstances, a diagnosis of Hashimoto thyroiditis is strongly supported by a positive tanned-cell titre for antibody to thyroglobulin > 1:2,500. Likewise, a positive agar-diffusion test shows a high correlation with Hashimoto thyroiditis, but only about one-half to two-thirds of the patients give a positive reading. The complement-fixation test gives a better correlation with the histology of the thyroid gland, and patients with Hashimoto thyroiditis are more clearly distinguished from those with simple or malignant goitres. A titre of > 1:32 is of clinical significance (Irvine, 1967).

When these three tests are used in combination, the sera of 90 per cent of patients with lymphadenoid goitre will give a significant thyroid complement-fixation titre, a high titre of antibody to thyroglobulin in the tanned-cell test or a positive diffusion test. Equivalent findings by any of these three tests are rare in patients with malignant goitre and in my experience have not occurred in patients with simple goitre. Strongly positive thyroid serology may rarely occur when Hashimoto thyroiditis and thyroid malignancy are both present within the same gland. Hirabayashi and Lindsay (1965) consider that thyroid cancer may induce chronic thyroiditis.

The clinical and serological diagnosis of Hashimoto thyroiditis should be confirmed histologically by needle biopsy of the goitre under local anaesthetic. The patient can be treated either with thyroxine alone or a short 10-day course of prednisolone in a dose of 60 mg./day, followed by thyroxine. So...
far seven patients with Hashimoto thyroiditis with goitres estimated to be greater than 60 grammes in size before treatment have been given such a course of prednisolone. The reduction in size of the goitre is very rapid and is apparent within a period of seven days. The prednisolone is then tailed off and thyroxine started in a dose of 0.2 or 0.3 mg/day. The response to thyroxine alone is often rather slow and may be incomplete and give rise to diagnostic uncertainties. The goitre may tend to increase a little after the course of prednisolone in spite of thyroxine therapy. Partial thyroidectomy is rarely required in patients with Hashimoto goitre.

The incidence of thyroid antibodies in control subjects rises with age and is higher in females than in males. In such subjects the presence of thyroid cytoplasmic antibody shows a statistical correlation with focal thyroiditis. The tendency for thyrotoxic patients to become myxcedematous after thyroidectomy can be correlated with the degree of lymphocytic infiltration in the operation specimen. Thyroid antibodies are of value as a prognostic guide to the risk of hypothyroidism in thyrotoxic patients in whom surgery is contemplated. In the presence of high titres of thyroid complement-fixing antibodies (C.F. > 1:32) the incidence of postoperative hypothyroidism in the first year is almost 25 per cent. (fig. 2). It has been said that high titres of thyroid antibodies might favour a natural remission of thyrotoxicosis over a period of a few years and that patients with such high titres should be treated with anti-thyroid drugs (Fulthorpe et al., 1961). This has not been found to be the case and indeed the converse may be true. The detection and titration of thyroid antibodies has not been found to correlate with the clinical response of thyrotoxic patients to treatment with radioactive iodine (Irvine and Stewart, 1967).

A third clinical use of thyroid antibodies is in the diagnosis of early primary hypothyroidism. In an equivocal case the presence of high titres of thyroid antibodies would favour a diagnosis of thyroid insufficiency. This may also prove helpful in distinguishing primary hypothyroidism from secondary thyroid failure due to hypopituitarism.
AUTO-IMMUNITY AND ENDOCRINE DISORDERS

SIGNIFICANCE OF THYROID ANTIBODIES IN PATHOGENESIS

Although thyroid complement-fixing (microsomal) antibody has been shown to be markedly cytotoxic to human thyroid cells in tissue culture (Irvine, 1962) there is no evidence that the transmission of this antibody in high titres across the placenta has any deleterious effect on the foetal thyroid. Likewise, the passive infusion of serum strongly positive for thyroid complement-fixing or thyroglobulin antibody into monkeys failed to produce any functional or histological damage in the animals' thyroid. Chronic thyroiditis can, however, be produced in experimental animals by repeated parenteral injection of thyroid antigen along with Freund's adjuvant.

With regard to thyroid-stimulating globulin a close correlation has been claimed by Carneiro and his colleagues (1966b) between the level of thyroid-stimulating globulin in the serum and the rate of iodine metabolism in the thyroid gland (fig. 3). There is good evidence that neonatal thyrotoxicosis is due to the placental transmission of thyroid-stimulating globulin (L.A.T.S.). Neonatal thyrotoxicosis is a self-limiting condition and the time course of its improvement is consistent with the biological life of thyroid-stimulating globulin. Injection of thyroid-stimulating globulin into experimental animals produces hyperplasia of the thyroid epithelium. It is tempting to speculate that thyroid-stimulating globulin is of fundamental importance in the etiology of thyrotoxicosis.

Lymphocytes may form antibody or be carriers of antibody. They are involved, for example, in the reactions of delayed hypersensitivity that are characteristic of graft versus host disease. Under the electron-microscope one can clearly see that in chronic thyroiditis lymphocytes penetrate through the basement membrane of the thyroid vesicle and come into close apposition with the thyroid cells (Irvine and Muir, 1963). If such a lymphocyte is
carrying or synthesizing thyroid antibody, then it can discharge its immunological missive directly at the target cell. In these circumstances the levels of the antibody in the serum may not be of primary importance but merely represent an overspill of excess antibody from the target tissue. Plasma cells may be found in similar situations.

**GENETIC ASPECTS**

The tendency to develop thyroid auto-immunity is familial and appears to be genetically rather than environmentally determined. Part of the evidence for this statement is based on twin studies; in thyrotoxicosis and in Hashimoto thyroiditis there is a high degree of concordance among uniovular twins (Irvine et al., 1962; Hassan et al., 1966). From family studies it would appear that the development of thyroid auto-immunity is controlled by a dominant autosomal gene with incomplete penetrance.

Sparkes and Motulsky (1963) and Failkow (1966) have drawn attention to the coexistence of Hashimoto thyroiditis and Turner's syndrome, and Williams and his colleagues (1964) have presented histological evidence of this association. It has been suggested that patients with X chromosome abnormalities may have an increased tendency to develop thyroid auto-immunity (Failkow, 1966; Valloton and Forbes, 1967).

Polani and his colleagues (1967) have carried out similar studies on 127 patients and 133 of their parents. Whereas in Klinefelter's syndrome (XXY) and in XX patients with pure ovarian dysgenesis, or Ullrich's syndrome, only a small proportion had traces of thyroid antibodies, the proportion of positive results and the titres rose progressively in groups of patients with Turner's syndrome (XO), mosaics with structurally normal, then abnormal X chromosomes, reaching a peak incidence of 65 per cent. in iso-X mosaics having one X chromosome with two long arms. The

<table>
<thead>
<tr>
<th>Percentage positive for antibody to</th>
<th>Thyroid</th>
<th>Stomach</th>
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<tbody>
<tr>
<td></td>
<td>Micro-</td>
<td>Thyro-</td>
</tr>
<tr>
<td></td>
<td>somal</td>
<td>globulin</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>344</td>
<td>37</td>
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<tr>
<td>Hashimoto goitre</td>
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<td>89</td>
</tr>
<tr>
<td>Primary hypothyroidism</td>
<td>185</td>
<td>57</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>174</td>
<td>26</td>
</tr>
<tr>
<td>Blood donors:</td>
<td></td>
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</tr>
<tr>
<td>12-65, M. and F.</td>
<td>1229</td>
<td>4</td>
</tr>
<tr>
<td>40-60, F.</td>
<td>142</td>
<td>15</td>
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</table>

Methods: Thyroid microsomal antibody—complement-fixation titre > 1:14.
Thyroglobulin antibody—tanned-cell hemagglutination titre > 1:25.
Gastric parietal microsomal antibody—indirect fluorescent antibody method.
Antibody to intrinsic factor—radio-immune assay.

Table I.—The incidence of thyrotoxicosis and gastric antibodies in the sera of patients with different forms of thyroid disease, Addisonian pernicious anaemia and in control subjects. (From Irvine, Davies and Sumerling, 1965.)
incidence of other types of antibody (e.g. A.N.F. and gastric parietal cell) was low
and the incidence of thyroid antibodies in the parents was normal for their age and
sex. The patients with significant titres of thyroid antibodies had decreased thyroid
reserve as determined by radio-iodine-uptake tests before and after T.S.H.

The reason for such an association between thyroid auto-immunity and
anomalies of the X chromosome is not yet clear, but it may be relevant that
in the normal population with increasing age an X chromosome may be
lost in a small proportion of cells. As already mentioned, the incidence of
thyroid auto-antibodies (and other types, such as gastric parietal-cell
antibodies) increases with age. The possibility that patients with Hashimoto
thyroiditis might have an increased deletion of the X chromosome is being
investigated.

ASSOCIATION BETWEEN THYROID DISEASE
AND ATROPHIC GASTRITIS

The recognition of the association between thyroiditis and gastritis led
to the detection of complement-fixing antibody specific for gastric mucosa
(Irvine et al., 1962). About 80 per cent. of patients with pernicious anaemia

![Fig. 4.—The titration of adrenal antibody in the sera of patients with primary adreno-
cortical insufficiency using the methods of indirect immunofluorescence and comple-
ment fixation. (From Irvine et al., 1967.)](image)

have parietal-cell antibody in the serum and about half have antibody to
intrinsic factor. Between a quarter and a third of patients with thyroid
auto-immune disease have parietal-cell antibody in the serum and 3 per
cent. have antibody to intrinsic factor. This 3 per cent. may seem low but is
highly significant, the incidence in the control subjects being zero (table I).
It correlates well with the finding of pernicious anaemia in 6 per cent. of this
thyroid population (Irvine et al., 1965).
ADDISON'S DISEASE

Adrenalitis can be induced experimentally in guinea-pigs by the injection of adrenal extract plus Freund's adjuvant. Antibodies specific for the secretory cells of adrenal cortex have been detected by complement fixation and by immuno-fluorescence; they occur only in patients with idiopathic Addison's disease and not in those in whom it is tuberculous (fig. 4).

Out of 235 patients with allegedly idiopathic Addison's disease studied in four laboratories (Irvine et al., 1967; Blizzard et al., 1967), the incidence of adrenal specific antibody was 66 per cent. in females and 38 per cent. in males.

There is yet no report of adrenal specific antibodies in any patient who was unequivocally known to have tuberculous destruction of the adrenals.

When patients with Addison's disease were studied for evidence of overt disease in other organs a high incidence of thyroid disorder, pernicious

<table>
<thead>
<tr>
<th>Type of adrenal insufficiency</th>
<th>Idiopathic</th>
<th>Probable idiopathic</th>
<th>Tuberculous</th>
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</thead>
<tbody>
<tr>
<td>Thyroid disease:</td>
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<td></td>
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<tr>
<td>Hypothyroidism</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Euthyroid goitre</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Eczema</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Schilder's disease</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Alopecia totalis</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total affected</td>
<td>8</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Total patients</td>
<td>12</td>
<td>23</td>
<td>16</td>
</tr>
</tbody>
</table>

Table II.—Diseases associated with primary adrenal insufficiency. (From Irvine et al., 1967.)

<table>
<thead>
<tr>
<th>Type of adrenal insufficiency</th>
<th>Gastric acid secretion in post-histamine hour (mEq HCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achlorhydria</td>
<td>&gt;0&lt;5</td>
</tr>
<tr>
<td>Idiopathic</td>
<td></td>
</tr>
<tr>
<td>Probable idiopathic</td>
<td>5</td>
</tr>
<tr>
<td>Tuberculous</td>
<td>—</td>
</tr>
</tbody>
</table>

* Including three patients with pernicious anemia.

Table III.—Gastric function in primary adrenal insufficiency. (From Irvine et al., 1967.)

anaemia, idiopathic hypoparathyroidism and diabetes mellitus was found in patients with idiopathic or probable idiopathic Addison's disease, but not in those with tuberculous destruction of the adrenal. Seven out of 12 of the idiopathic group and 11 out of 23 of the probable idiopathic group had
clinical and objective evidence of thyroid disease, pernicious anaemia, idiopathic hypoparathyroidism or diabetes mellitus, whilst these diseases were not found in any of the tuberculous patients (table II). Likewise, there was a high incidence of thyroid and gastric antibodies in the idiopathic and probable idiopathic groups but not in the patients with tuberculous adrenal insufficiency. In keeping with the gastric serology studies, patients with idiopathic Addison’s disease have been shown to have achlorhydria and hypochlorhydria more often than patients who have tuberculous destruction of the adrenals (table III).

