A STUDY OF THE SKELETAL MUSCLES OF SHEEP WITH SPECIAL REFERENCE TO SCRAPIE DISEASE

A Ph.D. THESIS SUBMITTED BY

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TEXT
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INTRODUCTION

This thesis describes an attempt to find out if specific changes occur in sheep affected with scrapie. In the expectation that a detailed study of a narrow field would be more productive than a study of many organs or systems, the work was limited in scope and represents but a small part of the broad, many-faceted approach to scrapie being conducted at the Moredun Research Institute.

In the past numerous observations on the symptoms and pathology of scrapie have invariably implicated the nervous or muscular systems or both as the primary site of the disease. However, both the macroscopic and microscopic findings have been inconclusive and their interpretation subject to controversy. The work described here forms part of an attempt to resolve some of the uncertainties. Using approximately the same series of cases, the author studied the skeletal muscle, while Wight (1958) investigated the spinal cord and Zlotnik (1958 a, b) the central nervous system.

Investigation into the condition of muscle in scrapie seemed particularly desirable in view of the reports of Bosanquet, Daniel and Parry (1956) and Parry (1957) in which these workers attributed all the symptoms manifest by sheep suffering from scrapie to primary muscular degeneration. Their contention was not refuted in subsequent reports by Palmer (1957) and Delez, Gustafson and Luttrell (1957) although their results were based on limited surveys. This view was particularly surprising in view of the mounting circumstantial evidence that scrapie was primarily a disease of the central nervous system,
an hypothesis which appeared to explain more adequately the symptomatology of the disease.

Early in the course of the work described here it became apparent that there was a lack of exact knowledge of the microscopic muscle structure of domestic animals. For this reason it became necessary to investigate the muscle structure of sheep in a variety of diseases and conditions other than scrapie. It was deemed desirable to record these findings in some detail in order to evaluate the changes found in the muscles of sheep suffering from scrapie.

Extrinsic ocular muscles were chosen for investigation primarily because they have been specifically implicated in the production of the symptoms of scrapie (Bosanquet et al. 1956, Delez et al. 1957). Although these muscles must undoubtedly be classified as skeletal muscles, available evidence indicates that structurally and in method and density of innervation they are distinctly different from limb muscles for example, consequently extrinsic ocular muscles are considered under separate headings.

An essential part of the investigation involved the examination of the sensory and motor innervation of muscles from scrapied sheep. It seemed not unreasonable to suppose that changes at the myoneural junction might provide an explanation for the occurrence of ataxia in cases in which degenerative cord and muscle changes were negligible. Such a lesion could also explain the atrophy seen in cases of scrapie. To determine the range of peripheral motor nerve changes that would be detectable and also to evaluate the efficiency of the examination methods used, denervation experiments were carried out on guinea pigs. This series provided information which was of value in the interpretation
of the motor end plate changes encountered in sheep.

The blood vessels, a major component of muscles, were also examined in some detail. In the course of a preliminary investigation it appeared that scrapied sheep might have a higher incidence of intramuscular arterial abnormalities than non-scrapied sheep. Since no comprehensive data on the pathology of the vascular system of sheep could be found, an investigation into the condition of the more numerous and accessible visceral arteries was initiated. The results of this survey and a consideration of the pathology of the intramuscular arteries are included in this report.
MATERIALS AND METHODS

SHEEP

A. Skeletal Muscles

The mature sheep used in this survey originated from several different areas of Scotland and Northern England and from flocks maintained at this Institute for breeding and transmission experiments. There were one hundred and twenty Cheviots and five Cheviot crosses, thirty-five Suffolks and one Suffolk cross, eleven Blackfaces and one Blackface cross, seven Swaledales, four Border Leicesters, two Wensleydales and one Dalesbred. One hundred and forty-five were females, ten were males and thirty-two were castrates. Observations were also made on tissues from four young lambs and fifteen foetuses.

Sixty sheep suffering from naturally occurring scrapie were selected by shepherds, owners or practicing veterinarians and were observed at this Institute for periods of up to five months. The preliminary diagnosis of scrapie based on clinical symptoms was confirmed in every case by examination of serial sections of the medulla as described by Zlotnik (1958). The average age of this group was 2.6 years and the range was 2 to 5 years.

The scrapie-producing agent isolated by Wilson, Anderson and Smith (1950) from a field case and maintained by subcutaneous or intracerebral passage in sheep was used to induce scrapie in an additional thirty-four experimental animals. Suspensions of infective brain or
spleen were inoculated subcutaneously or intracerebrally, and the sheep were kept under observation from the time that the first clinical signs of scrapie appeared. The clinical diagnosis was confirmed in every case by histological examination of the medulla. The average age of this group was 2.1 years and the range was 1 to 7 years.

Natural and experimental cases were, in most instances, killed when death seemed imminent, though a few in both groups were killed at an earlier stage when clinical symptoms were well established.

The control group of ninety-three animals were normal or were suffering from diseases other than scrapie (see Table III). Some were submitted to this Institute suspected of being affected with scrapie; however, careful clinical observation and post-mortem examination including histological study of the medulla proved this diagnosis to be incorrect. The average age of this group was 2.9 years, the range 1 to 7 years.

A thorough post-mortem examination of the skeletal muscle of sixty natural and thirty-four experimentally induced scrapie cases and seventy-six animals from the control group was carried out in the following manner. Carcasses were eviscerated and skinned, then left in a cool room or a cold box for from four to twenty-four hours to allow the muscle to set. During the gross examination each muscle was separated at its origin and insertion and carefully inspected for abnormalities. Next, each muscle was cut transversally or longitudinally into one-quarter to one-half inch thick slices and each slice examined. Samples of muscle selected for histological examination included any haemorrhagic portions and any areas which were pale or streaked.
Many random samples of apparently normal muscle were also taken and in all up to ninety-four samples were obtained from each animal. In some instances several samples were taken from a single muscle. The specimens were placed on thin pieces of cardboard and fixed in ten per cent formol-saline for at least four days. Many were post-fixed in formol-sublimate (900 c.c. saturated aqueous mercuric chloride and 100 c.c. of formalin) for 24 to 48 hours. All samples were dehydrated through alcohols, cleared in benzol or turpentine and embedded in paraffin with a melting point of 56 degrees centigrade. Sections seven to ten microns thick were cut and mounted on albuminized slides. Attempts were made to examine about half of the samples in the transverse and half in the longitudinal plane; preparations in both planes were often made from a single sample.

One section from each block was routinely stained with haematoxylin and eosin (H and E). In addition selected muscle sections were stained by several other methods, notably; Mallory's phosphotungstic acid haematoxylin (PTAH) (Mallory 1938), Giemsa's (Culling 1957), Kull's (Miller 1933), van Gieson's (Mallory 1938), von Kossa's (Culling 1957), Heidenhain's iron haematoxylin (Culling 1956), Lendrum's phloxine and tartrazine (Lendrum 1947), Perle's (Culling 1957), Gram's (Culling 1957), and a modification of Lendrum's kiton fast red stain (Lendrum 1949) involving the use of a counterstain of 2 per cent light green instead of the described lissamine in tartrazine (see Figs. 45, 46 and 50). Sections were dehydrated in alcohols, cleared in xylol and mounted in D.P.X. (Kirkpatrick and Lendrum 1941).

Muscle fibres in cross section were measured under high power magnification (X 485) by means of an ocular micrometer. In view of
the fixation artifacts produced at the edges of sections of muscle, these areas were ignored when making measurements. The measurement of all muscle fibres examined in this survey was the diameter recorded at right angles to the greatest diameter so that the abnormally large diameters encountered in fibres cut in slightly oblique sections were not included in the results. The diameters thus obtained were in fact found to be the least diameters except in specimens of very irregularly shaped fibres. Random sampling of fibres for measurement was accomplished by measuring all the wholly visible fibres in every fifth field in every fifth row. Camera lucida drawings were made using the one-sixth objective and a X6 ocular. The outlines were inked in and reduced photographically.

Motor nerve endings in muscle were studied in samples from fourteen naturally scrapied sheep, eight experimentally scrapied sheep and seventeen animals from the non-scarpia group. Several different methods of demonstration were utilized. Two modifications of Ranvier's gold chloride method were used. In one, pieces of fresh muscle 1 cm. x 1 cm. x 2 cm. were taken immediately after death, stripped of surface fascia and processed as described by Cole (1946). Frozen sections one hundred microns in thickness were then cut from the surface, floated onto slides and mounted in von Apathy's medium (Culling 1957). From five to ten well impregnated sections free from deep black discolouration could be obtained by this method. In the second modification six to eight small slivers of fresh muscle 2 mm. x 2 mm. x 1 cm. were processed in the same way and after washing briefly in water were dehydrated through 70, 96 and 100 per cent alcohols in three to four hours. They were then cleared in benzole and
embedded in paraffin over a period of four hours. Ribbons of sections twenty microns in thickness were mounted on albuminized slides, brought to water, counterstained for two minutes in 2 per cent toluidine blue, dehydrated, cleared in xylol and mounted in D.P.X.

Another method used with considerable success was Koelle's cholinesterase technique, as modified by Coupland and Holmes (1957) with Gross-Schultz's silver method (Culling 1957) (see Figs. 83 and 84). Fresh muscle, obtained at death, was fixed in formol-saline for three to six hours at 4 degrees centigrade. Frozen sections one hundred microns thick were then cut and processed through the substrate as indicated by Coupland and Holmes. Following this the sections were washed in water for ten minutes, fixed in formol-saline for eighteen hours, then impregnated by the silver method. Next the sections were usually dehydrated, cleared in xylol and mounted in D.P.X. In some specimens the nuclei were counterstained with 2 per cent methyl green for two minutes prior to dehydration. A few sections underwent silver impregnation, then were stained with Kay and Whitehead's Sudan IV stain (Lee 1950) and haematoxylin and mounted in von Apathy's medium.

A parallel series of frozen sections cut at 30 microns was prepared from all muscles processed for the purpose of showing motor nerve endings. These were stained by the Sudan IV method already mentioned without prior staining.

Random sampling of muscles for the gold chloride and cholinesterase methods was carried out. If five or fewer muscles were sampled, one or more of the following was used: gracilis, semitendinosus, semimembranosis, latissimus dorsi and trapezius. If more than five muscles were sampled, the supraspinatus, longissimus dorsi and/or gastrocnemius
were also included.

Other methods used to study nerve endings were: Holmes' (1947), Gross-Bielschowsky's (bulk) (Lee 1950) and Palmgren's (1955).

B. Extrinsic Ocular Muscles

The extrinsic ocular muscles chosen for examination were the two recti muscles and the oblique and retractor bulbi muscles. These were fixed in the usual way. At least two cross sections of each muscle were taken from fifteen natural and fifteen experimental scrapie cases, as well as from fifteen animals of the control group. In ten scrapied and ten control animals the innervation of these muscles was studied by the cholinesterase-silver method already described.

C. Arteries

The arterial survey was based on the examination of visceral arteries of sixty-seven mature sheep and fifteen foetuses. Observations on intramuscular arteries seen in the many muscles sampled were also included. Nineteen of the animals from which visceral arteries were obtained were suffering from scrapie contracted naturally and were part of the group of sixty used in the muscle survey. Eleven were from the experimental scrapie group. Of the remaining thirty-seven, twenty were from the non-scrapie group used in the muscle survey and seventeen were animals killed at the local abbatoir and destined for
human consumption. The late-stage foetuses were also obtained from this abbatoir. Of the non-scrupled group of thirty-seven, twenty-nine were clinically normal and five were suffering from various diseases.

For histological examination of visceral arteries, fifty mature animals and fifteen foetuses were sampled taking one block from the spleen, from each kidney, from the pancreas and from the heart. Two blocks were taken from the liver. From the seventeen animals killed at the abbatoir, spleen only was taken. From five of the fifty sheep more extensively sampled, tissue specimens containing six or more arteries were taken from each of the following areas: carotid and internal carotid, gastric mesentery, intestinal mesentery, subcutaneous tissue, femoral and iliac arteries. At least three samples of each aorta and additional blocks of heart were taken.

A number of spleens were fixed in distention. This was accomplished by tying the splenic artery to a blunted sixteen-gauge needle attached to a seventy inch length of tubing which was fastened to an inverted wash bottle of 10 per cent formalin fixative. The apparatus provided pressure estimated to be equivalent to the normal blood pressure in the aorta at the level of the splenic artery.

The tissues were fixed in formal-saline and in many cases post-fixed in formal-sublimate. They were dehydrated in alcohols, cleared in benzole and embedded in paraffin. Sections were cut at seven to ten microns. Many sections were stained with haematoxylin and eosin but at least one section from each block was stained by Gomori's aldehyde fuchsin (Culling 1957) in combination with haematoxylin and kiton fast red (Lendrum 1949) (see Figs. 77 and 78). Selected duplicate sections were stained by the Hart modification of Weigert's elastic
tissue stain (Mallory 1938), by von Kossa's method (Culling 1957), by Highman's modification of Benhold's stain (Lee 1950), by phloxine and tartrazine (Lendrum 1949), by Perle's method (Culling 1957), and by Gordon and Sweet's method (Culling 1957). Frozen sections of arteries, cut at twenty microns, were stained by Sudan IV (Kay and Whitehead's method, Lee 1950) and tested by the Schultz histochemical method (Serra 1946). Whole fixed aortas were stained for neutral fat by immersion in Herxheimer's Sudan IV solution (Mallory 1938) for five minutes, washed in 50 per cent alcohol for five minutes and then washed in water.

D. Diet

The dietary regimen of the sheep in this survey varied a great deal, however some classification was possible following a consideration of the diet over the six months prior to death.

The first group consisted of animals submitted to the Institute with a provisional diagnosis of scrapie. Definite information on previous diet was not obtained, but as they came from either hill or lowland farms, it could be presumed that they were fed according to general practices on such farms, i.e. on hill or permanent pasture for the summer, and on pasture plus turnips, or rarely, pellet supplement, for the winter. During the terminal period of observation in the Institute (see Tables I and II), these animals were put into small enclosures of permanent pasture or were kept inside. In both places they received hay ad libitum and, from October to April, ewe nut
supplement obtained from one of the leading national producers of pelleted feeds. The hay which consisted of baled grass originated from many sources and represented a pool periodically replenished by a local feed merchant. This group was made up of sixty-seven sheep (S-1 to 33, 35 to 38, 40 to 51, 57, 59, 60, C-1, 2, 10, 16, 22, 25, 26, 29, 33, 34, 37 to 39, 44, 72, 75).

The second group consisted of animals raised on Institute land and kept all the year round on permanent grass pasture. From the end of September until May they were fed pooled hay. Starting in October two weeks prior to tupping they received an additional daily supplement of three-quarters of a pound of ewe nuts. This supplement was reduced to one-half pound per head after tupping and increased to one pound two weeks before lambing was expected to start. A few in this group also received turnips over the winter months. There were eleven animals in this group (S-34, 39, 52 to 56, 58, and C-3 to 7, 45 to 48, 57, 60).

The sheep in the third group were mainly housed indoors, although a few periodically had access to permanent pasture. The diet consisted of pooled hay ad libitum plus ewe nut pellets at the rate of one-quarter to one-half pound per day. Seventy-five animals were included in this group (E-1 to 34, C-8, 9, 11 to 15, 17 to 21, 23, 24, 26, 27, 30 to 32, 35, 36, 41 to 43, 49 to 55, 58, 59, 62, 64, 66, 67, 70, 71, 73, 76).

The fourth group were on an experimental diet calculated to produce hypomagnesaemia. Over the six month period they were fed grass hay from a single farm and in May were given cut, fresh green grass ad libitum. One sheep died shortly after being fed the fresh
grass. This group consisted of two animals (C-63, 69).

The fifth group were on a diet designed to produce a low copper status. The grass hay and oats fed were from a farm where cereals grown were copper deficient, although no muscular diseases had occurred in the sheep on this farm. In addition, this group received 8 g. per day of a mineral and vitamin mixture consisting of 500 g. calcium orthophosphate, precipitated, (B.D.H.), 200 g. sodium chloride (B.D.H. analar), 100 g. Rovimix-E (Roche) and 4 g. Rovimix A50-D3 (Roche) given orally. Each week each animal received an individual dose of 20 c.c. of a 0.75 per cent solution of potassium iodide (analar). There were four animals in this group (C-56, 61, 68, 73).

GUINEA PIGS

Changes in motor innervation secondary to denervation were studied in six guinea pigs. The animals used were three month old males weighing 400 to 550 grams. The left, posterior thigh muscles were denervated by high sciatic neurectomy, a 5 m.m. piece of the nerve being removed through an incision made about 5 m.m. posterior to the trochanter major. The animals were killed by decapitation three, six, nine, twenty, thirty and forty days postoperatively. The semitendinosus, biceps femoris and semimembranosus muscles on both sides were removed at death and fixed in formol-saline kept at a temperature of 4 degrees centigrade for six hours. Frozen sections were cut one hundred microns thick and processed by the methods of Coupland and Holmes and Gross-Schultz
as previously described. Additional sections from each muscle, thirty microns thick were stained with Kay and Whitehead's Sudan IV and haematoxylin. Portions of the same muscles were subjected to further formalin fixation, embedded in paraffin and cut into transverse sections.

DEFINITIONS

The term "control" is applied to a particular case or cases originating from the non-scrapied group. A "normal control" is a member of this group which showed no clinical symptoms of any disease.

The following prefixes are used to indicate the origin of the specimens discussed. S- before a case number indicates a member of the naturally scrapied group, E- indicates a member of the experimentally scrapied group, and C- indicates a diseased or normal non-scrapied control animal.

The term "arteriosclerosis" in the following text is used to describe changes, that without active inflammation, affect smoothness, thickness, uniformity, homogeneity of tissue, elasticity of the vessel wall, or maintenance of a normal lumen (Fox 1933). The term "intimal sclerosis" is used to describe arteriosclerotic changes limited to or occurring predominantly in the intimal layer of the blood vessels.
SHEEP

A. Clinical Examination

Of the sixty sheep in this survey which contracted scrapie under field conditions none could be selected for description as a completely typical case. However, a composite series of symptoms which appeared with varying degrees of intensity in most cases can be described.

The commonest first sign of the disease was pruritis. Specific attention was seldom directed to the animal until the wool showed a brushed appearance or until breaking of the wool or alopecia became apparent. As the time passed, the rubbing against convenient objects became more vigorous and feeding was interrupted at increasingly shorter intervals so that the sheep could rub itself. The areas of the body most frequently rubbed, scratched or nibbled were the loin, rump, stifle, elbow, head and occasionally the lower leg or foot. At times, it appeared that these were merely the most accessible areas, yet the pattern of rubbing was noticeably consistent despite the position or height of the object being rubbed against. The rubbing response to pruritis generally continued as long as the animal could perform the necessary activity, but in some cases there appeared to be a reduction in the frequency of rubbing as the animal approached the terminal stages of the disease. Although the skin in the rubbed, pruritic areas was sometimes reddened and eventually became coarse, hardened and occasionally
focally infected, no evidence of primary change was seen.

The "scratch reflex", a term used in descriptions of scrapie to describe the pleasurable lip-smacking response to light scratching of the skin, was shown by several animals in this series. The difficulty in demonstrating this response in other cases lay, it seemed, in the difficulty encountered in keeping the animal pacified for a sufficiently long period, since manual restraint often abolished the reflex even in animals which otherwise gave a good response. Many of the cases were from large hill flocks which probably accounted for their fear of an approaching human. A few animals were quiet in this respect, but despite repeated attempts still exhibited no satisfactory response.

The second symptom which became apparent at variable periods in the course of the disease was an alteration in gait. This was manifested in several ways; the first sign of it was often only a slight sway in the hind quarters, or a persistently dragged fetlock. In cases where this sign became more prominent, there was either pronounced swaying of the hind quarters with a rotary progression of the hind legs which were frequently held in partial flexion, or an obvious ataxia as a result of which the sheep moved its hind legs rapidly sideways in order to retain its balance. Occasionally the front legs were affected, but pronounced anterior involvement was rare. Eventually all animals became recumbent one or more days prior to death. Frequently this recumbency did not appear to be the result of a progressive ataxia but incidental to it. Symptoms of locomotor disturbance did not appear to abate, and in some cases they progressed up to the time of death. Not all cases showed this change in a pronounced form.