From a clinical point of view it is important to make the differential diagnosis of the cause of primary adrenal failure. If adrenal antibodies are present it may be assumed that the patient has the auto-immune type of adrenal failure. Serological screening should be done for thyroid and gastric antibodies and particular attention be paid to the possibility that the patient may be suffering from other associated diseases. The serological studies will provide a good indication of subclinical disease in other tissues.

**IDIOPATHIC HYPOPARATHYROIDISM**

In idiopathic hypoparathyroidism a significantly high incidence of antibodies to parathyroid, thyroid, stomach and adrenal have been found in comparison with the incidence of these antibodies in control subjects (Blizzard et al., 1966). Likewise, clinical association between idiopathic hypoparathyroidism, thyroid disease and idiopathic Addison’s disease has been noted, particularly in children (Blizzard et al., 1967). Lesions of the parathyroid glands have not been reliably induced by experimental means using immunological techniques.

**DIABETES MELLITUS**

Auto-immune phenomena to pancreas or to insulin in diabetic subjects do not appear to be clearly established (Chetty and Watson, 1965). Mancini and his colleagues (1965) have reported finding antibodies to insulin in two patients who had diabetes mellitus but who had not been treated with insulin. Diabetes mellitus is associated with idiopathic adrenal insufficiency and is possibly more common in patients with thyroid disease than it is in control subjects. In the patients with idiopathic Addison’s disease the diabetes is predominantly insulin dependent (Irvine et al., 1967). Gastric antibodies are found more commonly in diabetic subjects, particularly young subjects, compared with the normal population of comparable age (Moore and Neilson, 1963). The evidence that diabetes mellitus is related to autoimmunity is indirect.

**TESTES**

Agglutination of motile spermatozoa in a man’s ejaculate can be due to the action of an auto-antibody against sperm. The agglutination prevents the spermatozoa from penetrating into the cervical mucus. The antibodies are present in the serum and also in the seminal plasma. They can be detec-
ted by agglutination in vitro and also by immunofluorescent procedures. The incidence of positive tests is predominantly in patients who have obstructed efferent seminiferous ducts. This leads to extravasation of sperm in the epididymis, which becomes infiltrated with macrophages, lymphocytes and plasma cells. It would therefore appear that auto-antibodies to sperm are not a primary cause of male infertility but are secondary to obstructed efferent ducts (Rümke, 1965).

**MECHANISMS OF AUTO-IMMUNITY IN ENDOCRINE DISEASE**

It used to be thought that in thyroid disease, for example, the basic defect was damage to the thyroid vesicle, either by trauma or by virus infection, with release of thyroglobulin. As a consequence, it was believed that a chain reaction was set up with antibody production and further thyroid damage. This concept has been disproved on two scores. Thyroglobulin has been demonstrated to be normally released into the thyroid lymphatics in the experimental animal (Roitt and Torrigiani, 1967), and damage to the thyroid gland by I31I in man produces only a transitory rise in thyroid antibody titres and not a chain reaction (Irvine, 1964). Likewise, an animal will only make antibodies to thyroid so long as injections of thyroid antigen with Freund’s adjuvant are continued. The puzzle about the auto-immune diseases is not that antibodies are made, but that they are made continuously.

A more likely hypothesis is that in this group of organ-specific auto-immune diseases there is a genetically determined defect in immunological tolerance (Irvine, 1964). For immunological tolerance to be maintained, there must be an immunological memory which recognizes substances that are constituents of the body’s own tissues and substances that do not belong to the body and are foreign. By virtue of this immunological memory, it may be postulated that clones of lymphocytes which tend to form antibodies against the body’s own tissues are normally destroyed. If this mechanism of immunological recognition for the deletion of abnormal clones of lymphocytes is defective then auto-antibodies against a particular constituent or group of constituents of the body may develop.

It has been shown that in experimental animals the thymus is essential for the development and maintenance of the immunological system; it is therefore tempting to speculate that some abnormality in the thymus may be responsible for the occurrence of certain patterns of auto-immunity. Irving and Sumerling (1965) studied the thymus radiologically in patients with different types of thyroid disease. A correlation was observed between the size of the thymus shadow on x-ray on the sagittal plane and the presence or absence of auto-antibodies in the patient’s serum. Enlargement of the thymic shadow was a common occurrence in patients showing auto-immunity and was not found in those in whom auto-antibodies were absent.

A second way of studying the thymus in patients with thyroid disease is to obtain a biopsy of the upper cornu of the thymus during a standard thyroidectomy with due respect to the parathyroids. The finding of germinal
centres in the medulla of the thymus is highly abnormal. The occurrence of thymic germinal centres again correlates with serological and histological evidence of auto-immunity. The pathology of the thymus is reminiscent of that seen in the strain of New Zealand mice that spontaneously develops auto-immune haemolytic anaemia and systemic lupus erythematosus (Burnet and Holmes, 1964).

Whether the changes in the thymus in patients with auto-immune thyroid diseases are simply another manifestation of an auto-immune phenomenon or whether a defect in thymic function might be of fundamental importance in the pathogenesis of these disorders remains unknown.

References

IMMUNOLOGICAL MECHANISMS IN THE PRODUCTION
OF DISEASE

W. J. Irvine

(1970) in 'A Companion to Medical Studies'
Ed. by R. Passmore and J.S. Robson.
Immunological mechanisms in the production of disease

The last chapter contains an account of the nature of hypersensitivity reactions. Here the concern is to discuss how these reactions cause disease in man. It is now more than fifty years since the role of anaphylaxis in diseases such as asthma, hay fever and food sensitivities was first discovered. Since then the immune mechanisms responsible for transfusion reactions, haemolytic disease of the newborn and other blood disorders have been elucidated. The discovery during the last fifteen years that aberrant immunological reactions are also the probable cause of many important diseases, previously labelled as idiopathic, has been one of the most exciting events in modern medicine. Immune reactions are implicated in the apparently spontaneous atrophy of various tissues such as the thyroid, parathyroid and adrenal glands and the gastric mucosa, either individually or collectively. They may also be involved in the progressive destruction of organs, such as the kidneys i.e., glomerulonephritis and in certain conditions of the liver. The changes produced in these tissues are basically inflammatory in nature and the conditions are therefore referred to as chronic thyroiditis, gastritis, etc. Other inflammatory diseases of these tissues, mainly of known infective aetiology are given the same general name, but are not relevant to the mechanisms discussed here.

The present chapter is based on the classification of hypersensitivity reactions given in table 22.2, p. 22.19. The major diseases that may arise from each of the four main types of hypersensitivity are considered in turn. Disorders of connective tissue in which aberrant immunological mechanisms may play an important role are described on p. 30.9.

The rare group of diseases which arise when one or more parts of the immunological system fail to develop is also described, together with some disorders in which immunoglobulins are present in the blood in excess. An account is also given of drug hypersensitivity.

Type I or anaphylactic reactions

Systemic anaphylaxis occurring in animals is described on p. 22.19. In man it may be induced by a foreign protein, e.g. antitetanus or antipertussis horse serum, sometimes by drugs such as penicillin or local anaesthetics and very occasionally by streptomycin, PAS, iodine-containing contrast media used in radiography and insect stings. Anaphylactic shock is fortunately rare and it is often fatal. Within 30 min of receiving an injection of the offending serum or drug, the patient collapses with wheezing and a feeling of tightness over the chest; the blood pressure falls, urticaria, cyanosis and convulsions follow and death may occur from cardiac arrest in a few minutes.

More limited reactions, local anaphylaxis, caused by antigens gaining access to the tissues via the respiratory and gastrointestinal tracts, are much more frequent. Common allergens are present in strawberries, nuts, egg, fish, pollens, dandruff, milk and fragments of insects; aspirin has also been implicated.

Respiratory symptoms include hay fever, rhinitis and asthma arising from bronchiolar constriction. In the skin there may be urticaria (nettle rash or hives), oedema, purpura or eczema. Oedema sometimes involves the eyelids, lips, tongue and larynx, when it is called angioneurotic oedema. Common gastrointestinal symptoms are dyspepsia, vomiting and diarrhoea. In severe cases headache, fever, joint pains and circulatory collapse may develop.

Sudden death of babies in their cot is sometimes due to aspiration of cow's milk which induces a local anaphylactic response in the lungs followed by asphyxia due to pulmonary oedema. The severe oedema of the lung in lobar pneumonia due to the pneumococcus has also been ascribed to type I allergy; this may explain the severity of the reaction of the tissues to a bacteria from which no soluble toxic factor can be identified (p. 18.69).

A local reaction occurs in a person who is generally sensitized, and this would be revealed if sufficient of the antigen were absorbed. It is not known, however, why one person with general sensitization exposed to an antigen present in the inspired air experiences asthma, whereas another exposed to the same antigen develops only hay fever. This is hard to explain as a quantitative dosage effect, and there may be some as yet unknown local predisposing factors. IgE is raised in the blood in persons subject to anaphylactic reactions, sometimes as much as sixfold and it is possible that its concentration
23.2 Immunological mechanisms and disease

may be related to the severity of the reactions. Allergens which invoke IgE do so in extremely low concentrations of < 1 pg/ml.

Hypersensitivity reactions in any one patient may not be limited to any one type. Thus in patients with asthma associated with pulmonary Aspergillus fumigatus not only are reaginic antibodies present but other types of antibodies associated with type III reactions are also involved (p. 23.6).

One of the curious features about reagin is that its known actions are harmful and of no obvious benefit to the individual or the species. The concentration of IgE however has been found to be raised in Ethiopian children, especially those infested with Ascaris lumbricoides. It remains to be established whether this is responsible for effective protection against worms.

DESENSITIZATION

This is achieved by repeated small subcutaneous injections of the antigen to which the patient is hypersensitive over a period of a few months. The dose is slowly increased during the course, as the injection of a large quantity of antigen at the start would carry a risk of an anaphylactic reaction, particularly if accidentally given intravenously. The antibody which develops as a result of this treatment is sometimes referred to as 'blocking antibody' as it blocks antigen from contact with reaginic antibody coating tissue cells. The titre of blocking antibody can be readily followed in the serum by means of agglutination tests with red cells coated with the appropriate antigen (p. 22.15).

The main problem associated with this treatment, particularly in local anaphylaxis, e.g. hay fever and asthma, is that it is frequently difficult to be sure which of a number of possible antigens is responsible for the hypersensitivity. The patient is usually tested by an intradermal injection or prick through a drop of antigen on the skin, with a panel of different antigens; even if the panel contains an antigen to which the patient is sensitive, cross reactions frequently occur which make it difficult to arrive at a precise diagnosis. Furthermore, the precise chemical nature of the hypersensitivity-inducing antigens is not known, and the methods employed in making them for use in desensitization procedures are relatively crude, the end product inevitably containing contaminating material of no value in desensitization. When reproducible and precise in vitro methods of antigen assay are eventually developed, considerable advances in this field of therapy should be possible.

Drugs that are used to ameliorate the anaphylactic response are described on p. 24.4.

Type II cytolytic or cytotoxic reactions

In this type of hypersensitivity, antibody receptors combine with either an antigenic component of a tissue cell or with an antigen or hapten which has become intimately attached to tissue cells. The antibody is usually of the classical IgG or IgM type. When they combine with antigens of invading micro-organisms they immobilize them.

Transfusion reactions involve the lysis of red cells by antibody and by complement. Following incompatible transfusion (vol. 1, p. 26.19) lysis may occur intravascularly or in the spleen where the sensitized cells are more readily destroyed with or without the participation of complement. Following blood transfusion, type I reactions, e.g. urticaria, oedema, bronchospasm, may also occur and are possibly due to traces of soluble foreign antigens in the donor's plasma reacting with reaginic antibody in that of the recipient or vice versa.

Haemolytic disease of the newborn occurs at birth or within the first two or three days of life and the rhesus (Rh) factor is the sensitizing antigen (vol. 1, p. 26.20).