The third sign, which was often apparent early in the disease,
was a loss of condition of the animal. At first it was manifest only as a general loss of bloom on the fleece, but soon, particularly in shorn animals, the spinous processes and wings of the ilia became noticeably prominent as the surrounding muscles were reduced in volume. Some individuals seemed to be more resistant to this change than others, but none of the sheep included in this survey gained in condition. The rate at which condition was lost was very variable; in cases where emaciation was rapid the sheep became extremely thin.

Hyperesthesia or an altered reaction to stimuli was frequently but not consistently seen. Increased excitability manifested by elevation of the head, quick jerky movements and bulging or deviation of the eyes were the most frequent signs, but a few animals exhibited a generalized trembling in the presence of humans.

The apparent severity of the three major signs: pruritis, locomotor difficulty and loss of condition were arbitrarily rated (Table I). The most prominent symptom observed during the terminal, designated period was noted and is recorded in Table I.

Suffolks, as a group tended to show more prominent signs of ataxia than the other breeds studied. In fourteen out of twenty-four Suffolks the locomotor disturbances were considered the predominant symptom as compared to nine animals which showed a more prominent pruritic response. Severe hyperesthesia was not a characteristic sign of the disease in this breed. Among the twenty-three Cheviots and predominantly Cheviot crosses, signs of pruritis predominated in sixteen; only one was markedly ataxic. The naturally scrapied Cheviots provided all but one of the cases showing severe hyperesthesia or quivering. Of the three Border Leicesters, pruritis predominated in two, locomotor
difficulties in one. Scrapie in the Dale breeds (Swaledale, Dalesbred and Wenslydale) was expressed clinically in the pruritic form in eight out of ten animals while locomotor disturbances predominated in one. As a rule the symptoms in the Dale breeds were much less intense than in Suffolks or Cheviots.

Loss of condition observed between one inspection and the next was relatively slight, and was always overshadowed by the other symptoms. Suffolks as a group showed a slower loss of condition than other breeds and were less emaciated at the time of death. The Cheviot group showed the most rapid loss of condition, followed by the Border Leicesters. At their best the sheep of the Dale breeds were not well fleshed, and terminally they were very emaciated.

An estimate of the extent of emaciation existing when the animals reached the terminal state was made (Table I); however, the extent of carcass atrophy seen on post-mortem examination (Table IV, column 2) probably represents a more accurate evaluation of this change.

Within the group of thirty-four experimentally induced cases of scrapie the symptoms of the disease were comparable to the symptoms in the naturally affected group, although slight differences were observed.

In the experimentally induced cases pruritis was always present and was expressed in a manner similar to that seen in the natural group, however, in the terminal stages, the rubbing frequently abated as the animals became listless and lethargic. Pruritis was considered the predominant sign in twenty-three of the thirty-four animals (Table II).

Locomotor disturbances seen in this group consisted of partial flexion of the hind legs and a spraddled, rotary progression. Dragging
of the fetlock was also common and caused the animals to stumble frequently. Severe ataxia was seen in only two animals, but these two were the most severely ataxic animals in the entire survey (cases E-8 and 16). Locomotor disturbance was considered the predominant symptom in eleven of the thirty-four cases.

The loss of condition seen in the experimental group was no different from that of the natural group, but clinical emaciation was often extreme.

Hyperaesthesia or altered responses, were frequently present, but were not outstanding characteristics of the experimentally induced group. However, all members of the group appeared to go through a stage of altered reaction. The terminal lethargy so often seen might perhaps be considered a form of hypoaesthesia. A satisfactory scratch reflex could seldom be elicited in these animals.

In the control group of seventy-six sheep, symptoms varied greatly. Individual manifestations depended on the health status of the animal concerned (Table III). Thirty-nine sheep showed no symptoms whatever. Although the intense rubbing associated with advanced scrapie was not seen, many animals casually rubbed convenient objects from time to time. Three animals from the control group exhibited a very satisfactory scratch reflex (C-16, 38, 76). Locomotor disturbances were seen in six (C-28, 29, 39, 40, 68, 76), and terminal recumbency was not uncommon in those control animals which were in the final stages of some intercurrent disease.
B. Post-Mortem Examination

1. Skeletal Muscles

Macroscopic Examination

(a) Atrophy

An estimate of carcass condition in all the animals in this survey was made and is recorded in Tables IV, V and VI, column 2. The category chosen depended upon both the general appearance of the musculature and two specific features. The first of these was the depth of the depression of the supraspinatus, infraspinatus and longissimus dorsi muscles below the corresponding bony spinous processes, and the second was the amount and texture of the intramuscular and subcutaneous fat.

Six of the ninety-four scraped and twenty-one of the seventy-six control animals showed no apparent alteration in fat cover (-). Forty scraped and thirty-three control sheep showed some slight wasting of the muscles and an apparent reduction in the quantity but not in the quality of fat (+). A moderate but quite noticeable reduction of both elements was observed in twenty-eight scraped and fifteen control animals and in many of these the fat deposits showed definite signs of reduction and replacement by gelatinous material (++). Sheep in this category had muscles that were pale and occasionally soft but not flabby. In the final group of fourteen scraped and seven control animals
the macroscopic atrophy of the muscles was severe and no visible fat remained (+++). The muscles were pale, usually flabby, and were separated by oedema and gelatinous connective tissue. In the control animals the oedema was not as obvious as in the scrapied ones.

There was no apparent difference between the sheep with natural and those with experimental scrapie.

A characteristic of those carcasses that were very emaciated was that rigor mortis was absent or incomplete even after several hours in a cool room. A feature of one experimental and eight natural cases of scrapie was the presence of single or multiple sero-sanguinous pockets in the layer of subcutaneous fat over the back. The lesions varied from small foci to large, multilocular cavities extending from the sacral to the cervical regions.

(b) Other Lesions

Changes in the muscle mostly minute but visible macroscopically were seen in eight out of sixty natural scrapie cases, in three out of thirty-four experimentally induced cases, and in nineteen of seventy-six control animals. The appearance of these lesions varied considerably and it was not unusual to see two quite different types of lesion in a single animal or even in a single muscle. In some animals two or more lesions were found which varied only slightly from one another and represented a graded series of macroscopic changes.

Experience provided an explanation of some of the changes observed. The small, white, space-occupying focal lesions caused by Cysticercus ovis could soon be distinguished by the naked eye from the lesions
in which the muscle fibres were more intimately involved. Parasitic infestation was encountered in three muscles from two scrapied animals (S-52, 57) and in two muscles from two control sheep (C-33, 51).

A second group of lesions which could be quite readily distinguished macroscopically resembled infarcts. They had a central mass of necrotic muscle surrounded by a haemorrhagic and/or pale zone of reaction. In one animal (C-75) the proximal heads of the upper thigh muscles were extensively involved, and the width of the surrounding reactive zones varied from one to three or four millimeters wide. Similar but solitary lesions of only two to three centimeters in diameter were also seen (C-67, 68). Such lesions were found in animals of the control group only and were present in thirteen muscles from three cases.

The remainder of the macroscopic lesions had no specific features. They were mainly soft, pale, poorly defined foci which could be found only by careful examination of the sliced muscles. In a few the foci were made more conspicuous by the presence of haemorrhages and occasionally by large or small transverse ruptures of muscle bundles. A small proportion of the pale foci contained numbers of minute chalky-white streaks which in some samples were sufficiently numerous to give the muscle an opaque, creamy appearance. During the cutting of these samples inequalities in resistance and texture gave the impression that there was considerable variation in the amount of fibrous tissue present in the lesions. The foci were generally small and solitary, but in some muscles they were multiple, and in a few they coalesced to form irregular, pale patches occupying up to two-thirds of a single small muscle. Such lesions accounted for the majority of the muscle changes found. Twenty-seven
muscles from seven scraped animals (S-19, 27, 53, 55, E-8, 25, 27) and ninety-three muscles from eighteen control animals (C-33, 45 to 50, 52, 56, 60, 61, 67, 68, 72 to 76) were affected with this type of lesion.

A second type of lesion which lacked specific features was that made conspicuous by the large amounts of fat which apparently replaced some of the muscle bundles. These lesions could easily be recognized macroscopically by their very fatty appearance and the absence of all but a few scattered, apparently normal muscle bundles. This change was seen in four muscles from two scraped animals (S-58, S-60) and in one muscle from a control animal (C-48).

To summarize, the macroscopic features of muscle lesions showed considerable variation in appearance but the majority were small and circumscribed. They were found in 11.7 per cent of the scraped animals and 25 per cent of the controls.

In addition to the focal lesions, described above, two scraped and three control animals had extensive rupture of the abdominal muscles. The ruptured muscles were distributed as follows: Case S-53 - bilateral but incomplete rupture of the bellies of the recti muscles and unilateral, incomplete rupture of the proximal ends of the internal and external oblique muscles. Case S-55 - unilateral and incomplete rupture of the bellies of the rectus muscle, and the internal and external oblique muscles. Case C-46 - bilateral, complete rupture of the rectus muscles, bilateral, incomplete rupture of the internal and external oblique muscles. Case C-49 - unilateral and incomplete rupture of the internal and external oblique muscles. Case C-76 - complete rupture of one rectus muscle, incomplete rupture of the other, and unilateral incomplete
rupture of the internal and external oblique muscles.

In the first four cases, the ruptures appeared to be relatively recent, and incompletely organized hemorrhagic clots adhered to the torn, contracted ends of the muscles; over the torn area the viscera was supported by skin and subcutaneous tissue. In one case (C-76), the torn muscles were very fibrotic and adhesions between skin and muscles and between muscles were extensive.

**Microscopic Examination**

It was considered essential that all changes found in the muscles be described in detail in order to provide sufficient information for valid comparisons. The microscopic changes observed could be broadly divided into the following groups: (a) simple atrophy (b) simple hypertrophy (c) degenerative changes of muscle fibres. (d) inflammatory changes (e) infarcts (f) other changes (g) neuro-muscular spindles.

(a) **Simple Atrophy**

Normal Control Sheep

The muscle samples taken from sheep which appeared clinically normal and in good bodily condition (-) showed great variations in fibre diameter (Fig. 1). Despite these variations, no single diameter appeared to be represented by an unusually large population and there
appeared to be a progressive and gradual decrease in the numbers of larger than normal or smaller than normal fibres to the extremes of the range represented. Measured diameters of six hundred fibres taken from several muscles produced a relatively consistent "normal" curve when frequency was plotted against diameter. In Fig. 2 the heavy line represents the curve of fibre diameter frequency of a trapezius muscle of a mature Cheviot of the control group (C-17). The mean was 22.14 ± 0.387, and the range of fibre diameter was three to fifty microns. Muscles from mature, normal Suffolk sheep had a slightly higher mean fibre diameter (26.2 microns) with a greater range (3-80 microns), while those from the Dale breeds gave fibre diameter values similar to those of the Cheviot breed.

E maciated Control Sheep

Muscles from (++) emaciated, non-scraped animals, particularly those suffering from Johne's disease (C-25), peritoneal abscess (C-16), or neoplasm (C-52), showed considerable reduction in fibre diameter, although in some muscles a scattering of larger fibres was seen. Fig. 3 represents camera lucida drawings of three muscles from a normal control Cheviot (C-14); Fig. 4 shows the equivalent muscles from an emaciated, three year old Cheviot suffering from a large peritoneal abscess (C-16). It will be seen that, with regard to fibre diameter, the three abnormal muscles each exhibits atrophy in a somewhat different manner. Some single muscles from other emaciated animals of the same group all appeared comparatively alike. Thus, the trapezius, supraspinatus,
semimembranosus and rectus femoris muscles tended to retain a population of large fibres while the longissimus dorsi, latissimus dorsi, semitendinosus and the bipinnate muscles of the lower limbs showed fibres of a more uniform small diameter. Other muscles were less constant or showed an intermediate picture as shown in Fig. 4,B. Six hundred measurements of fibres from the trapezius of a very emaciated Cheviot (case C-16) produced a lopsided frequency curve with a mean of 13.44 microns and a second rise in frequency at about 29 microns (Fig.2). When, from the same muscle, six hundred more fibres over fourteen microns in diameter were measured, it was appreciated that this rise was a second curve. The diameters ranged from 2.5 - 40 microns.

Scrapied Sheep

In the scrapie group, the picture was very similar to that seen in the emaciated controls. The same muscles tended to retain a number of fibres of large diameter (Figs. 5 and 6). The trapezius from a very emaciated, scrapied Cheviot animal (S-9) produced a double curve similar to that seen in case C-16 (Fig.2). The large population of small fibres was concentrated further to the left at about 10 microns and the low rise to the right of this again proved to be a second curve centered at about 28 microns; the range was 2 - 40 microns. As in the emaciated controls there was a disparity of fibre size between adjacent muscles (Fig.7) and in a few cases between different areas of a single sample (Figs. 8 and 9). A sharply defined difference between adjacent primary bundles was not seen in any control animals but was observed in emaciated,
scraped sheep (Figs. 10 and 11). In these two muscles a group of large fibres in one or two primary bundles was particularly prominent because adjacent primary bundles were composed of uniformly small fibres. The least diameter of the large fibres in one case slightly exceeded the largest diameter found in a comparable normal muscle, however, both large and small fibres exhibited similar staining properties. All fibres appeared to be intact and normal when viewed in longitudinal sections.

Muscles from two cases (S-58, 60) and also some type IV lesions showed highly irregular patterns of fibre diameter. These specimens will be described and considered under the headings of hypertrophy and type IV and type V degeneration.

As the muscle fibres were progressively reduced in diameter in emaciation their histological appearance changed remarkably little. The number of nuclei present in the sarcolemma increased slightly in the early stages, but this was evident only in longitudinal sections where they formed chains of three to twelve nuclei. The shape of the nuclei was not altered at any stage. In some fibres striations were visible as long as muscle structure could be identified. Granular or hyaline changes were not seen except in the distribution described as type I change. When the least diameter of the smallest fibres fell below 10 microns the fibres often became progressively more angular; although the histological texture did not appear altered the impression gained was that the angularity was a result of an increased malleability at this stage of atrophy (Fig.12). In (+++) emaciated carcasses fibres whose diameter was less than that of a red blood cell were seen but the eventual fate of these fibres could not be accurately determined. No
small fibres of this type were found undergoing erosion or replacement by macrophages.

(b) **Simple Hypertrophy**

Absolute hypertrophy was not generally a feature of the muscles examined from either scraped or control animals, although a few fibres of up to 80 microns in least diameter were seen in otherwise atrophic muscles. Four trapezius muscles from two slightly emaciated, scraped animals did contain some very large fibres, but since this hypertrophy was associated with degenerative changes these specimens are considered under the heading of type V degeneration.

(c) **Degenerative Changes in Muscle Fibres**

The histological appearance of the over 1,400 muscles sampled in this survey presented a number of abnormalities as well as atrophy and hypertrophy. The microscopic changes are classified under seven categories on the basis of differences of distribution within a sample and differences in morphology. In some of the groups the distinguishing histological features reflected the macroscopic appearance. However, some of the macroscopic differences described proved on histological examination to be only minor variations of otherwise comparable lesions of muscle degeneration. The numerical designation does not necessarily denote a progressive increase in the severity of change.
The following criteria of muscle fibre degeneration were used:

(i) Replacement of the sarcoplasm and myofibrils of portions of a muscle fibre by nuclei.

(ii) Transverse division of a fibre resulting in the formation of fragments with contracted ends.

(iii) Granular, vacuolar or hyaline degeneration. These types of degeneration are included only if it was evident that the continuity of the myofibrils was interrupted, or if the hyalinized area lacked cross striations, or if the fibres were swollen and contained prominent longitudinal striae.

(iv) The absence of sarcolemmal nuclei over a considerable portion of a fibre or, when viewed in cross section, of a group of several fibres.

**Type I**

This category was based on histological findings and had no macroscopic counterpart. The lesions consisted of single scattered, degenerated fibres. In the majority of animals in this survey type I change was the only change seen (see Tables IV, V, VI). This type of lesion occurred in all but one of the animals from which more than ten random samples were examined; these samples included muscles which appeared to be completely normal when examined macroscopically. The most important feature of this type of change was the number and distribution of degenerating fibres within the samples. The usual finding was from one to three, with a rare maximum of ten, degenerated fibres in samples
which contained from five to twenty thousand visible fibres, i.e. about two abnormal fibres per half inch square section. In some isolated fibres a nuclear reaction had been stimulated, and the nuclei either replaced the cytoplasm or surrounded an angular mass of normal or degenerating cytoplasm. In these latter fibres a ring of nuclei one or two deep often surrounded a central mass of cytoplasm containing a sarcocyst (Fig.13). Occasionally the nuclei appeared to be vesicular but more often they were elongated, small and dark staining (Fig.14). An occasional macrophage was visible in the centre or at the edges of these fibres. Some of the fibres contained an area of hyaline or granulo-fatty degeneration adjacent to the cellular portions. Such areas appeared swollen, flat and pale, or were strongly eosinophilic in haematoxylin and eosin preparations, cross striations were absent or greatly distorted and longitudinal striations were often prominent. In some hyaline areas nuclei were not visible. In longitudinal sections type I changes seldom involved more than a short segment of a single fibre, although nuclei of adjacent normal fibres sometimes appeared more numerous where the damaged and normal fibres touched. The diameter of the fibres varied greatly, some appeared reduced in size while others were distended and packed with nuclei. Type I changes were found in every body muscle regularly sampled.

Analysis of the incidence of type I changes revealed no apparent differences between breeds of sheep or between sexes. There was, however, a distinct increase in incidence with progressive aging when this variable only was considered. Thus, about twenty-nine per cent of muscle samples from the twenty-five animals aged one year contained examples of type I change (see Table VII) whereas fifty-three per cent
of the samples from the twenty-eight animals aged three years had similar lesions. The number of animals over three years old was small, but in them the incidence of type I changes remained high.

When scrapied and control groups were compared with respect to type I changes it was found that the control group had a somewhat higher incidence (45% vs. 36%) but when these figures were corrected for age (Table VII) the incidences was similar in both groups.

To summarize: the widely distributed type I changes of single fibre degeneration were visible only under the microscope. Scrapied and control animals were apparently equally affected, and the number of fibres involved increased with age.

Type II

Type II changes of focal atrophy and degeneration had no macroscopic counterpart. The lesions consisted of a diffuse increase of sarcolemmal nuclei over part or all of a sample while ten or more fibres per section showed evidence of cytoplasmic damage. The cellular appearance (Fig.15) was due to an enlargement and multiplication of nuclei at the borders of fibres (Fig.16) although few fibres were completely ringed with nuclei. Those fibres that were surrounded by nuclei showed additional changes of either hyaline or granular cytoplasmic degeneration. A few fibres were undergoing cellular replacement by macrophages or proliferating muscle nuclei. It was unusual to see more than two damaged fibres in any one primary bundle and many primary bundles contained no abnormal fibres.
Although there was a variable reduction in fibre diameter most fibres had normal striations and staining reactions. Occasionally aggregations of lymphocytes, macrophages and eosinophils were visible in the connective tissue surrounding primary bundles and particularly was this so around small vessels. Although connective tissue appeared to be somewhat condensed there was no absolute increase. Type II changes were seen on histological examination of four muscle samples selected at random from one natural and two experimental cases of scrapie (S-12, E-6, 7). The lesions were distributed unilaterally in the sternocephalic, semitendinosus, semimembranosus and longissimus dorsi muscles.

In summary: type II lesions could be seen only on microscopic examination. They were confined to a very few muscles from three out of ninety-four scrapied sheep and were not seen in muscles from animals of the control group.

**Type III**

Fibrotic changes of type III were without macroscopic counterpart in four out of seven samples. The other three samples showed small, pale foci which were firm when cut and which were visible to the naked eye.

The predominant and distinguishing histological feature of type III lesions was the very obvious increase in connective tissue; this however, did not cause any serious distortion of the architecture of the muscle. Thickening of the endomyseal connective tissue was directly
continuous with the heavy perimyseal bands; the increase in these heavier bands was particularly noticeable where it formed thick perineural and perivascular layers (Fig.17). Scattered throughout the lesions were occasional muscle fibres which were undergoing fragmentary degeneration or cellular replacement by large vesicular nuclei. In some examples fifty or sixty fibres were involved. This degenerative process appeared similar to that described in type I.

Within the heavy perimyseal connective tissue bands of two samples (C-33) there were a number of small, straight muscle fibres which appeared to have escaped the usual muscle bundle grouping. Some of these fibres were disorientated up to ninety degrees and were visible as longitudinal fibres in a transverse section (Fig.18). Inflammatory round cells or polymorphonuclear cells were not seen. Arterial changes were found within four examples of type III lesions (cases S-14, C-33). Organized thrombi were visible in three arteries (Fig. 81) while another contained a relatively large, fibrous intimal plaque. Two cases (S-14, E-10) provided several examples of sarcoplasmic masses and another (C-33) showed numerous striated muscle-fibre annulets. These specimens will be described separately under their respective headings.