Autoimmune haemolytic anaemias are a third example of a type II reaction; in these the red blood cell destruction is due to damage to the cells resulting from adsorption of autoantibodies arising spontaneously. The presence of adsorbed antibodies on the surface of the red cells can be demonstrated by the direct or indirect antiglobulin test (fig. 23.1). The removal of red blood cells from the circulation is probably brought about by the antibodies affecting their surface properties and by damage to the metabolism. The incomplete type of autoantibody causes their removal largely by the spleen; agglutinating and complement-fixing antibodies lead to removal by the reticuloendothelial system, the liver playing an important role by virtue of its size. An important factor in determining the filtering action of the spleen and liver is the intensity with which the antibody-affected
cells undergo autoagglutination. In addition antibodies which are demonstrably lytic in vitro and present in sufficient titres may cause intravascular haemolysis. Erythroagglutocytosis is important as a means of disposing of cells coated with complement-fixing antibody.

The study of blood disorders associated with cytopenia exemplifies another pathological immunological mechanism. Tissue cells may be rendered temporarily susceptible to the cytotoxic action of antibody and complement by adsorption of an antigen (e.g. a bacterial product) or a hapten (e.g. a drug) to their surface. Reaction of the bacterial antibody or antibody to the cell-drug complex then results in the destruction of the cell. Reference has been made to the generalized purpura due to thrombocytopenia which may develop following the administration of the drug apranol (sedormid, p. 22.20). Quinidine, aspirin, PAS, phenylbutazone, thiazides, stilbophen, phenazone, tetracyclines, streptomycin, thiourea derivatives, troxideone, phenobarbitone, oestrogens, sulphanylimides and other drugs may induce purpura in the same way, but do so much less frequently. A comparable disorder giving rise to haemolytic anaemia may follow the use of drugs showing an affinity for the red blood cells; these include stilbophen, phenacitin, PAS, isoniazid, sulphasalazine, phenothiazines and penicillins. A similar mechanism involving the white cells may be the basis of one form of drug-induced agranulocytosis, e.g. phenothiazines, PAS, thiouracil, diuretic and anti-diabetic sulphanylimides and colchicine. Methylxop also occasionally causes haemolytic anaemia; the antibody responsible appears to react with one of the Rh antigens on the patient's red blood cells which then become antigenic.

A group of disorders characterized by IgG antibodies in the serum, which show a high degree of organ specificity, include some forms of thyroid disease, i.e. thyroiditis, chronic thyroiditis or Hashimoto's disease (plate 23.4, facing p. 23.6) and primary thyroid atrophy, gastric atrophy (plate 23.5b) associated with pernicious anaemia and certain cases of adrenal atrophy (plate 23.5b), parathyroid atrophy and premature gonadal failure. Thus four different IgG antibodies that react with different components of thyroid cells or their secretions have been described; these are antibody to thyroglobulin (plate 23.4a, facing p. 23.5), antibody to an iodine-free component of thyroid colloid, complement fixing antibody reactive with the microsomal fraction of thyroid cells (plate 23.4b) and long acting thyroid stimulating substance. Gastric antibodies include those reacting with cytoplasm of oxyctic cells (plate 23.2a) and at least two reacting with different sites on intrinsic factor. Some patients with idiopathic adrenal atrophy have antibodies reactive with steroid-producing cells in the gonads (plate 23.2c) as well as those in the adrenal cortex. There is a clinical and even more pronounced immunological overlap between the diseases in this group. Thus 20–30 per cent of patients with thyroid autoimmune disease have gastric oxyctic cell antibodies in the serum as well as thyroid antibodies; over half of the patients with idiopathic primary adrenal atrophy have thyroid, gastric or parathyroid antibodies in the serum, the majority also having IgG antibodies specific for adrenocortical cells (plate 23.2b).

Although the presence of these specific IgG antibodies can be taken as evidence that the patient's immunological mechanisms are disturbed, it does not prove that they are responsible for any of the pathological features of the diseases. However, type II reactions may be important in this respect since thyroid microsomal antibodies, for example, are highly cytotoxic to human thyroid cells in tissue culture provided complement is present (plate 23.4a & b, facing p. 23.5). Nevertheless, thyroid disease has never been transferred successfully in experimental animals by means of this particular thyroid IgG antibody although this may be due to the antibody having difficulty in gaining access to the antigen in vivo. If, however, the animal's thyroid is previously damaged by irradiation, the infusion of thyroid microsomal antibodies may then augment thyroid damage. Likewise, although thyroid, gastric and adrenal antibodies are all capable of crossing the placenta into the foetal circulation, there is no evidence that, with the exception of LATS, this does the foetal organs any harm. As is described on p. 23.7, type IV reactions are also possibly concerned with the pathogenesis of autoimmune diseases.

THE LONG ACTING THYROID HORMONE (LATS) AND THYROTOXICOSIS

In 1957 Adams and Purves in New Zealand were studying a bioassay method for thyrotropic hormone (TSH) in mice. In this test (vol. 1, p. 25.4) TSH is injected into a suitably prepared animal, organically bound 131I is released from the thyroid into the blood, and the increase in plasma radioactivity is recorded. The peak rise in blood 131I occurs about three hours after the TSH is given (fig. 23.2). However, when the plasma of patients with thyrotoxicosis was injected, the time course for the plasma radioactivity was much more prolonged with the peak occurring in about 12 hours. The substance responsible for this late release was named long acting thyroid stimulator (LATS). Preparations of LATS were made from the glands of patients with thyrotoxicosis and on injection into rats these caused hyperplasia of the thyroid epithelial cells and also increased the uptake of 131I by human thyroid cells in tissue culture. LATS was shown to be an immunoglobulin of the IgG type. In man the occurrence of LATS in the plasma correlates closely with thyrotoxicosis and seldom occurs in any patient who is not thyrotoxic or who has not been thyrotoxic in the past. Its transmission across the placenta correlates with thyrotoxicosis in the newborn. In the absence of associated
23.4 Immunological mechanisms and disease

thyrotoxicosis, LATS does not occur in chronic goitrous thyroiditis nor is it found in primary hypothyroidism, although both conditions are associated with a high incidence of other thyroid autoantibodies. Furthermore, in thyrotoxic patients before treatment has been initiated, there is a correlation between the level of LATS in the plasma and the severity of the thyroid overactivity (fig. 23.3). There was initial hesitancy in accepting LATS as an autoantibody due to the notion that antibodies must necessarily be damaging or inhibitory as implied by the terms cytotoxic and cytolytic.

Other examples of stimulation by antibodies as part of type II hypersensitivity reactions include the transformation of lymphocytes into lymphoblasts under the influence of antilymphocytic serum. This, however, is more immediately relevant to antigen recognition than to any specific disease process.

THE KIDNEY AND HYPERSENSITIVITY REACTIONS

There are two different and distinct mechanisms whereby the host's antibody response may cause glomerulonephritis, which may lead to progressive renal failure:

1. the patient may produce antibodies capable of reacting with his own glomerular capillary basement membrane (GBM) antigen/s (a type II reaction), and
2. the patient may produce antibodies capable of reacting with exogenous or nonglomerular endogenous antigens with the formation of circulating antigen-antibody complexes which may become trapped in the renal glomeruli (a type III reaction (p. 23.4).

Both of these processes serve to induce an antigen-antibody reaction in the glomeruli in which inflammation occurs. The degree of glomerular injury appears to be related directly to the quantity and quality of antibody and/or antigen involved. There is no evidence that delayed or cellular hypersensitivity (type IV reaction) is important in the genesis of glomerulonephritis. Mononuclear cells, the hallmark of delayed sensitivity, are inconspicuous if indeed they participate at all.

These two mechanisms have distinctive immuno-histochemical and morphological characteristics, making it possible to differentiate them in biopsy specimens. In lesions induced by anti-GBM antibody (type II reaction), antibody and complement are arranged in a uniform pattern along the inner aspect of the GBM and are readily detected by EM or immunofluorescence (plate 23.6a, facing p. 23.8). In type III reactions circulating nonglomerular antigen-antibody complexes, plus complement, accumulate in discrete irregular granules along the outer aspect of the GBM beneath the epithelial cells. These deposits are readily demonstrable by immunofluorescence or EM (plate 23.6b) and may even be visible in light microscopy.

Glomerulonephritis can be produced in animals by injecting heterologous, homologous or autologous GBM.
Positive reactions in the indirect immunofluorescence test for autoantibodies to various antigens; the sera of patients with conditions referred to in the text have been used. The disease conditions shown in parenthesis are those in which the occurrence of the corresponding antibody in the serum is typical, but these antibodies may occur with a lesser incidence also in other related conditions and in control subjects.

In the indirect immunofluorescence test, unfixed air-dried sections are prepared from snap frozen tissue. The sections are treated with the test serum, washed and treated with antihuman γ-globulin (mono- or polyvalent) conjugated with fluorescein isothiocyanate. After further washing, the sections are examined by ultraviolet fluorescence microscopy.

(a) Human thyroglobulin in the colloid of thyroid vesicles ($\times 210$).
(b) Cytoplasm of human thyroid epithelial cells ($\times 210$).
(Tyroid autoimmune diseases: thyrotoxicosis; chronic thyroiditis (Hashimoto's disease); primary atrophic hypothyroidism.)
(a) Human gastric parietal cells (x 240). (Addisonian pernicious anaemia.)

(c) Theca cells of a Graafian follicle in human ovary (x 140). From Irvine W.J. et al. (1968) Lancet ii, 883. (Premature ovarian failure associated with idiopathic adrenal atrophy.)

(b) Cytoplasm of the secretory cells of human adrenal glands (x 220). (Idiopathic adrenal atrophy.)

Plate 23.3.

(a) Thymus myoid cells (p. 23.5) (× 630).  
(b) Heart muscle (× 810).  
(c) Skeletal muscle of calf (× 810).

All three tissues possess a common antigen. (Myasthenia gravis).

(d) Mitochondria in rat kidney tubules (× 260). (Primary biliary cirrhosis and active chronic hepatitis.)

(e) Nuclei in rat kidney (× 260). (Systemic lupus erythematosus.)
(a) Human thyroid cells trypsinized to get them into suspension and then cultured _in vitro_ in 10 per cent normal human serum. The cells spread out as a monolayer on the coverslip (_x 1100_).

(b) Same as above but a few minutes after changing the culture medium to 10 per cent serum from a patient with chronic thyroiditis (Hashimoto's disease) (_x 1100_).

A comparable cytotoxic effect, specific for thyroid cells, has not been demonstrated with cells that have not been previously subjected to enzyme treatment. This illustrates that thyroid specific antibodies may be damaging to human thyroid cells provided they can gain access to the thyroid antigen which is in relation to the cell membranes.

antigens. Antigens cross-reactive or identical with GBM antigens are present in the urine of all healthy mammals studied. These may be extracted from the urine of normal rabbits and used to induce nephritis similar to that produced by autologous, homologous and heterologous antigens. In such nephritic animals, little anti-GBM antibody can be found in the circulation, but if they are nephrectomized it accumulates in the serum, presumably as a result of the removal of the target antigen. Anti-GBM antibodies can also be readily eluted from the kidneys of these animals. Glomerulonephritis, induced by immunizing an animal with GBM, may be considered autoimmune in the sense that it is the host's antibody reacting with his own GBM which is the cause of the disease; the fact that the original immunizing antigen need not be autologous is irrelevant.

An important step in demonstrating the participation of anti-GBM antibodies in human renal disease was their isolation in 1967 from the serum and kidneys of patients with glomerulonephritis. Acid elution of homogenates of kidneys from such patients demonstrated not only the presence of anti-GBM antibodies but also their localization in the affected organ. In other patients anti-GBM antibodies were detected in the serum only after total nephrectomy undertaken prior to renal transplantation. The appearance of circulating antibodies after nephrectomy parallels the experimental observations in animals and indicates how efficiently the GBM, exposed to the circulation by the fenestrae, removes circulating anti-GBM antibodies. As little as 6 mg of globulin eluted from human kidneys with anti-GBM type nephritis when injected into monkeys cause immediately a severe, progressive glomerulonephritis.