Type III lesions were found in five muscles from three of ninety-four scrapied sheep (S-14, E-10, 27) and in two muscles from one of seventy-six control animals (C-33). These changes were seen to be distributed unilaterally in the semitendinosus, biceps femoris, rectus femoris and longissimus dorsi muscles.

In summary: fibrotic foci classified as type III were seen on both macroscopical and microscopical examination. The seven examples were found in muscles from three out of ninety-four scrapied sheep and
in one out of seventy-six control animals.

Type IV

The dystrophic lesions included under type IV were the histological counterparts of most of the macroscopically visible foci. This category included some of the apparently fibrotic lesions, all of the soft, pale lesions and all of those lesions which contained opaque flecks. The general process appeared to be the same in all cases, and the predominant macroscopic feature reflected one of the various stages of a single process. Sixteen muscles from four young lambs were classified as having type IV lesions.

The predominant and consistent microscopic feature of all the muscles included in this group was the presence of many transversely fragmented fibres and the formation of broad contracted muscle fragments. In longitudinal section these fragments were usually separated along the length of the fibre by segments of a collapsed sarcolemmal tube. In most cases the process occurred only in single fibres or small groups of fibres but in a few muscles all fibres were involved.

A consideration of the variable proportions of degeneration, fibrosis and regeneration within type IV lesions suggested that some lesions were of longer duration than others. These variations were classified arbitrarily beginning with those lesions in which only degeneration of muscle fibres had occurred.

(1) In the grossly haemorrhagic muscles (cases C-45, 60, 61, 73, 74), the degenerative process was uncomplicated. In the muscles
involved degeneration was extensive but selectivity was still evident (Fig. 19). The sarcolemmal nuclei in both the contracted fragments and the empty tubes appeared normal in size and number (Fig. 20) and cross striations were retained in many fragments. Some of the fragments contained vacuoles but when these were stained with Sudan IV in frozen sections no fat was seen. Groups of free red blood cells, which stained normally and appeared healthy, were distributed between fibres and in perimyseal spaces; in a few single fibres or groups of fibres the sarcolemmal sheaths appeared to be completely severed allowing red blood cells to fill the gaps between the contracted fragments.

In two muscles from one case (C-74), a slightly different type of early degeneration was visible in a few scattered fibres; there was a transverse division of the fibre between prominent cross striations (discoid degeneration). The divided muscle had not contracted (see Fig. 40) and in other respects the fibre showed remarkably little change although dark brown granules, similar to haemosiderin in degenerating blood clots, were scattered throughout the two samples. The capillaries were very congested and a few had ruptured, producing focal haemorrhages. Within the same samples the more usual contracted fragments and empty sarcolemmal tubes were also present.

(ii) The muscles included in the second group contained foci which were pale and soft when viewed macroscopically (cases S-19, 53, 55, E-25, O-46 to 50, 52, 56, 61, 67, 68, 72, 73, 76). In these lesions the sarcolemmal nuclei were prominent; there was an apparent general increase in number, selected fibres showed a great increase and in some examples nuclei had replaced the muscle substance. In all sections a few eosinophilic, fragmented fibres around which there
was no sarcolemmal cell increase (Fig. 21) were seen. Contracted fragments seen in paraffin sections often contained small vacuoles and in frozen sections these were demonstrated to be fat droplets. With phosphotungstic acid haematoxylin stains the fragments were tan or blue and most were moderately phloxinophilic. Cross striations were usually absent although longitudinal clefts were often prominent. Within some lesions unfragmented fibres varied greatly in diameter (Fig. 21) although the cross striations appeared normal. Some of the very small fibres in some lesions were basophilic, had central nuclei and appeared to be undergoing regeneration (Fig. 22). However, central nuclei were not confined to these small fibres but were often numerous in fibres of normal size. Other small fibres were neither basophilic, nor contained central nuclei, and were embedded in a variable thickness of fibrous tissue. Elsewhere within these lesions the variable amounts of endomyseal and perimyseal connective tissue bore little relation to the state of the proliferating nuclei or to the age of the lesion, although some very fibrotic lesions did appear older. In many samples in this group muscle fibres were replaced by large fat cells which distorted the muscle architecture. In preparations stained by von Kossa's technique there was no evidence of calcification within most lesions of this age group, although a few did contain occasional fibres packed with calcareous granules.

(iii) A slightly different variation that was perhaps of a similar age to the above, was seen in muscles which contained macroscopic, opaque streaks (cases S-19, 27, 53, C-50). The muscle lesions seen in the four lambs (born of cases S-54, 58, C-68, 73) were also classified as of this type. The essential features were a granular calcification
of degenerating fibres and a remarkable increase in sarcolemmal nuclei in certain degenerate fibres. Fifteen of the sixteen muscles from two cases (S-19, 27) and the sixteen muscles from the four lambs appeared to represent the least complicated picture in that every fibre showing evidence of degeneration was packed with calcium (Fig.23) or with large nuclei (Fig.24). The fibres themselves were neither smaller nor larger than normal fibres. Multinucleated cells were visible in most samples, but basophilic regenerating fibres were seen only in the lambs' muscles. Hyaline fibres were extremely rare and there was little or no increase in fibrous tissue (Fig.25). In five muscles from three cases (S-19, 53, C-50) essentially the same processes were found, but normal and degenerate fibres were reduced in size and embedded in varying quantities of connective tissue (Fig.26). Replacement of muscle by fat was obvious in three of these muscles and a few small, basophilic fibres with central nuclei were seen in two muscles.

A somewhat different variation was seen in one muscle (case C-46) where foci of lymphocytes and macrophages surrounded or displaced muscle fibres of reduced diameter (Fig.27). In other aspects this lesion was similar to other type IV muscle lesions.

(iv) Grossly fibrotic muscles from one case (C-76) probably represented a later stage of the general process seen in other type IV lesions. Scattered, fragmented fibres which were not surrounded by sarcolemmal nuclei and in many cases appeared to lack such nuclei were embedded in narrow bands of dense connective tissue, as indeed were all the fibres present in the areas involved. The diameter of the fibres was extremely variable (Fig.28), and no regenerating fibres
were seen. Fat stores to some extent distorted the muscle architecture and appeared to have replaced degenerated fibres. Calcified fibres were not seen.

(v) A final variation which appeared to contain essentially the same elements of degeneration as other type IV lesions was the focal fatty lesion seen in one case (C-48). Although only occasional muscle fibres showed definite evidence of recent degenerative change, the prominent feature of the lesion was replacement of fibres by rows of fat cells (Fig. 29). Although the normal muscle pattern was not severely distorted in this lesion there appeared to be some general increase in connective tissue. Sarcolemmal nuclei appeared more numerous than normal but shrunken or calcified fibres were not observed.

Some animals showed very uniform type IV lesions in all the muscles involved but most showed two or more variations in different muscles.

In several type IV lesions focal areas provided evidence of a circulatory disturbance sufficiently severe to cause necrosis of muscle fibres. These areas of necrosis could only be identified in places where patches or islands of several fibres completely lacked sarcolemmal nuclei. Surrounding these islands of large fibres was a variable layer of connective tissue, which in some cases contained small, apparently regenerating muscle fibres, though many of these did not contain central nuclei (Fig. 30). This appearance was invariably associated with areas of dense haemorrhage which in some cases appeared to be undergoing degeneration and organization. Fibre necrosis of this type was seen adjacent to the sites of gross rupture of nine
abdominal muscles from four cases (S-53, 55, C-46, 49), in a rhomboideus muscle from one case (S-53), and in a semimembranosus muscle from another case (C-61). In all the muscles mentioned above, uncomplicated type IV lesions were seen in other areas.

Generally there was good correlation between the macroscopic and microscopic appearances. Several areas showing questionable gross lesions proved to be normal, and no additional, purely microscopic type IV lesions were seen in forty-three macroscopically normal samples from animals showing type IV changes.

In all, one hundred and twelve muscles from four naturally scrapied animals, one experimentally scrapied animal and sixteen controls were considered examples of type IV degeneration. The distribution of lesions within the carcasses varied considerably as indicated by the fact that twenty-eight different muscles were involved. The frequency with which the various muscles were involved in mature sheep is indicated in the following list.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>latissimus dorsi</td>
<td>21</td>
</tr>
<tr>
<td>rectus abdominus</td>
<td>15</td>
</tr>
<tr>
<td>gluteus profundus</td>
<td>9</td>
</tr>
<tr>
<td>obliquus internus</td>
<td>6</td>
</tr>
<tr>
<td>vastus lateralis</td>
<td>6</td>
</tr>
<tr>
<td>triceps</td>
<td>5</td>
</tr>
<tr>
<td>obliquus externus</td>
<td>5</td>
</tr>
<tr>
<td>rectus femoris</td>
<td>4</td>
</tr>
<tr>
<td>extensor carpi radialis</td>
<td>4</td>
</tr>
<tr>
<td>supraspinatus</td>
<td>4</td>
</tr>
<tr>
<td>semimembranosus</td>
<td>3</td>
</tr>
<tr>
<td>gastrocnemius</td>
<td>2</td>
</tr>
<tr>
<td>semitendinosus</td>
<td>2</td>
</tr>
<tr>
<td>sartorius</td>
<td>2</td>
</tr>
<tr>
<td>infraspinatus</td>
<td>2</td>
</tr>
<tr>
<td>extensor carpi ulnaris</td>
<td>2</td>
</tr>
<tr>
<td>sup. digital flexor</td>
<td>2</td>
</tr>
<tr>
<td>intercostal</td>
<td>1</td>
</tr>
<tr>
<td>biceps brachii</td>
<td>1</td>
</tr>
<tr>
<td>sternocleidomastoide</td>
<td>1</td>
</tr>
<tr>
<td>trapezius</td>
<td>1</td>
</tr>
<tr>
<td>serratus</td>
<td>1</td>
</tr>
<tr>
<td>Muscle</td>
<td>Count</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td>subscapularis</td>
<td>3</td>
</tr>
<tr>
<td>pectoralis superficialis</td>
<td>1</td>
</tr>
<tr>
<td>rhomboideus</td>
<td>3</td>
</tr>
<tr>
<td>vastus intermedius</td>
<td>1</td>
</tr>
</tbody>
</table>

Seventy-two samples were bilateral pairs of muscles, while forty were unilateral specimens.

In summary: type IV lesions characterized by transverse fragmentation of muscle fibres were seen in one hundred and twelve muscles from twenty-one mature animals. These muscles were from five out of ninety-four scrapied sheep and from sixteen out of seventy-six controls. Lesions histologically similar were seen in muscles from four young lambs.

**Type V**

This category comprised the four trapezius muscles from two naturally scrapied sheep (cases 5-58, 60) and these represented four out of five of the muscles which were macroscopically streaked with fat. Histologically these muscles presented an unusual appearance, namely mixed hypertrophy and atrophy of the fibres which were surrounded by massive deposits of fat. In cross section the architecture of the muscle was barely recognizable. In some places the remnants of a primary bundle appeared to be represented by three or four fibres of variable size embedded in a mass of fat cells, while in an adjacent area, a very large, undivided group of large fibres occupied an area much greater than that occupied by a normal primary bundle and this too was surrounded by fat. Although some groups of six to thirty fibres were all very large and other similar groups were uniformly very small, the more usual picture was one of haphazard mixture of the two as well
as a few fibres of normal size (Fig. 31). In one case (C-60) the groups of fibres varied in extremes and within foci of connective tissue, groups of minute, barely recognizable fibres were seen. In longitudinal sections, the small fibres were very tortuous and some appeared to end in a strand of connective tissue (Fig. 32). A few fibres of all sizes were undergoing hyaline change or cellular replacement.

Visible in cross sections of muscles from both cases, but more pronounced in case C-60, were many large fibres which were partially or completely divided longitudinally by thin strands of connective tissue (Fig. 33). In longitudinal sections it appeared that these fine septae were incomplete in both planes since the septae in the longitudinal clefts between the myofibrils could be traced only a short distance before they vanished and the myofibrils were again undivided (Fig. 34). In the fibres where this division was incomplete, one or more small capillaries were frequently seen running up the centre of the muscle fibre. Central nuclei were numerous within both divided and undivided muscle fibres.

This process of fibre splitting, which could be identified only where it was incomplete, seemingly explained the origins of other groups of two to twelve irregularly shaped fibres separated by very fine septae and surrounded by heavier connective tissue (Fig. 33). The least diameter of fibres showing no signs of division was as great as 140 microns (compared to about 80 microns for the largest fibres in a comparable normal muscle - see Fig. 33) and incompletely divided fibres were found with a least diameter of up to about 260 microns.

To summarize: type V lesions characterized by mixed atrophy and hypertrophy and by massive interstitial fat deposition were found in
four muscles from two out of ninety-four scrapied sheep, but were not found in control animals.

(d) **Inflammatory Changes**

This category included three varieties of lesions which appeared to fall under the general heading of myositis; their macroscopic counterparts appeared as pale, firm foci.

(i) The first type consisted of a focal accumulation of inflammatory cells around either a parasitic cyst tentatively identified as *Cysticercus ovis* or, in foci where an intact parasite was not seen, around a central mass of necrotic debris. Adjacent to the parasite or debris was a layer of lymphocytes, macrophages and eosinophils supported in a meshwork of collagenous fibrils. On the inner aspect of this zone was a narrow, dense, often eccentric layer of fibroblasts (Fig.35). Beyond this very cellular layer, eosinophils lay packed between widely separated muscle fibres; this zone was sometimes very broad, particularly around those foci that contained debris only. The muscle fibres themselves showed little or no evidence of degeneration although in the more central portions muscle fibres of reduced diameter occasionally appeared to be constricted by strands of connective tissue. Parasitic myositis was seen in two muscles from two scrapied sheep and in two muscles from two control sheep (cases S-52, 57, C-33, 51).

(ii) The second variety of myositis was in many respects similar to
the first but was associated with a central colony of bacteria resembling
Actinomyces sp., which were surrounded by radiating club-like structures
(Fig. 36). Outside this central zone was a narrow band composed of
lymphocytes, macrophages and occasionally a few giant cells, supported
in a collagenous net. Beyond this band was a variable zone of eosinophils
which extended longitudinally between widely separated muscle fibres;
these fibres showed little or no alteration from normal. In some areas
the eosinophil zones of adjacent microscopic mycotic foci coalesced
within a larger macroscopic focus. Atrophy of muscles adjacent to the
central areas was not observed. By appropriate staining methods it
was demonstrated that the central mass contained a few long, beaded,
Gram-positive filaments; the radiating clubs were acid fast. Mycotic
myositis was seen in five muscles from one case (C-76).

(iii) The third variety of myositis contained no visible centre and
consisted of a focus of inflammatory cells and oedema which had infiltrated
between the muscle fibres. Within the lesion the cellularity was diffuse
and not as prominent as in the previous types of myositis; lymphocytes
and neutrophils predominated. Although a few of the muscle fibres
appeared to be undergoing hyaline degeneration and cross striations
were not readily visible, very few fibres showed much definite evidence
of degenerative change. The connective tissue elements did seem to be
more prominent than in normal muscle but this effect appeared to be the
result of the oedematous separation of the connective tissue fibres.
The vascular elements appeared normal, although a few red blood cells
were visible. The variety of non-specific myositis described here was
seen in one muscle (case S-57).
Unilateral lesions of myositis were seen in the supraspinatus, infraspinatus, longissimus dorsi, semimembranosus and semitendinosus muscles.

In review: three variations of myositic foci were seen in a very few muscles from six animals. Of the sheep involved three were from the scrapied group of ninety-four animals and three were from the control group of seventy-six animals.

(e) Infarcts

Muscles containing infarcts or simulated infarcts are included in this category. Fifteen muscles from three control animals (C-67, 68, 75) showed this type of lesion which was the microscopic counterpart of the necrotic, circumscribed, macroscopic lesions. The lesions from two cases (C-67, 68) were less than three centimetres in diameter; they consisted of a central, anuclear zone surrounded by a very narrow cellular zone, surrounded in turn by normal muscle (Fig. 37). The cellular zone was made up of prominent, multiplying muscle nuclei, fibroblasts and quite a large number of lymphocytes and neutrophils. In both cases there were a large number of crystals and degenerating red blood cells, within the central necrotic zone and this material appeared to be spreading the dead muscle fibres. In case C-67 the crystals were about 4 to 16 microns across while in case C-68 the diameter of the granules was less than 1 micron. Examination of the clinical histories of these two animals revealed that two and three days previously both had been given intramuscular injections of antibiotics. Sheep C-67 had received
Chloromycetin into the triceps muscle and sheep C-68 had received penicillin into the supraspinatus muscle.

Histological examination of muscles from the third animal (C-75) revealed many typical infarcts. The central anuclear zone contained red blood cells and many arteries and veins in the area were distended and packed with erythrocytes. Surrounding the central area was a zone of intense cellularity, the inner aspect of which was made up largely of fibroblasts and a few lymphocytes and neutrophils. The outer part of this zone consisted chiefly of numerous, large muscle nuclei; occasionally small, basophilic fibres with central nuclei could be seen penetrating into the necrotic area. Distal to this highly cellular layer was a broader, less cellular zone in which the muscle fibres were undergoing two changes - atrophy and fragmentation (Fig.38) with occasional examples of cellular replacement. A few of the fibres were calcified. Towards the outer borders of this zone the fibres became progressively more normal in appearance and the apparent increase in connective tissue observed in the more proximal areas disappeared. The outer limits of this transitional zone were impossible to define.

Other infarcted muscles from the same animal were similar in appearance except that at least two apparently more advanced stages of organization were visible. In these latter instances the very cellular zone was less well defined and contained few or no neutrophils, although lymphocytes were more numerous and macrophages were visible. Fine, basophilic, regenerating fibres were more numerous in all but the central areas and the architecture of the muscle was distorted by erratic, moderately heavy connective tissue bands containing many large fat cells. These septae, composed of fibrous tissue, fat, and, peripherally, some
regenerating muscle fibres, had divided the necrotic muscle masses into irregular islands of ten to fifty anuclear fibres; these bundles however, still retained their form in the centre, while the outer fibres of the islands were calcified or were undergoing erosion and cellular replacement. Organized thrombi were observed in three veins and nerves were surrounded by heavy perineural connective tissue bands.

Bilateral infarcts in the interspinatus, gluteus profundus, semimembranosus, biceps femoris, and vastus lateralis muscles and unilateral infarcts in the vastus intermedius and deep and superficial digital flexor muscles were observed.

To summarize: infarcts associated on the one hand with crystalline antibiotics and on the other with total vascular occlusion by red blood cells were seen in the muscles of three control animals only.

The degenerative changes observed in muscles from scrapied and non-scarepied sheep are analysed in Table VIII.

(f) **Other Changes**

This rather non-specific category includes a number of features which were observed but which bore no relationship to those described in the various groupings nor to each other. Round fibres, cross striations and sarcocysts were seen in virtually all preparations and to avoid needless repetition are described here. On the other hand striated muscle-fibre annulets and sarcoplasmic masses were seen so rarely that they could hardly be included among characteristics of any group; it is however, deemed important to describe them in some detail.
Round Fibres

In a number of muscles samples from scrapied sheep there were rounded muscle fibres which were in many cases more strongly eosinophilic than their neighbours. Occasionally Coenheim's fields were not readily discernible in cross sectional preparations. These fibres were usually solitary, but in some samples several hundred were grouped together. Examination of normal muscles and muscles from sheep suffering from diseases other than scrapie revealed many similar fibres, although there was considerable variation from muscle to muscle and between different sheep. Animals showing some degree of emaciation and animals less than one year old appeared to have more rounded fibres than did normal sheep.

In normal sheep a slightly different manifestation was presented by the muscles which were directly attached to roughened bone surfaces and were pale and fatty, e.g. the vastus intermedius and pectineus muscles. In cross sections of these muscles almost all the fibres were rounded and there were many internal nuclei. The findings were similar in scrapied and non-scrapied animals.

In an attempt to obtain more information about rounded fibres, fresh frozen and fixed frozen sections were compared with processed muscle from the same sample. It was found that the greatest proportion of rounded fibres occurred in the fresh frozen samples, a lesser number in the fixed samples and fewest in the processed preparations. Those fibres in the fresh frozen sections which were not rounded were less sharply angular than similar fibres in the processed sections.