In one patient, circulating anti-GBM antibodies which were demonstrated after nephrectomy fell in a linear manner and were no longer detectable one day after a renal transplant. An immediate and persistent glomerulonephritis developed in the transplanted kidney. These observations show not only that these antibodies are reactive in vivo but also that they can damage the renal glomerulus. It appears that patients forming anti-GBM antibodies may not be good candidates for renal transplantation since they are likely to reproduce in the transplant the same nephritic changes already suffered by their own kidneys. When patients with this form of glomerulonephritis require organ replacement, nephrectomy followed by a period of maintenance by haemodialysis and immunosuppression until the antibodies disappear from the circulation would seem to be a wise preliminary procedure.

MISCELLANEOUS OTHER CONDITIONS

Recent studies in pemphigus, a serious and fortunately uncommon disease of the skin characterized by bullous eruptions, show that autoimmunity is almost certainly concerned in its pathogenesis. The reaction of serum immunoglobulins with intercellular antigen peculiar to squamous epithelium can be demonstrated in frozen sections of skin obtained from man or laboratory animals. The titre of the antibody to the intercellular antigen is proportional to the severity of the disease. The antigen, a heat-labile water-insoluble substance, lies between squamous epithelial cells at a site corresponding to the lesion of the disease (plate 23.2d). The pathogenesis, however, cannot be attributed solely to autoimmunity; injection of serum from patients with pemphigus into the skin of monkeys produces intercellular fixation of antibody but not the fully developed disease. If the antibody is a cause rather than a result of the disease, additional features must be involved, such as increased tissue permeability allowing access of complement or other mediators of inflammation to the intercellular spaces of squamous epithelium.

In contrast to the diseases described above that are characterized by the occurrence of organ-specific antibodies, certain forms of liver disease, notably primary biliary cirrhosis (plate 23.5d, facing p. 23.6) and active chronic hepatitis, are associated with antibodies in the serum that are reactive with mitochondria (plate 23.3d) and with smooth muscle. These antibodies are not specific for any particular organ and clearly their role in producing liver damage cannot be of great significance, although other immune mechanisms not so readily demonstrated may well be. Antinuclear antibodies, frequently called antinuclear factors (ANF), are reactive against components of tissue nuclei and their reactivity likewise does not show any precise organ specificity (plate 23.3e). The occurrence of ANFs in the serum is characteristic of systemic lupus erythematosus, with a lesser incidence in other connective tissue disorders (p. 30.12).

Myasthenia gravis, a disorder affecting neuromuscular function (p. 4.5) and commonly associated with enlargement of the thymus is also of particular interest. There is a high incidence of IgG antibodies in the serum which react with A or 1 bands of skeletal muscle (vol. I, p. 15.4) and which cross-react with cardiac muscle and certain cells in the medulla of the thymus that are derived from muscle cells present at an early stage in foetal development (plate 23.3f, b & c). The site of reactivity of the antibody with skeletal muscle cannot explain the block of neuromuscular transmission which is the characteristic functional abnormality in the disorder. In myasthenia the thymic medulla is frequently the site of germinal centre formation (plate 23.5e), which is abnormal, and recent studies have shown that a neuromuscular-blocking factor which is not immunological may be produced by the gland under these conditions. As described later under type IV reactions, the thymitis may be immunologically induced.
23.6 Immunological mechanisms and disease

Antigens may be shared between an exogenous, e.g. bacterial, factor and a normal constituent of the body's tissues. The formation of antibodies to the exogenous factor would then result in these antibodies cross-reacting with the body constituent. Such a mechanism may be a factor, for example, in the pathogenesis of rheumatic fever, where there is evidence that antigen in the human cardiac myofibrils may be shared with components of certain streptococci (p. 30.10). A similar mechanism may occur in relation to ulcerative colitis where there is evidence that an antigen of a type of Esch. coli (i.e. 014) is shared with a constituent of the mucosal cells of human colon.

Type III or toxic complex reactions

Serum sickness, first described by Von Pirquet and Shick in 1905, is the classical example in man. The reaction may occur 8–12 days after a single injection of an antigen, which is usually horse serum, e.g. antitetanus. It consists of skin rashes, fever, joint pains and often swelling of the regional lymph nodes. The symptoms vary greatly, as do their duration. The illness is seldom serious, though it may give rise to much discomfort. Now that antitoxins and other horse serum preparations are much less used in medicine, serum sickness is not commonly seen. However, many drug reactions, especially those involving the penicillins and sulphonamides, are probably due to toxic complexes formed by drug haptns reacting with specific antibodies, formed in response to previous exposure to the drug.

As mentioned on p. 23.4, type III reaction has been implicated in some forms of glomerulonephritis in man. Further evidence for this comes from studies on a highly inbred strain of New Zealand black (NZB) mice. These animals frequently develop a characteristic glomerulonephritis and they are also infected chronically with one or more viruses. The glomerulonephritis is associated with the formation of antinuclear antibodies and complexes of these antibodies with nuclear antigens and complement can be demonstrated in the glomeruli. The primary abnormality in this experimental model, which closely resembles lupus erythematosus in man, may well be the presence of nuclear antigen, but whether this is derived from the infecting viruses or from abnormal catabolism of the host's own nuclear material, perhaps as a result of the infection, is uncertain. Thus the relation between the infection and the renal disease is not clear. This disorder, however, is a good example of glomeruli trapping an antigen–antibody complex with a consequent focal inflammatory reaction. The size and shape of the complex in relation to the local capillary circulation would seem to be at least one factor determining the probability of trapping.

In man, glomerular lesions arise in association with systemic lupus erythematosus (SLE, p. 30.12), streptococcal infections (p. 18.69) and quartan malaria. In these conditions the presence of a focal antigen–antibody complex in the glomeruli can be demonstrated and the antigens involved in the complexes are identified or suspected (plate 23.6a, facing p. 23.8). In SLE, antinuclear antibodies have been found in high titres in affected kidneys, and complexes of nuclear antigens, host immunoglobulin and complement demonstrated in the glomeruli. During exacerbations of the renal disease, antinuclear antibodies have been found in the circulation, an event which is almost certain to lead to the formation of complexes. In poststreptococcal glomerulonephritis, host immunoglobulins and complement can readily be detected in deposits in the glomeruli. Evidence for the presence of streptococcal antigens is less strong, but suggestive. Similarly immunoglobulins and complement have been found in characteristic complexes in the glomeruli in cases of quartan malaria associated with glomerular inflammation. These complexes can be demonstrated also in the circulation and may be presumed to contain antigen derived from the malarial parasites.

In addition to these three entities in which the nature of the antigen is at least partly known, granular deposits containing host immunoglobulin and complement and resembling antigen–antibody complexes have been found along the basement membrane of the glomeruli in a number of cases of children and adults with acute and chronic renal disorders in which there is little hint as to the antigens involved. Endogenous or autoantigens and many infectious agents could result in the formation of such complexes. Immunological analysis of tissue obtained by percutaneous renal biopsy from a sufficient number of cases of glomerulonephritis may in the future provide estimates of the frequency with which different antigens cause the disease.

The source and supply of antigen is relevant to the course of any disease arising from a type III hypersensitivity reaction. If the antigen has been given in a single dose, as in serum sickness, the lesion should quickly regress. If, however, the antigen is given repeatedly, as in penicillin treatment, or is endogenous, as is the case in lupus erythematosus or intractable streptococcal infection, then the lesions persist or recur.

Reference has been made to the tendency for some individuals to produce reagins with the development of asthma when various antigens are inhaled (p. 23.1). However, under certain circumstances a type III response develops in the tissues around bronchi and alveoli on exposure to certain organic dusts. The spores of Aspergillus fumigatus are able to grow in the lungs and provide a persisting source of antigen. In some individuals, asthma is associated with a type I reaction, but in others, circulating antibodies are found and a type III reaction develops with extensive patchy involvement of the pulmonary
Plate 23.5: Histology of autoimmune diseases.

(a) A field from the thyroid in a case of chronic thyroiditis (Hashimoto's disease). Atrophy of the parenchymal tissue is seen; the remaining thyroid follicles are small and lined by large cells with very eosinophilic cytoplasm (Askanazy cells). Much of the tissue is replaced by lymphoid follicles and aggregations of lymphocytes. Haem. & eosin (x 50).

(b) An adrenal gland in primary adrenocortical atrophy; the upper half of the field shows a narrowed cortex with only a few groups of parenchymal cells; much of the tissue is fibrovascular connective tissue infiltrated by lymphocytes. The lower half consists of unaffected medulla. Haem. & eosin (x 85).

(c) Gastric mucosa from the body of the stomach in a case of atrophic gastritis. Note the absence of parietal and zymogenic cells, the paucity of glands and the increase of lymphoid tissue with infiltration of the lamina propria by small mononuclear cells. Haem. & eosin (x 100).

(d) Primary biliary cirrhosis showing abnormal hepatic architecture; nodules of regenerating liver tissue, bands of fibrous tissue infiltrated by lymphocytes and plasma cells, and canaliculi distended with plugs of bile, indicating biliary obstruction, are seen. Haem. & eosin (x 95).

(e) Chronic thymitis. Section of thymic medulla showing the presence of a lymphoid follicle together with three Hassall's corpuscles. Haem. & eosin (x 170).

(f) A field from a salivary gland in a case of Sjögren's disease. Several dilated ducts are seen; there is severe atrophy of the glandular tissue, with replacement by lymphoid tissue. Residual glands are present at the periphery of the field. Haem. & eosin (x 95).
tissue analogous to an Arthus reaction. Similar reactions to the inhalation of certain species of actinomycetes from mouldy hay gives rise to a diffuse infiltrative pulmonary disease known as Farmer's lung; exposure to other antigens, e.g. dust from mushrooms or pigeon droppings, leads to similar allergic occupational hazards.

**Type IV or delayed cellular hypersensitivity reactions**

**MICROBIAL HYPERSENSITIVITY**
The classical example is the tuberculin reaction which is described on p. 21.17 and p. 22.18. Similar inflammatory reactions occur in other chronic microbial infections in which the parasites may be intracellular, e.g. leprosy and brucellosis; they may be found also in fungal and viral infections, e.g. histoplasmosis, coccidiomycosis and psittacosis, and in helminthic infestation and Leishmaniasis. It is difficult to assess the significance of these reactions for the host although it is possible they represent a mild and delayed defence mechanism. On the other hand, unlike the more violent anaphylactic reactions, they do not constitute in themselves disease processes. Decreased delayed sensitivity is characteristic of sarcoidosis but the explanation for this is unknown (p. 21.20). Type IV reactions may also be responsible for the reaction of tissues to silica and beryllium (p. 21.20) in which granulomatous lesions are found in the lungs. Graft rejection also possesses features in common with type IV reactions, i.e. infiltration with mononuclear cells and failure of transfer by serum.

**CONTACT DERMATITIS**
This may occur in sensitized persons after exposure to a variety of substances, plants, household and industrial chemicals and drugs. These substances must be soluble and penetrate tissue. Primulas and, in America, poison ivy are two plants which have a bad reputation in this respect. As most of the chemicals which cause contact dermatitis are not themselves antigens, they must first form compounds with skin proteins, and these then cause the sensitization. The hypersensitivity of the skin is generalized and may be detected by the application of a small patch test containing the allergen anywhere on the body. The delayed reaction takes up to 48 hours to develop, and it is important to distinguish it from reactions due to direct irritation or toxicity. For many years it was believed that the allergy was confined to the cells of the skin, and that it spread over the body surface by continuity. This has been shown to be incorrect by experiments on guinea-pigs in which skin sensitization was tested after isolation of an area of skin from nervous lymphatic and vascular connections. Sensitization occurs only when lymph drainage to the regional lymph nodes is intact and the allergy is conveyed to the whole skin by immunologically competent cells in the blood stream.

Transfer of contact dermatitis has been achieved in animals and man with cells from the thoracic duct and spleen.

**AUTOIMMUNE DISEASE**
The evidence that type IV reactions are involved in some of the autoimmune diseases in man is largely indirect. In many of these conditions there is a marked accumulation of mononuclear cells in the thyroid, adrenal or parathyroid glands, gastric or colonic mucosa and liver or thymus (plates 23.5a-e) which is similar if not identical with that found in delayed hypersensitivity reactions. Provided adjuvants are used, comparable histological lesions, e.g. thyroiditis, gastritis, adenitis, orchitis, thymitis and encephalomyelitis, can be induced in experimental animals by the injection of suitable antigens and can be transferred from an affected to a normal animal by means of lymphocytes, though not by serum. The animals also develop positive skin tests for the antigen which are of the delayed hypersensitivity type. Furthermore the development of experimental thyroiditis or of allergic encephalomyelitis, can be prevented by prior treatment of the animal with antilymphocytic serum (p. 22.3).

An experimental model of myasthenia gravis can be produced in laboratory animals by immunizing with skeletal muscle extracts together with Freund's adjuvant. This produces an infiltration of lymphocytes in the thymic medulla and antibodies in the serum reactive with the A or I bands of skeletal muscle. The production of the block in neuromuscular transmission is dependent on the presence of the thymus although skeletal muscle antibodies may be produced with or without the thymus.

In man, the experimental opportunities are more limited. However, the lymphocytes from a patient with the autoimmune type of thyroiditis proliferate in vitro under the stimulus of thyroid antigen. Likewise, lymphocytes from a patient with autoimmune chronic inflammation of the adrenal glands give in vitro reactions indicating that they have been specifically sensitized for adrenal cortex. The leucocytes, but not the serum, from a proportion of patients with ulcerative colitis can exert a specific cytotoxic effect on colon cells in vitro, provided complement is present in the system.

It is not known whether type II reactions operate synergistically with type IV reactions in contributing to the origin or manifestations of autoimmune diseases. One of the difficulties is that techniques for study of humoral antibodies are far more advanced than those for the study of cellular immunity.

**Rheumatoid arthritis** is another disorder about which there has been even more speculation on the role of autoimmune delayed hypersensitivity reactions. Rheumatoid factor (p. 30.12) is an IgM immunoglobulin and can be detected in moderate or high titres in the sera of the
majority of patients with rheumatoid arthritis, while such
titres do not occur in nonrheumatoid subjects. However,
apart from its importance in drawing attention to the
possible role of autoimmunity in the pathogenesis of
rheumatoid arthritis, the rheumatoid factor has not yet
been implicated in the pathogenesis of the disease. The
development of typical rheumatoid lesions in subjects
with congenital hypogammaglobulinaemia shows that
neither circulating antibodies nor plasma cells, which
give rise to them, can play a basic pathogenetic role. It
has been suggested that the rheumatoid state might result
from delayed hypersensitivity to one or more products of
inflammation. The histopathology of the synovial mem¬
brane with its diffuse infiltration of plasma cells and the
aggregations of lymphocytes with formation of germinal
centres could then be interpreted as the immunological
response to the local persistence of antigen. The nature of
this antigen is not known and various claims for the
implication of mycoplasma or other agents have not been
adequately substantiated. Other indirect hints that
delayed hypersensitivity may be important in the patho¬
genesis of rheumatoid arthritis include the demonstration
that an arthritis virtually indistinguishable from rheuma¬
toid arthritis can be induced in rabbits by the intra¬
articular injection of fibrin, if the animals have been
previously made sensitive by intradermal injection of
fibrin in Freund’s adjuvant.

Sjögren’s disease or syndrome is a chronic benign
disorder which occurs in middle-aged females with
involvement of the uveal tract and the salivary glands. In
about one half of patients, this is associated with some
form of connective tissue disease, usually rheumatoid
arthritis or, less commonly, systemic lupus erythematosus.
The lacrimal and salivary glands show extensive chronic
inflammatory cell infiltration and acinar atrophy with
failure of secretion (plate 23.5f). Antibodies to the sali¬
vary duct cell cytoplasm have been demonstrated in the
serum of such patients. Rheumatoid factor and antinu¬
clear factors are also commonly present in the serum.
Although the lesion in the salivary glands produced by
immunological techniques in animals do not exactly
resemble those seen in the human disease, delayed hyper¬
sensitivity reactions are more probably involved in the
pathogenesis than are type II reactions.

Why should these immune reactions occur?
Type I reactions occur in individuals who are so con¬
stitutionally disposed, and the nature of the reaction to
the allergen depends largely on the immunological re¬
activity of the subject. This also appears to dictate
whether a type I reaction, leading for example to asthma,
or a type III reaction, leading to an allergic pulmonary
disease, occurs. In the case of type I reactions the basic
abnormality is the capacity to produce reagins and is
probably inherited, but at present it is impossible to fit
any genetic hypothesis to the existing data. The genetic
basis for an inherited disease may be either a single gene
or it may be multifactorial. Individuals affected by type I
reactions tend to develop the disorder in childhood if
both parents are allergic subjects but do so at a much
later age in the absence of a family history. Since only a
small proportion of children in allergic families develop
the disease, penetration is clearly incomplete. Environ¬
mental influences may also be expected to contribute
substantially. More accurate information on the in¬
heritance of this type of hypersensitivity reaction is further
complicated by the difficulties in diagnosis. All forms of
bronchospasm are not allergic in origin and likewise there
is clinical difficulty in defining allergic skin reactions, par¬
ticularly in infants. The problem of inheritance and its
role in human allergy is likely to be further advanced
only when IgE reagent antibody is more readily detectable
and the specificity of the antigens determined.

The formation of autoantibodies (type II and type IV
reaction) must be considered in terms of the general
theory of antibody formation and tolerance. It is reason¬
able to suppose that the consequences of antigenic
stimulation depend on the same factors for autoantigens
as for exogenous or foreign antigen, and it is not neces¬
sary to invoke any special mechanism whereby the animal
or individual ‘recognized’ their own antigens as being
part of ‘self’.

Autoantigens may be considered in two groups:
(1) those to which tolerance is not normally fully
established, and
(2) those in which antibody formation is a result of
breakdown of a previously established tolerance.

Antigens in the first group, if suitably extracted and
injected in the correct dosage, give rise to antibody for¬
novation in normal animals; antigens in the second group
do not do this. The distinction between these two groups
is not absolute and there are important genetic factors
which determine the ease with which animals form anti¬
body to autoantigens; adjuvants also facilitate auto¬
antibody formation. Furthermore, immunization with
related or chemically modified antigens may cause
autoantibody formation when the autologous antigen
does not.

The clearest example of an autoantigen of the first
type is one which has been sequestered anatomically,
i.e. it has never met the animal’s reticuloendothelial
system. Good examples of this are antigens in the anterior
chamber of the eye which lacks lymphatic and vascular
connections. Antigens in such special sites do not give
rise to any immune response but will do so if injected
parenterally. Thus, if the lens of the eye is damaged by
trauma, leakage of antigen from the lens into the cir¬
culation may occur and antibodies are formed. This is
probably the basis of sympathetic ophthalmia which may
Plate 23.6.

(a) This is an electron micrograph of part of a human renal glomerulus in a case of poststreptococcal proliferative glomerulonephritis. Situated on the capillary basement membrane (BM) and protruding from it into an epithelial cell (E) is a large conical deposit (D) of granular material which is an antigen-antibody complex (× 25,000).

(b) This electron micrograph shows part of a human renal glomerulus; the capillary basement membrane (BM) is thickened over much of its course by linear, dark deposits (D) along its sub-endothelial aspect. This may represent deposition of anti-GBM antibody (× 6000).
follow a perforating injury of the globe in which the uveal tract is involved. After some weeks, a diffuse lymphocytic infiltration may develop in the uveal tissues of the injured eye. At any time from about the third week to several years following the trauma, a similar lesion may appear in the other eye. Antiuveal antibodies have been identified in patients with such injuries and extracts of uveal tissue elicit a positive intradermal hypersensitivity test in these patients.

The commonest form of sequestration of antigen is in the tissue cells, although this cannot be as absolute as in the above example. Many cells of the body break down and are replaced, and it is clear that a pre-existing state of tolerance has broken down. Presumably these antibodies developed primarily because of changes in the antibody-forming system itself, but how this immunological homeostasis is upset is poorly understood. It is possible that new clones of lymphocytes may develop as a result of random somatic mutation. Under physiological circumstances, any newly formed clone of lymphocytes that reacts with one of the body’s own tissues (and to which constituent the body has become immunologically tolerant) should be deleted. Therefore, it is necessary to postulate not only a mutation which produces the aberrant clone but also a failure of the deletion of the clone when it arises. Furthermore, any theory of autoimmune disease must account for the occurrence of the formation of autoantibodies not in a random manner but in groups. Thus in systemic lupus erythematosus, antibodies are formed not only to a considerable variety of different nuclear components but to multiple determinants in a single component such as DNA-nucleoprotein. In other diseases, notably in the organ-specific group of autoimmune disorders, i.e. chronic thyroiditis, pernicious anaemia, primary Addison’s disease and hypoparathyroidism, there is both clinical and immunological overlap. Thus patients with idiopathic Addison’s disease not only have a high incidence of antibodies specific for the cytoplasm of adrenocortical cells but also have antibodies specific for thyroid cytoplasm or gastric parietal cells. The multiplicity of antibodies involved makes it unlikely that the primary defect in this group of diseases is an abnormality of the many tissue constituents and there is no evidence that the antigens are shared between these different tissues. Further, when tissue is damaged, e.g. in myocardial infarction or in burns, there is only a transitory rise in antibodies to the myocardium or skin respectively, which does not lead to a progressive autoimmune disorder. Likewise, infection of the thyroid or tests by mumps virus and the destruction of the adrenal gland by tuberculosis are not associated with continual antibody formation against the respective tissues. One of the unexplained characteristics of the autoimmune disease is that the antibodies are continually formed over periods of many years. In autoimmune thyroid disease and in Addisonian pernicious anaemia, family studies show that the tendency to produce thyroid and gastric auto-antibodies is controlled by an autosomal gene with incomplete penetrance showing a sex-controlled bias. It is probable therefore that both the organ-specific group of autoimmune diseases, e.g., thyroid, adrenal, etc., and the organ-nonspecific group, e.g. SLE, are genetically determined disorders of immunological tolerance. This hypothesis is compatible with observations in experimental animals.

Autoantigens

Autoantibodies are formed to the surface antigens of circulating cells, as is the case in some forms of haemolytic anaemia, it is clear that a pre-existing state of tolerance has broken down. Presumably these antibodies developed primarily because of changes in the antibody-forming system itself, but how this immunological homeostasis is upset is poorly understood. It is possible that new clones of lymphocytes may develop as a result of random somatic mutation. Under physiological circumstances, any newly formed clone of lymphocytes that reacts with one of the body’s own tissues (and to which constituent the body has become immunologically tolerant) should be deleted. Therefore, it is necessary to postulate not only a mutation which produces the aberrant clone but also a failure of the deletion of the clone when it arises. Furthermore, any theory of autoimmune disease must account for the occurrence of the formation of autoantibodies not in a random manner but in groups. Thus in systemic lupus erythematosus, antibodies are formed not only to a considerable variety of different nuclear components but to multiple determinants in a single component such as DNA-nucleoprotein. In other diseases, notably in the organ-specific group of autoimmune disorders, i.e. chronic thyroiditis, pernicious anaemia, primary Addison’s disease and hypoparathyroidism, there is both clinical and immunological overlap. Thus patients with idiopathic Addison’s disease not only have a high incidence of antibodies specific for the cytoplasm of adrenocortical cells but also have antibodies specific for thyroid cytoplasm or gastric parietal cells. The multiplicity of antibodies involved makes it unlikely that the primary defect in this group of diseases is an abnormality of the many tissue constituents and there is no evidence that the antigens are shared between these different tissues. Further, when tissue is damaged, e.g. in myocardial infarction or in burns, there is only a transitory rise in antibodies to the myocardium or skin respectively, which does not lead to a progressive autoimmune disorder. Likewise, infection of the thyroid or tests by mumps virus and the destruction of the adrenal gland by tuberculosis are not associated with continual antibody formation against the respective tissues. One of the unexplained characteristics of the autoimmune disease is that the antibodies are continually formed over periods of many years. In autoimmune thyroid disease and in Addisonian pernicious anaemia, family studies show that the tendency to produce thyroid and gastric auto-antibodies is controlled by an autosomal gene with incomplete penetrance showing a sex-controlled bias. It is probable therefore that both the organ-specific group of autoimmune diseases, e.g., thyroid, adrenal, etc., and the organ-nonspecific group, e.g. SLE, are genetically determined disorders of immunological tolerance. This hypothesis is compatible with observations in experimental animals.