With haematoxylin and eosin the staining reaction of rounded fibres was variable. In muscles undergoing obvious atrophy, a majority
of the rounded fibres did not stain differently from normal fibres unless extreme care was used during the staining process. This applied also to the rounded fibres in such muscles as the vastus intermedius and the pectineus. A few of the rounded fibres in both these categories and also a few rounded fibres in normal muscle retained the eosin very strongly.

With phosphotungstic acid haematoxylin and Heidenhain's iron haematoxylin the rounded fibres did not give a distinctive reaction, although many rounded fibres took the second stain very strongly. Eosin-methylene blue and Giemsa's stains gave a similar picture to haematoxylin and eosin.

In muscles showing type IV degeneration, particularly in the earliest stages prior to nuclear proliferation, many strongly eosinophilic, enlarged, round fibres were visible among adjacent empty sarcolemmal tubes (Fig.19). In longitudinal sections these were identified as contracted, broken fragments of fibres in the early stages of degeneration (Fig.20); many of the fibres contained vacuoles.

**Cross Striations**

The cross striations of sheep muscle were studied in sections four microns thick using Kull's stain and also the kiton fast red - light green combination already described.

The appearance of the bands and lines and the intensity with which these structures stained varied considerably in individual fibres from apparently normal muscles. In many muscles satisfactory preparations
of cross striations could not be made from existing paraffin blocks. Non-degenerated fibres in kiton fast red preparations usually appeared as alternate bands of red (dark or Q-band) and green (light or J-band). The Q band was divided by the light green Qh line and the J band by the dark blue Z line, although this latter line was not always discernible (Fig.39). In contrast, fibres which were obviously contracted, as judged by the length of the sarcomere and the width of the fibres, appeared simply as narrow alternating bands of red and green.

In muscle samples showing definite evidence of degeneration, some fibres lacked the two thin lines, in spite of the fact that sarcomeres appeared to be the same length as those in adjacent fibres which did contain the Qh line (Fig.39). In affected fibres the Q band was solid red and was perhaps less well defined than in adjacent normal fibres, and the J band was an uninterrupted light green. In some fibres taken from several degenerate muscles a similar appearance was associated with the few sarcomeres adjacent to more obviously degenerated myofibrils (Fig.40a). In these, the Qh and Z lines were not lost within a single sarcomere but often disappeared on one side of the fibre two or three sarcomeres short of their disappearance on the other side of the fibre (Fig.40a). The Z line appeared to be the first to go; in some fibres the Qh line remained in a portion of the fibre until the myofibrils could no longer be identified.

In contrast many fibres showing more obvious signs of degeneration such as fragmentation and nuclear replacement, retained clearly marked Qh and Z lines as long as myofibrils were visible. This was particularly apparent in one case (C-74) with discoid degeneration (Fig.40b) but could also be detected in other cases which had acute, fragmentary degeneration
(0-45, 60, 61, 73).

The absence of Z lines alone was not considered of any significance as it often appeared to be the result of imperfect staining. The absence of both the Z line and the Qh line in fibres apparently not in extreme relaxation or contraction, appeared to be a more frequent phenomenon in muscles showing extensive fragmentation, particularly in the muscles from four lambs, but occasional fibres in several normal muscles had a similar appearance.

The presence or absence of striations in haematoxylin and eosin preparations is recorded with the observations on the various types of degeneration. It must also be recorded that cross striations were not visible in all muscle fibres from apparently normal muscles from normal control sheep.

Sarcocysts

In the course of examination of sheep in this survey, sarcocysts, some very large (Fig. 41) were seen in skeletal muscle in very variable numbers. In some animals, particularly those reared in a confined environment (e.g. the experimental group) the infestation reached phenomenal proportions. In one animal one and a half years old, a cross section of sternocephalisus muscle revealed as many as ninety Meischer's tubes in one high power field. This amounted to the involvement, at a single level, of approximately one in eight fibres. Although this animal was rather more lethargic than most, no other symptoms were seen which were not attributable to scrapie. Muscles from every
animal sampled contained sarcocysts, although in some animals two or three samples had to be searched before one of these bodies was found. Extremes of infection were seen in both scrapied and control animals although the majority of samples fell midway in the range.

A small proportion of the parasites was surrounded by a cellular reaction or, more usually, the infected muscle fibres were surrounded by a cuff of lymphocytes and a few eosinophils (Fig.42). Reactions of this type were numerous in a few animals, but one or two cuffs of cells were seen in a high proportion of the sheep examined. One natural and two experimental scrapie cases and two control sheep exhibited a great many examples of tissue reaction to the sarcocysts and while one of these suffered from a massive infestation it appeared that the relative number of fibres containing sarcocysts had no bearing on the proportion of infested fibres surrounded by reactive cells. In many instances the muscle fibres containing the parasites appeared normal despite a considerable reactive cuff while in other examples the fibres showed signs of degeneration as demonstrated by basophilic cytoplasm and proliferation of sarcolemmal nuclei. Except for a very few examples, the Rainey's corpuscles were clearly visible and the few Meischer's tubes observed in stages of degeneration were not always reactive.

Striated Muscle-Fibre Annulets

This phenomenon (Germain-Ringbinde) was seen in the semitendinosus and biceps femoris muscles from one hind limb of an animal from the
control group (case C-33) although it was not encountered during the examination of over fourteen hundred muscle samples from the limbs and bodies of one hundred and twenty-one sheep. The annulets in the affected tissues were seen in macroscopically visible fibrotic lesions and, on histological examination, were found to be numerous in the narrow transitional zone which existed between complete replacement fibrosis and the relatively normal muscle in a type III lesion. In form, these annulets were predominantly of the simple type with the circling fibrils lying in a circumferential, subsarcolemmal position (Fig.45) but several examples were seen in which the circling fibrils had split the main muscle fibre into two or more divisions which were identified in cross section (Fig.43a). Central nuclei, which were apparently normal, were seen in a few of the circling fibrils. The ring often seemed to be a separate entity (Fig.44), although in some cases the myofibrils of the vertical muscle fibre appeared to be continuous with those of the annulet (Fig.43b).

The figures here described appeared to be most numerous in the semitendinosus muscle. An exhaustive search under high power of samples of this muscle from ten other sheep was undertaken, but nothing even slightly resembling circling fibrils was found.

Sarcoplasmic Masses

Two specimens of type III microscopic lesions from two scrapied sheep (S-14, E-10) presented, in transverse sections, several examples of muscle fibres which contained a small number of myofibrils at one
side of the muscle fibre. The balance of the space was occupied by finely granular sarcoplasm, and occasionally a second group of myofibrils. When a myofibril stain such as kiton fast red was used (Fig. 46) this sarcoplasm was seen to contain many very fine red granules. In the surrounding area several fibres contained rarified zones of varying size in which the myofibrils appeared fewer in number yet there was no cellular reaction in any of these fibres.

(g) Neuro-Muscular Spindles

In the course of examination of skeletal muscles from scraped and normal sheep many neuromuscular spindles were encountered and examined. Since some differences appeared to exist between spindles in normal muscle and those in muscles from scraped animals, it was deemed advisable to make a more exact comparison.

Normal Spindles

In order to establish a basis for comparison, fifty spindles from ten normal control sheep were examined and measured in detail. The precautions taken during fibre measurement were again observed. Any spindles in which one or more intrafusal muscle fibres did not contain a central nucleus or nuclear bag, were discarded, thus the polar portions of the spindles, and the terminal, tapered parts of both spindles and muscle fibres were generally avoided.

The range of spindle diameter in the normal group was 60-190 microns, average 100 microns. The number of fibres per spindle was 4-9, average 6.4, and the intrafusal fibre diameter ranged from 4-25
microns, average 13 microns. In muscle samples from all the normal control animals included in the survey the intrafusal spaces of the spindles contained no visible material; evidence of muscle fibre degeneration as defined for extrafusal fibres i.e. granular degeneration, fragmentation or cellular replacement, was not observed. About two per cent of the spindles examined were complex in structure.

Spindles from Scrapied Sheep

Fifty spindles from twelve scrapied animals were measured in the manner already described and the usual precautions were observed. The spindle diameters ranged from 40-300 microns, average 100 microns, and the number of fibres per spindle was 3-10, average 6.2. The fibre diameter ranged from 3-26 microns, average 11.5 microns. The intrafusal space of many spindles obtained from scrapied animals contained a globular, proteinaceous material which was eosinophilic with haematoxylin and eosin and did not stain with kiton fast red. This material had an appearance very similar to the serum clot in many veins and arteries. In the scrapied group three spindles with diameters greater than 200 microns were from a single animal; the muscles and fat of this animal were very oedematous. No visible abnormalities were recorded in the intrafusal fibres and complex spindles were observed in a frequency similar to that of the normal group.

Spindles in Degenerate Muscle

Because some evidence of intrafusal fibre degeneration was observed in those muscles showing type IV degeneration, a third group of fifty spindles was selected from muscles showing definite evidence of type IV change. The usual precautions were taken; in addition, only those spindles containing one or more muscle fibres were included since
some spindles from areas which may have been infarcted contained no fibres. The range of spindle diameter was 50-220 microns, average 101 microns. The number of muscle fibres ranged from 1-9, average 4.6 and the fibre diameter was 4-30 microns, average 11.8 microns. A comparison of the three groups has been made in Table IX.

Since the type IV group contained spindles from both scrapied and control animals, many of the spindles contained proteinaceous material and two were distended to over 200 microns. Twenty-eight of the fifty examples in this group also had a somewhat thickened capsule. Surprisingly, the average diameter was not less than normal. Within the spindles there was no increase of fibrous tissue but the degenerative muscle changes seen in some spindles explained why the number of fibres was reduced. The most frequent change was fragmentation and the formation of empty sarcolemmal sheaths alternating with contracted fragments (Figs. 47 and 48); a few examples showed granular breakdown only. Complex spindles appeared in the usual proportion, but in one muscle from this group a single spindle unit contained seven separate bundles of intrafusal fibres (Fig.49).