Genetic factors are of paramount importance in the occurrence of spontaneous autoimmune disease in experimental animals. Thus NZB mice, which develop autoimmune haemolytic anaemia and diseases which resemble systemic lupus erythematosus and glomerulonephritis, transmit the condition to their offspring. Mating these mice with other strains produces variants in the disease picture. In addition, in experimental animals Freund’s adjuvants have a mutagenic effect on antibody-forming cells and act as a substitute for the genetic predisposition. The critics of the central-defect hypothesis argue that what is inherited in these animals is a susceptibility to virus infection and that chronicity of tissue damage is consistent with the nature of virus infection. Such a virus might show predilection for certain tissues or could be responsible for the abnormal behaviour of the antibody-forming system by invading the reticuloendothelial system.

Is the formation of autoantibodies always an abnormal process? A significant proportion of the population, especially females, develop thyroid and gastric circulating autoantibodies as they grow older. This is associated with subclinical chronic inflammation and a variable degree of atrophy of the corresponding tissues. This has given rise to the view that the development of autoimmunity is part of the ageing process (fig. 23.4).

As already mentioned, invading micro-organisms may sometimes carry antigens which are shared by certain tissues of the host, e.g. some group A β-haemolytic streptococci and cardiac antigens, group A type 12 β-haemolytic streptococci and renal antigens, and certain forms of E. coli 014 and colon antigen. If the appropriate forbidden clone has developed in the host, antibody formed in response to these antigens could give rise to
Drug hypersensitivity

Adverse reactions to drugs are described throughout this volume where the individual drugs are described, and in chap. 32. While these reactions may be due to overdosage, drug interaction, unwanted but recognized pharmacological actions or secondary effects, in many instances the reaction is unpredictable and differs from the usual pharmacological effect. Such idiosyncrasies usually occur in only a small minority of individuals exposed to the agent. These reactions can occasionally be attributed to a genetically determined defect or abnormality in molecular structure of a body constituent or enzyme and are described under the topic of pharmacogenetics (p. 319). A larger number, however, are believed to be due to hypersensitivity in the immunological sense.

In some instances the immunological basis of the reaction has been established by the presence of an antibody demonstrated in vitro or by means of passive transfer. These have been referred to under the different types of immunological mechanisms believed to be involved. In many other cases the immunological mechanism is unknown. While these reactions are widely accepted as being allergic it is possible that a few may be shown subsequently to arise from genetic or other metabolic abnormalities which have not yet been revealed.

Hypersensitivity reactions to drugs are more prone to occur in children than in adults, and in females rather than males. Patients who exhibit reaginic reactions to other antigens appear to be particularly susceptible. The prolonged use of a multiplicity of drugs increases the severity and incidence of reactions. In those sensitized, severe reactions may be induced by very small doses. There is no clear relationship between reactions and chemical structure but cross sensitization between two or more drugs may occur. The possibility that hypersensitivity is due to a contaminant of a preparation of a drug, as has been suggested in the case of penicillin, rather than to the drug itself should also be kept in mind (p. 20.27).

In addition to anaphylactic and serum sickness-like reactions and to cytopenic blood reactions already described (pp. 23.1 & 3), the clinical manifestations of drug hypersensitivity are extremely varied but commonly affect the skin, liver, kidneys and connective tissues.

Skin reactions

These include pruritus, urticaria, the so-called exanthematous reactions such as generalized or local erythema and other circumscribed or bullous eruptions. They are caused by a large number of drugs which include phenazone and its related compounds, barbiturates, sulphonamides, phenylbutazone, thiazides, chloroquin, quinine, phenothiazines and sulphonylureas. Exfoliative dermatitis, in which the skin becomes inflamed with exfoliation
THE KIDNEY

Some drugs may also induce photosensitivity, which suggest hypersensitivity, but again the evidence is circumstantial.

The possible role of drugs in inducing hypersensitivity reactions in polyarteritis nodosa and systemic lupus erythematosus is described on p. 30.12. Retroperitoneal fibrosis is a rare reaction to methysergide (p. 13.7).

The Kidney, Liver

Proteinuria due to drugs, including phenindione, sulphonamides, PAS, methadione, penicillamine, bismuth, mercury and gold.

THE LIVER

Intrahepatic cholestasis may be caused by a number of C-17 alkyl substituted testosterone derivatives including methyl testosterone and some oral contraceptives, e.g. norethisterone and norethynodrel. The mechanism of this reaction is uncertain, but it may represent an intoxication rather than a hypersensitivity reaction. On the other hand, the phenothiazines, nitrofurantoin and chlorpropamide induce an allergic intrahepatic cholestasis in which overgrowth of villi in the biliary canaliculi occurs and in which there is an infiltration of inflammatory cells.

Hepatic cell necrosis occurs in a small number of patients taking monoamine oxidase inhibitors, sulphonamides, phenindione, cincophen, PAS and erythromycin estolate. In some cases this reaction is associated with other features such as fever, joint pains, skin rashes, etc.

Drug hypersensitivity


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<tr>
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<th>Cellular</th>
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<td>Melanoma infection</td>
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<td>Autosomal-recessive</td>
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Fig. 23.5. A functional classification of the immunity deficiency states. Examples of nonspecific mechanisms are indicated but defects are not included. There is reason to believe that the eleven numbered parameters vary independently in these patients. After Soothill J.F. (1968) Clinical Aspects of Immunology, 2nd Edition, ed. Gell and Coombs. Oxford: Blackwell Scientific Publications.
Phenytoin and troxidone may also induce fever, enlargement of lymph nodes and skin rashes.

**Immunological deficiency diseases in man**

So important are the immunological mechanisms for survival that it is not surprising that patients are rarely seen suffering from defects of the mechanisms that lead to deficiencies. However, deficiencies of the hormonal and cellular components of the immunological system may arise and occur separately or together. Table 23.1 summarizes some of the features of the deficiencies and fig. 23.5 provides a more elaborate analysis of possible conditions.

**DEFICIENCY OF HUMORAL IMMUNITY**

A condition known as hypo- or agammaglobulinaemia arises as a sex-linked hereditary disorder. The lack of immunoglobulins is not absolute but the patients fail to respond to antigenic stimuli. However, delayed hypersensitivity and the capacity to react to a homograft are normal. It is not incompatible with survival for many years, though the patients are very susceptible to bacterial infections, particularly to pyogenic cocci. The analysis shown in fig. 23.5 is at present largely speculative but as the different immunoglobulins have different functions, deficiencies might be expected to produce different clinical pictures. Thus IgM deficiency is possibly associated with meningococcal meningitis and lack of IgA, which is secreted by mucous membranes, with gastrointestinal or respiratory tract infections. One possible explanation of such syndromes is abnormal functioning of the regulator genes determining Ig synthesis.

Maternal IgG passes across the placenta to the foetus during the third trimester of pregnancy. Premature babies may thus have some degree of hypogammaglobulinaemia and prophylactic IgG treatment may reduce the incidence of infections. Immunoglobulobulin deficiency may arise also in adults as a result of abnormal metabolism of serum proteins and this appears to occur in renal failure with uraemia where susceptibility to infection is increased. There is also evidence that in some patients the immunoglobulins are present in normal concentrations but are functionally defective (dysgammaglobulinaemia).

**DEFICIENCY OF CELLULAR IMMUNITY**

This condition is analogous to that which develops in thymectomized neonatal mice (vol. 1, p. 27.19). There may be severe lymphocytopenia and a predominance of reticulum cells in the lymphoid tissue, and the patient's lymphocytes are unable to respond by transformation, proliferation or differentiation following stimulation with antigens in vitro. On account of the deficiency in delayed hypersensitivity, affected children cannot reject skin homografts and do not respond in the normal way to antigens such as monilia. The child fails to thrive and there is wasting and diarrhoea. Moniliasis and viral infections arise and survival is usually brief. In a few individuals the lymphocyte count may be normal, but the cells are functionally incompetent (dyslymphocytosis).

**COMBINED DEFICIENCY STATES**

Several types have been described in which there are combinations of agammaglobulinaemia and delayed hypersensitivity. In one hereditary type there is incomplete descent of the thymus gland embryologically; histologically the thymus shows general hypoplasia and disturbed structure, with a predominance of epithelial cells and reticulum cells, and absence of Hassall's corpuscles. Di George and his colleagues in 1968 described a distinct group of patients with isolated lymphopenia in whom multiple malformations resulted from a suppressed development of the third and fourth branchial pouches. In the first few days of life there is tetany attributable to hypoparathyroidism; if the infants survive this critical phase, lymphocytic incompetence is manifested later. The recognition of this type of immunological deficiency is of practical as well as theoretical importance as it should be correctable by replacement of the thymus. This in fact was demonstrated later the same year in two cases who were given transplants of fragments of human foetal thymus.

**Abnormal immunoglobulin synthesis**

In various proliferative and neoplastic disorders, mainly of the reticuloendothelial system, discrete components of immunoglobulins (M-proteins) may appear in the plasma. M-proteins are remarkably homogeneous. In an individual patient they belong to only one of the five immunoglobulin classes IgG, IgA, IgM, IgD or IgE, and contain light chains of either K or L type, never mixtures. Myeloma (p. 28.4) and Waldenstrom's macroglobulinaemia are the most important of these conditions and when associated with these diseases the proteins may be designated G-, A-, or D-myeloma proteins or M-macroglobulins (Waldenstrom). Abnormal proteins may occur also in the urine in these disorders. Bence Jones protein is found in the urine of about 50 per cent of patients with myeloma and 15 per cent of those with macroglobulinaemia. It consists of light chains only, usually dimerized, and in an individual patient these are either all K- or all L-chains (p. 22.11).

Heavy chain disease is a very rare disorder that occurs in association with rapidly progressive lymphomatous tumours. A dimer of the γ heavy chain which resembles the Fe fragment is present in the plasma and urine. No diseases with abnormal production of the heavy chains of immunoglobulins other than IgG have yet been described.

M-proteins are either abnormal proteins not present in the body in health or the result of excessive synthesis of
a homogeneous protein which in health constitutes only a small proportion of normal protein molecules. The cellular basis for the homogeneity of M-proteins is probably increased synthesis by a single group or clone of lymphoid cells. Patients with abnormal immunoglobulin synthesis have an increased susceptibility to bacterial infections and particularly to bacterial pneumonia. This is partially the result of an impaired capacity to synthesize normal immunoglobulins and functional antibodies.

FURTHER READING


PROBLEMS OF AUTOIMMUNITY AS A CAUSE OF DISEASE

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"Advances in Medicine" Royal College of Physicians, London,
My purpose is to try and get the role of autoimmunity in disease into perspective. In so doing it is necessary to steer a rational course between the over enthusiastic claims of some and the unnecessarily severe scepticism of others.

There are four types of immunological reaction:

Type 1 - anaphylactic, reaginic-dependent
Type 2 - humoral antibody (IgG, IgM)
Type 3 - immune complex
Type 4 - delayed (cellular) hypersensitivity

In type 1 (anaphylactic or reagin-dependent), free antigen reacts with antibody passively sensitising the cell surface. While this reaction is important in such conditions as bronchial asthma, urticaria and hay fever, it has little relevance to autoimmunity and it will not be considered further. Types 2, 3 and 4 have all been implicated in autoimmune disease. In type 2 reactions, antibody in the circulation or produced locally by plasma cells reacts with antigen on the cell surface or elsewhere in the tissues. In type 3 reactions, antigen and antibody reacting in moderate antigen excess form complexes which, together with complement, are damaging to the tissues in which they are deposited. In type 4 reactions, immunologically competent mononuclear cells that have been modified in a specific manner are the effector mechanism while the target may be antigen on the surface of cells or otherwise distributed in the tissues (figure 1).

Type 2 Reactions:

The clearest example of a type 2 reaction as a cause of disease occurs in autoimmune haemolytic anaemia (see Dacie, 1957). In this condition, the red cell destruction is due to damage to the erythrocytes as a result of adsorption of autoantibodies to the cell surface. The presence of adsorbed antibodies on the surface of the red cells can be demonstrated by the antiglobulin test, otherwise known as the Coombs test (figure 2). When antihuman globulin is added to a suspension of the patient's red cells that
Antigens

Liberation of histamine and other pharmacologically active substances

Antibody

Site of involvement of complement

Mechanisms in mononuclear cells directed against two specificities

Fig. 1. The four types of immune reaction (from Coombs & Gell, 1968).
Fig. 2. Detection of antibodies on red cells (direct Coombs test).
have antibody on their surface, agglutination of the cells occurs. The "warm type" of red cell antibody causes removal of erythocytes largely by the spleen. On the other hand, agglutinating and complement fixing types of red cell antibodies lead to removal of RBC's by the reticuloendothelial system and notably by the liver. The severity of haemolytic anaemia can be assessed by labelling RBC's with $^{51}$Cr. Whereas transfused RBC's normally survive in the circulation with a half-life of about 25 days, in autoimmune haemolytic anaemia it has been estimated that normal RBC's survive in the circulation with a half-life of as little as 5 days. Autoimmune haemolytic anaemia was the first condition described as being autoimmune and is one of the clearest examples of autoimmunity as an important mechanism in the cause of disease.

Circulating antibodies reactive in vitro with components of tissues other than red cells are a common occurrence in a wide range of diseases in man, but their role in causing these diseases is less clear. With reference to type 2 reactions, the role of autoantibodies might be subdivided into four headings (Table I). Other examples of IgG antibodies being directly pathogenic include antibody to glomerular basement membrane in certain types of experimental glomerulonephritis in animals and possibly in Goodpasture's syndrome in man (Dixon, 1968). In these forms of glomerulonephritis, antibody to the glomerular basement membrane (GBM) is laid down in a linear form along the basement membrane. This can be demonstrated by immunofluorescence or by electronmicroscopy. Infusion of such antibodies into experimental animals rapidly induces glomerulonephritis and under certain circumstances this mechanism may result in damage to a kidney transplant in a patient suffering from glomerulonephritis.

The long-acting thyroid stimulator does seem to be a likely cause of thyrotoxicosis in some cases and the transfer of this IgG immunoglobulin across the placenta is the most probable explanation
<table>
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<td><strong>HUMORAL ANTIBODIES IN AUTOIMMUNE DISEASE (TYPE 2 REACTIONS)</strong></td>
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1. **Pathogenic per se**
   - e.g. red cell antibodies in autoimmune haemolytic anaemia,
   - long-acting thyroid stimulator in thyrotoxicosis,
   - GBM antibody in Goodpasture's syndrome and experimental glomerulonephritis.

2. **Only weakly pathogenic**
   - intrinsic factor antibody in gastric juice in pernicious anaemia.

3. **Pathogenic but only when combined with delayed hypersensitivity or other forms of tissue damage**
   - testicular antibodies in experimental orchitis,
   - thyroid complement fixing antibody in chronic thyroiditis.

4. **Harmless by products (indicators of other more significant immunological reactions)**
   - mitochondrial antibodies in primary biliary cirrhosis and active chronic hepatitis,
   - antibodies to A or I bands in skeletal muscle in myasthenia gravis.
for neonatal hyperthyroidism (Munro et al, 1967).

In other instances, current evidence would suggest that IgG antibodies may only have a weakly pathogenic effect. Thus, the occurrence of intrinsic factor antibody I in the circulation of a patient shows a strong correlation with Addisonian pernicious anaemia, but the role of the antibody in causing the severe gastric atrophy characteristic of this condition is not clear. For example, pernicious anaemia may occur in patients with marked hypogammaglobulinaemia. However, intrinsic factor antibody has been shown to be present in the gastric juice of some patients with pernicious anaemia and it may block the effectiveness of what little intrinsic factor the stomach is still capable of secreting and hence precipitate malabsorption of vitamin $B_{12}$ (Roitt & Doniach, 1969).

In yet other circumstances, humoral IgG antibodies may only be pathogenic when combined with type 4 reactions (i.e. delayed cellular hypersensitivity) or other forms of tissue damage. Patricia Brown and her colleagues (1967) have shown that in experimental orchitis in guinea pigs, neither circulating antibody nor delayed hypersensitivity alone is sufficient to produce lesions but that both types of immune reaction are necessary. Again complement fixing antibodies to thyroid secretory epithelium are a frequent occurrence in autoimmune thyroid disease and they seem to have little or no pathogenic role by themselves. These antibodies can be transmitted across the placenta with no evidence of damage to the foetal thyroid. Although at least one of these antibodies can bring about rapid destruction of trypsinised human thyroid cells in vitro in the presence of complement (Irvine, 1962), in vivo it seems to have difficulty in getting at the intracellular antigens.

Finally, in yet other situations, antibodies detectable in the serum and often useful diagnostically, seem to have no discernable pathogenic role whatsoever. For example, mitochondrial antibodies, that have no tissue specificity, occur in patients with active chronic hepatitis, in primary biliary cirrhosis and in cryptogenic cirrhosis (Doniach et al,
Again, antibody to thyroglobulin would seem to have little to do with the pathogenesis of chronic thyroiditis; it is harmless to thyroid cells in tissue culture and its transfusion into animals produces at best a weak granulocytic reaction in the thyroid (Karensen & Godal, 1969). Weir and Elson (1969) have produced evidence which suggests that IgM antibodies may be important physiological scavengers rather than be implicated in the pathogenesis of autoimmune disease.

In summary, circulating or locally produced antibodies involved in type 2 reactions may be directly responsible for certain diseases, while in other diseases such antibodies, although abundantly present and useful in diagnosis, seem to have little or nothing to do with pathogenesis.

Type 4 Reactions:

It is appropriate to consider type 4 reactions next because type 2 and type 4 reactions are frequently associated one with the other in human disease and in experimental autoimmune disease. Type 4 reactions are those mediated by specifically modified immologically competent lymphocytes and in which free antibody plays no part. The evidence for the importance of type 4 reactions (referred to as delayed cellular hypersensitivity) can be summarised as follows:

1. Histology of the affected organ shows lymphocytic infiltration and atrophy.
2. Certain diseases (such as thyroiditis, adrenalitis, allergic encephalomyelitis, gastritis) can be produced in animals by methods that favour cellular hypersensitivity. Such methods include the use of a mycobacterial adjuvant and the use of the intradermal or foot pad route.
3. Where it has been attempted these diseases cannot be induced in neonatally thymectomised animals, but can be in bursectomised birds. Type 4 reactions are mediated by thymus dependent
lymphocytes while some type 2 reactions are not affected by absence of the thymus in the neonatal period but are dependent on the bursa of Fabricius in birds or its equivalent in higher species.

4. Positive in vitro tests supposedly for cellular type hypersensitivity have been obtained with the leucocytes of patients or of experimental animals with certain autoimmune diseases. These tests include:-

(a) Uptake of specific antigen by sensitised lymphocytes
(b) Antigen-induced blast transformation of these cells
(c) Inhibition of macrophage migration by specific antigen
   (David & Paterson, 1965)
(d) Production by sensitised lymphocytes of a specific cytotoxic effect on myelin, colon cells or thyroid epithelial cells in tissue culture (e.g. Perlmann & Broberger, 1963, in relation to ulcerative colitis).

5. Finally, certain diseases induced immunologically in experimental animals have been transferred to normal animals by means of lymphocytes alone (e.g. experimental adrenalitis, Levine & Wenk, 1968).

The frequent association of type 2 and type 4 reactions has already been alluded to - i.e. those mediated by humoral antibodies and those mediated by lymphocytes. It has also been mentioned that, while type 4 cellular hypersensitivity reactions are likely to be more important in pathogenesis, type 2 reactions may play some synergistic role or perhaps have no pathogenic function at all. Figure 3 indicates why this association may occur. The immunological system can be broadly subdivided into two divisions - cellular immunity which is thymus dependent and humoral immunity which is not dependent on the thymus but which, as already mentioned, is dependent on the bursa of Fabricius in birds or its equivalent in higher species (Good & Finstad, 1969).

However, there are certain antigens that will only give rise to humoral antibodies if the thymus is present during the neonatal period. For
Possible Relationship Between Type 2 and Type 4 Immune Reactions

T-LYMPHOCYTE

+ antigen
(± macrophage)

co-operation

B-LYMPHOCYTE

+ thymus dependent antigen

+ thymus independent antigen

Delayed (cellular) hypersensitivity
(type 4 reaction)

the humoral antibody synthesis
(type 2 reaction)

Fig. 3. Possible relationship between type 2 and type 4 immune reactions.
bursa dependent lymphocytes to respond to these antigens there is evidence that the co-operation of thymus dependent lymphocytes is required (Roitt et al, 1969). It may be because of this phenomenon that humoral antibodies with or without a role in pathogenesis are so frequently found in conjunction with delayed hypersensitivity (type 4) reactions.

Immune mechanism may simply be part of a chain of events that leads to the final end product, i.e. the clinical manifestation of disease. Experimental myasthenia gravis is an example. Here one can induce a thymitis by immunisation schedules using thymus or skeletal muscle. Circulating antibodies reactive with the A or I bands of skeletal muscle are produced but by themselves would appear to have no effect on neuromuscular transmission. These antibodies seem to be another example of non-pathogenic type 2 immune reactions. There is evidence that the thymus, which is the site of immunologically induced thymitis, releases a substance that is not an antibody but which impairs neuromuscular transmission in a manner characteristic of myasthenia gravis (Goldstein & Whittingham, 1966; Kalden et al, 1969).

**Type 3 (Immune Complex) Reactions:**

Type 3 reactions are induced by the deposition of antigen-antibody complexes in the tissues causing inflammation via mediators such as complement and polymorphonuclear leucocytes. Perhaps the best example of disease induced by this mechanism is to be found in some cases of glomerulonephritis. Thus, in the single clinical entity of glomerulonephritis there are at least two autoimmune pathogenic mechanisms and, in all probability, numerous aetiological factors capable of initiating each. As we saw previously, in glomerulonephritis induced by a type 2 reaction the patient or experimental animal produces or is given antibodies reactive with his own glomerular basement
membrane (GBM). In glomerulonephritis produced by a type 3 reaction the specificity of the antibodies is irrelevant; what is important is that antigen-antibody complexes of appropriate size are present in the circulation and are trapped in the glomerular capillary walls or filter. In experimental situations in which quantitation is possible, the degree of glomerular injury appears to be directly related to the quantity and quality of antibody and/or antigen involved. There is no indication that type 4 reactions play any part in glomerulonephritis; mononuclear cells, the hallmark of type 4 reactions, are conspicuously absent in this condition.

The only property of antigen-antibody complexes presently known to affect their localisation in vessels is size. Small complexes in great antigen excess tend to remain in the circulation whereas frank precipitates formed at equivalence or at antibody excess are rapidly taken up and disposed of by phagocytes. The intermediate sized complexes formed in moderate antigen excess are still soluble but large enough to react with complement and are the most likely to be trapped in vessel walls and so induce inflammation. The vessel walls of the renal glomeruli are particularly vulnerable. Morphologically, the trapped antigen-antibody complex appears under the electron microscope as heaped up lesions on the epithelial surface of the glomerular basement membrane in contrast to the linear deposits seen in type 2 reactions involving GBM. With immunofluorescence, the staining is seen to be distinctly granular in a type 3 reaction (Dixon, 1969).

Type 3 reactions may be related to autoimmunity or they may not be. The antigen involved may be autogenous or it may be quite foreign to the body. Thus, post-streptococcal glomerulonephritis, at least as it is seen in children, is caused by the deposition of circulating non-glomerular antigen-antibody complexes. The best known example of type 3 immune reactions as a cause of spontaneously occurring
glomerulonephritis is that associated with lupus erythematosus in man and that which occurs in NZB/W hybrid mice. Both these diseases are associated with the formation of antinuclear antibodies, particularly with DNA and the deposition of these antibodies, nuclear antigens and complement in the glomeruli (Koffler et al, 1967).

Immunosuppression:

Further evidence that immunological mechanisms may play some part in the pathogenesis of certain diseases is that immunosuppressive treatment may be beneficial. The use of corticosteroids in systemic lupus erythematosus, autoimmune haemolytic anaemia and liver disease is well established. Some corticosteroids have also been shown to be effective in restoring acid secretion in some patients with pernicious anaemia. In 1963, the effectiveness of 6-mercaptopurine in suppressing the primary antibody response and allograft rejection in experimental animals was demonstrated. Soon afterwards, the same drug was applied to the treatment of human autoimmune diseases, with limited success in autoimmune acquired haemolytic anaemia and chronic hepatitis. In the form of azathioprine and combined corticosteroid drugs (a regime which had proved successful in maintaining renal homografts) successful suppression of systemic lupus erythematosus and of rheumatoid arthritis has been reported in about 70% of cases. The use of antilymphocytic antibody in autoimmune disease has been reviewed by Denman (1969).

Why Should Autoimmune Reactions Occur?

Finally, we should consider why it is that autoimmune reactions occur (Table II). There are some antigens, such as those of the lens of the eye, that do not normally come into contact with the immunological system so that there has been no opportunity for immunological tolerance or self-recognition to develop. Thus, injury to an eye may lead to the escape of antigen with antibody formation as a physiological consequence
### TABLE II

**WHY SHOULD AUTOIMMUNE REACTIONS OCCUR?**

1. **Release of antigens to which tolerance is not normally fully established**
   
e.g. sympathetic ophthalmia.

2. **Antigens to which an immune response is a result of breakdown of previously established tolerance**
   
e.g. ? organ-specific autoimmune diseases
   
   (chronic thyroiditis, idio. Addison's, P.A.) and ? in non-organ-specific autoimmune diseases (S.L.E.).

3. **Invading micro-organisms may share antigen with certain host tissue**
   
e.g. ulcerative colitis, rheumatic carditis.

4. **Chemicals or viruses may change tissue constituents to render them antigenic**
   
e.g. sedormid purpura.
and lead to immunological damage to the other eye. A further example of this is male sperm. If this is extravasated following blockage of the vasa efferens it may induce antibody formation (Rumke, 1965). This mechanism would not seem to be a common cause in terms of the whole spectrum of autoimmune diseases.

Secondly, there may be a defect in immunological tolerance either occurring spontaneously (possibly by somatic mutation or genetically) or induced by some exogenous factor. The immunological system is a most intricate and precise system whereby substances foreign to the body are clearly distinguished from the body's own constituents. It would therefore be strange if this system was not liable to breakdown so that errors were made as to what is part of the body and what is not. On present evidence, it would seem that the bulk of the autoimmune diseases may come under this category with the organ-specific (but interrelated) disorders of autoimmune thyroid disease, Addisonian pernicious anaemia, idiopathic Addison's disease, idiopathic hypoparathyroidism and autoimmune ovarian failure at one end of the spectrum and with the non-organ-specific diseases such as lupus erythematosus at the other end with Sjogren's disease and the autoimmune liver diseases perhaps coming somewhere in between (Irvine, 1964; Roitt & Doniach, 1965).

In a hypersensitive host, invading micro-organisms may share antigen with certain of the host tissues and induce the formation of antibodies against these tissues. Possibly this may be an effect of antigen dosage. Thus some group A beta-haemolytic streptococci share antigen with cardiac antigen, which may be relevant to rheumatic carditis. Likewise, the sharing of antigen between E. coli 014 and colon epithelium may be relevant in the pathogenesis of ulcerative colitis (Asherson & Holborow, 1966). Alternatively, tolerance may be broken by an exogenous agent acting as a hapten when linked to a tissue component, such as occurs in sedormid thrombocytopenic purpura (Ackroyd, 1964).
When the breakdown of immunological tolerance seems to be spontaneous one asks whether it is genetically determined, whether it occurs by spontaneous mutation or whether it might be induced by some agent such as a virus that has so far escaped recognition at least in the human situation. A characteristic feature of the diseases in this group is that the autoantibodies are formed continually, at least over a period of years. Thus, if a tissue is damaged - such as occurs in myocardial infarction, burns, etc. - in general the corresponding antibodies only appear transitorily. One of the clearest examples of this is idiopathic autoimmune adrenal failure compared to tuberculous destruction of the adrenal. Idiopathic adrenal failure is associated with type 2 and type 4 immune reactions specific for the adrenal, while circulating adrenal antibodies and delayed hypersensitivity reactions against adrenal antigen are conspicuously absent in tuberculous destruction of the gland (figure 4) (Irvine et al, 1967; Nerup et al, 1969). The association of idiopathic Addison's disease with other organ-specific autoimmune disorders (Irvine, 1970) would tend to count against a random spontaneous mutation in the immune system as being the likely explanation. It is known that there is a genetic factor operating in the autoimmune diseases of disordered immunological tolerance, but the exact mode of inheritance escapes clear definition.

The genetic factor could operate as a built-in defect in the immune system so that, usually later rather than sooner, tolerance breaks down according to a certain pattern. Indeed, the formation of certain organ-specific autoantibodies seems to be a function of ageing, especially in females. Alternatively, what may be genetically determined is susceptibility to an exogenous agent such as a virus.

Figure 5 is a synopsis of what may be happening in the NZB/W mice which have been so extensively studied as a model of disseminated lupus erythematosus. Genetic factors are clearly important in this strain, but
Fig. 4. Adrenal specific antibodies (IgG) in the sera of patients with idiopathic (autoimmune) adrenal insufficiency compared to their absence in tuberculous adrenal destruction (from Irvine et al, 1967).
AUTOIMMUNITY IN NZB MICE

Vertically transmitted virus

? Certain tissue cells rendered antigenic
? Hyper-reactive lymphoid system

R.B.C.  Nuclei  Thymus

Autoimmune Haemolytic Anaemia (type 2 reaction)

Release of nuclear material + formation of antinuclear antibodies

Ag-Ab complexes in serum

Glomerulonephritis (type 3 reaction)

Thymitis (? type 4 reaction)

Fig. 5. Suggested role for autoimmunity in NZB and NZB/W mice.
the following evidence strongly points to a genetic susceptibility to a particular type of virus that is vertically transmitted from mother to unborn young. Thus, autoimmune disease with positive Coombs and LE cell tests can be transmitted to other strains of mice by grafts of thymus from autoimmune strains, by inocula of spleen cells and most significantly by cell-free extracts from spleen. Moreover, the development of positive Coombs tests can be significantly delayed by vaccinating baby NZB mice with formaldehyde inactivated cell-free filtrates of older NZB mouse spleens (Mellors, 1969). The virus may change various tissue components to make them antigenic or it may render the lymphoid system hyper-reactive. To recapitulate what has been said above, IgG antibodies to RBC's would then induce haemolytic anaemia by type 2 reaction; damage to cell nuclei may cause release of nuclear antigens and the production of antinuclear antibodies with the formation of immune complexes. In turn, these, in the presence of excess antigen and with the aid of complement, may get trapped in the renal glomeruli with consequent glomerulonephritis induced by a type 3 immune reaction. Finally, much attention has been given to abnormalities of the thymus as a cause of autoimmune disease, particularly in the NZB model. However, if a normal thymus is transplanted into an NZB mouse, it also develops lymphocytic infiltration in the thymic medulla (Helyer & Howie, 1963) suggesting that the thymus is simply another target organ for autoimmune reactions, possibly mediated by a type 4 mechanism. Neonatal thymectomy does not prevent the onset of Coombs positivity in NZB mice (Howie, 1969) and, with the exception of myasthenia gravis, thymectomy in man would appear to be of little benefit in adults with diseases associated with autoimmunity (Irvine, 1968).

However, what holds for mice may not hold for men. Whether or not there is an exogenous factor operating in man is a question that remains unanswered. Also unanswered is the question as to why immunological tolerance should break down according to certain patterns that we as
clinicians recognise as inter-related diseases in which immune mechanisms seem to play an important part.
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CHAPTER XII

WORK SUBMITTED IN SUPPORT OF THE THESIS
CLINICAL AND EXPERIMENTAL IMMUNOLOGY

Edited by W. J. Irvine

CLINICAL AND EXPERIMENTAL IMMUNOLOGY is an international journal. The aim of the journal is to integrate the clinical and laboratory aspects of immunology.

Along with IMMUNOLOGY it is an official journal of the British Society for Immunology. Published by Blackwell Scientific Publications Limited (Oxford and Edinburgh), it was started in 1966 with myself as Editor-in-chief and an international Editorial Board. The journal rapidly became established as a major publication in immunology. This is manifest from the growth of the journal from a quarterly issue in 1966, to bimonthly in 1967, to nine issues per annum in 1968 and finally to monthly in 1969 and thereafter. The journal expanded in 1970 to allow publication of 1800 pages in two volumes each of six issues. In 1971 it will reach its optimum size of 2000 pages per annum.

The statistics for the journal are shown in Fig. 1. The mean publication time has been maintained throughout at 6 months with a range 3-10½ months in 1969 (Fig. 2). The circulation of the journal continues to rise satisfactorily.
Clinical and Experimental Immunology is published quarterly, each issue consisting of about 100 pages. Four issues form one volume. The annual subscription is £5 (U.S.A. and Canada $17.50); price per number 30s. (U.S.A. and Canada $5).

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Business matters, including correspondence and remittances relating to subscriptions, back numbers and reprints, should be sent to the publishers: Blackwell Scientific Publications, 24-25 Broad Street, Oxford.
EDITORIAL

The advances in immunology in relation to clinical medicine have been so rapid and expansive in recent years that a medium for publication of original papers in this line of study has become necessary. As in other subjects, many of the initial clues have come from clinical observation and subsequent advance has been achieved by a combination of continued clinical study with the support of more readily controlled and precise experiments in the research laboratory. It is the aim of *Clinical and Experimental Immunology* to encourage greater integration between clinical observation and fundamental laboratory research.

The subject of immunology has many ramifications and there are already in existence a number of excellent journals which, over the years, have become concerned with some particular aspect. This new Journal has been inaugurated to provide a forum for articles that are concerned with immunological phenomena in relation to disease. In addition to papers describing the incidence of antibodies and their value in diagnosis in different disease states, the Journal will publish articles which are concerned with methods of treatment of these conditions by altering the patient's immunological status. Of equal interest to the Journal are papers which deal with the fundamental nature of the disorder in immunological homoeostasis that is manifest in a wide range of disease states, or which help to elucidate the possible role of immunological mechanisms in pathogenesis. In the study of the natural history of diseases associated with autoimmunity it is important to be able to detect the presence of a pathogenic process in the subclinical state. The application of immunological techniques to the assay of hormones and other body secretions is therefore highly relevant to the interests of this Journal. The interests of the Journal are, in fact, well represented by the contents of this first issue.

Some people feel that there are already too many scientific journals and wonder why it is necessary to introduce yet another. However, if there is to be progress in research, or in any other line of significant human activity, there must be change with growth and development in some areas and quiescence in others. Those responsible for the production of scientific journals must adapt themselves to the needs of the present time by either altering the format and interests of current journals or by introducing new ones. The justification for each journal must be the need which it supplies. Scientific papers relevant to the interests of a sufficiently large group of research workers should be made readily available, but beyond this the aid of a computer is required if a fully comprehensive survey of the literature in any particular field is to be achieved.

The period between the receipt of a paper in the Editorial Office and its eventual publication is not always satisfactory to either the authors or readers. How to reduce this time delay is an editorial problem. Information Exchange Group Number 5 (National Institutes of Health, U.S.A.) provides a means whereby unedited scientific papers on immunopathology are precirculated and may be referred to as a 'personal communication'. It may be that scientific journals should themselves be precirculated in off-set typing at the stage of acceptance of the paper by the journal for publication. The scientific information would
then be available with a minimum of delay, although the final printing may take up to another 6 months or longer. It is my view that this would be a useful service, but whether it would be practicable to put it into effect is still under discussion.

Research is not limited by geographic boundaries and, therefore, my policy has been to make this Journal international. This policy is reflected in the Editorial Board. In establishing this Journal I also felt that it should be associated with a scientific society. Such an association should put the Journal on a surer basis and help to integrate the Journal with existing publications in the field of immunology. The British Society for Immunology has agreed to sponsor Clinical and Experimental Immunology as a sister journal to Immunology and to encourage its international character.

There are many scientific problems that are still to be solved concerning the immunological aspects of human disease and it is hoped that the pages of Clinical and Experimental Immunology will make stimulating reading both to clinicians and to immunologists in the years to come.

W. J. Irvine
Fig. 1 Statistics for the Journal CLINICAL AND EXPERIMENTAL IMMUNOLOGY for 1966 to 1969.
Fig. 2  Distribution curve for the time interval in months between receipt and distribution for papers received in 1969.