In three muscle samples taken from two scrapied animals showing type III changes, a few spindles showed another type of alteration. This was an obvious fibrosis of the spindle sheath, of the intrafusal space and of the spindle nerve (Fig.50). In one control animal (case 6-76) from which other muscles were undergoing focal myositis a similar picture was presented by the muscle spindles of one sample. In all four sections there was obvious fibrosis of perimyseal structures generally but the connective tissue was particularly dense around spindles and nerves; intrafusal fibres appeared normal. In both the
two scrapied sheep and also in the control animal fibrotic spindles were limited to one or two samples from each of the three animals, although the number of samples examined histologically was ninety-two, twenty and eight respectively.
2. Motor Innervation of Skeletal Muscle

The motor innervation of muscles from sheep of both the control and scraped groups was investigated by several different methods. To facilitate comparison the muscles examined were divided into three groups: (a) Muscles macroscopically free of focal lesions and obtained from both scraped and control animals; these specimens were examined by the gold chloride method. (b) Muscles macroscopically free of focal lesions and obtained from both scraped and control animals; these specimens were examined by the cholinesterase-silver method. (c) Muscles which contained macroscopically visible lesions. These were taken from both scraped and control animals and were examined by several methods.

Animals in all stages of emaciation were included in all three groups; selection of cases for the groups was exercised only to the extent that slightly and greatly emaciated cases were included in each category. In order to facilitate comparisons of methods several muscles from four animals (two scraped, two controls) were sampled for both (a) and (b) groups.

(a) For the first series, the two modifications of the gold chloride method already described were used. The specimens examined in this way included twenty-two muscles from seven control animals (C-32, 33, 34, 35, 36, 39, 40) and thirty-nine muscles from nine scraped animals (E-23, 24, S-29, 30, 31, 32, 33, 34, 36). Two adjacent samples
from each muscle were processed as described above. The first was blocked in paraffin, and twenty-four serial sections, each twenty microns thick, were cut, mounted and counterstained with toluidin blue. By using this method the appearance of the black axons and end plates could be correlated directly with the condition of the muscle fibres, whose sarcolemmal nuclei stained a contrasting light blue. Cross striations were marked by a granular deposition of gold (Figs. 51 and 52). These preparations were compared with those from adjacent samples. The latter preparations were made by cutting five frozen sections, one hundred microns thick and staining by the other modification of the gold chloride method. In this case the black axons and end plates contrasted with the light pink to red muscle fibres (Figs. 53 and 54). Nuclei were not generally visible. These gold chloride preparations were compared with paraffin cross sections stained by haematoxylin and eosin, and longitudinal frozen sections from the same muscles stained with Sudan IV. From this first series over fifty thousand end plates from scraped animals and over thirty thousand end plates from control animals were examined.

(b) In the second series the basic method used was that of Koelle for demonstrating cholinesterase in motor end plates and the described silver stain was superadded. Twenty-three muscles from six control animals (C-39, 40, 42, 43, 44, 62) and thirty-five muscles from seven scraped animals (E-25, 27, 33, S-34, 36, 52, 57) were examined. By this method the end plates showed less detail than in gold preparations, but the black axons were clearer and more uniform and contrasted well against the yellow to tan muscle fibres. When the preparations were counterstained with methyl green the green nuclei did not interfere
with the generally clear precise picture; as many as six thousand five hundred complete end plates were visible in a single section. As in series (a) parallel samples of the same muscles were also examined in paraffin cross sections and in Sudan IV stained frozen sections.

Muscles from control animals were also examined by the Gross-Bielschowsky (bulk) method and by Holmes' method. (cases C-30, 32, 38, 40).

Considerable pleomorphism of end plates was observed in all sections prepared from normal control animals. By the gold chloride methods the usual appearance of the teliodendria i.e. terminal ramifications, was that of a coarse round or ovoid ring, often divided into unequal sections by one to four traversing, aurophilic septae (Fig.54). Attached to the distal part of the circumference of the ring were several short projections which gave the end plate the appearance of a shortened hand with short, stubby fingers (Fig.51). Variations were many; in a few endings two incompletely separated rings were visible, while in others the terminal axon ended in several branches rather than a ring (Fig.54). In side view a distinct Doyeres' hillock was seen in many endings projecting from the surface of the muscle fibre (Fig.54). By gold methods terminal axons were often irregular and varicose and in less well impregnated preparations they were marked by a row of fine black granules. Axons were of the same calibre as the terminal rings and this, and the irregularity of the axons, were undesirable features of this method. In several endings in normal muscle the axons divided at variable distances from the end plate and both branches proceeded to intermingle in the terminal ring (see Fig.84). Sub-terminal branches of axons going to separate endings or lying free on the muscle fibre surface were not seen in normal muscle.
In sections stained with Sudan IV no neutral fat was observed in nerve sheaths and the orange myelin could be traced to the end plate nuclei.

In preparations stained for cholinesterase a very similar picture was presented except that the ringed or "fern leaf" teliodendria were, if anything, even coarser, while the terminal axons were dense, uniform and thinner than those demonstrated by the gold chloride methods. The finger-like projections on the terminal rings were not prominent but pleomorphism appeared just as frequently in the end plates and the variations were similar. A comparison of the two methods in samples from the same muscles revealed that in gold chloride frozen sections the size of the terminal apparatus was about twice that of the same structure in the gold chloride-paraffin section preparations, and that by the cholinesterase method the terminations were comparable in size to those in gold chloride paraffin sections.

By the Gross-Bielschowsky and Holmes methods the finer, terminal endings of the sheeps' teliodendria were seen to consist of two to five terminal branches within the end plates. These terminal branches remained separate and were associated with six to ten vesicular sole plate nuclei which were clearly visible in these preparations (see Fig. 65). By these methods the endings were somewhat larger in size than those seen in gold chloride paraffin sections.

In addition to pleomorphism of shape, end plates in all preparations examined in this survey showed some variation in size of adjacent end plates (Fig. 55). Smaller plates were, as a rule, associated with smaller muscle fibres and in this respect there was good correlation between the cross sectional appearance of the muscles and the nerve ending.
preparations (Figs. 56 and 57). Occasionally exceptions were found in which two adjacent muscle fibres approximately equal in diameter were innervated by two end plates obviously unequal in size. This discrepancy did not reach extremes and even in an intensive search few examples were found. In muscle samples from emaciated control animals, the discrepancy in size between adjacent end plates was more obvious, as was the greater number of small fibres seen in cross sections (Fig. 57). There appeared to be no increase in the number of fibres innervated by disproportionately small end plates. In gold chloride preparations, a greater number of the motor end plates appeared to lose their ring structure and assume the appearance of a progressively shorter rod with progressively fewer and shorter side branches as the process of atrophy progressed (Fig. 53). In cholinesterase preparations the rings became smaller and more elongated. However, the most striking feature of atrophy was not the appearance of individual endings, but the relative increase in the number of endings in a single preparation. This condensation was also reflected in the crenation of the axons (Fig. 58) which at times pursued very tortuous courses between the muscle fibres.

For reasons to be discussed later the following criteria were adopted as evidence of degeneration or impairment of function:

(i) Fragmentation of terminal axons in gold chloride and silver preparations.

(ii) Neutral fat in myelin sheaths in Sudan IV preparations.

(iii) Axons without endings or endings without axons, provided that these did not appear at the top or bottom of the sections, and provided impregnation was adequate.

(iv) Frequent disparity in the size of the end plates when compared to the diameters of muscle fibres.
In the two series examination of muscles from scrapied animals revealed no significant differences from muscles from the control group. Understandably, many of these animals showed the changes associated with atrophy but the criteria of degeneration were not fulfilled.

(c) The study of motor innervation in muscles showing macroscopic evidence of degeneration included nine muscles from four scrapied sheep and ten muscles from four control animals. The histological types of degeneration represented were: type III (C-33) - two muscles, type IV (C-46, 61, E-25, S-27, 53) - fourteen muscles, type V (S-58) - one muscle and infarction (C-75) - two muscles.

Type III lesions were investigated by the gold chloride method in paraffin sections and by Holmes' method. Apparently normal end plates were seen in the two muscles, but the very fibrotic zones contained some unusual features as far as peripheral nerves were concerned. In gold chloride preparations nerve sheaths, which contained no aurophilic material, could be traced in serial sections from identifiable nerve trunks to several very small muscle fibres which were embedded in connective tissue. The vesicular nuclei of the end plate appeared normal, and so did the striations of the muscle fibres. In silver preparations, although empty nerve sheaths could not be identified with any certainty, no nerve tissue could be found in the comparable fibrotic area. However, in the transitional zone between the fibrotic and normal areas nerve fibres were abundant, and two further abnormalities were observed. The first of these was the presence of very coarse teliodendria which in some cases appeared to form a complete ring (Fig.59) rather than the expected fine, separate branches (see Fig.65). This gave the endings
an appearance similar to that seen in gold chloride preparations. The second abnormality was the presence, prior to contact with the end plates, of sub-terminal branches of axons. In one example an irregular sub-terminal branch gave rise to a second sub-terminal branch (Fig. 60). In sections stained by the Sudan IV method no neutral fat was seen in large or small nerve trunks.

Type IV lesions were investigated by the Coupland and Holmes-Gross-Schultz method, by Gross-Bielschowsky (bulk) method and by Holmes' method. In these preparations the appearance of the motor nerves and end plates was variable and for purposes of description they will be divided into two groups.

In the two muscles sampled from one case (C-46) and one of three muscles sampled from another (S-53), there was abundant evidence of degeneration. Within the damaged areas which, in these muscles, contained variable amounts of fibrous tissue, axon fragmentation involving all nerve fibres was visible. In one muscle no small bundles could be identified and in the larger trunks only small fragments of axons remained. In the other two muscles, the terminal axons were marked by only occasional argentophilic fragments, while the small trunks contained more easily identified short fragments (Figs. 61 and 62). Many fibres in the larger trunks appeared to be intact but a few were interrupted by short breaks in continuity. When stained with Sudan IV sections of these three muscles showed neutral fat in nerve trunks of all sizes, although finer terminals could only occasionally be located by this feature.

When stained by the cholinesterase method the end plates in these same areas showed a consistent alteration; this consisted of a loss of definite form and a finely granular appearance (Fig. 63) when
compared to the normal shape and crystalline appearance of end plates in undamaged areas of the same sections. In the centre of such a damaged area these fine clusters of granules covered a variable area, and in some cases an end plate appeared to be reduced to a very few granules. Nuclei, identified by the superadded methyl green stain, appeared numerous and normal or slightly fatter than normal; the groups of vesicular sole plate nuclei could be identified even though the copper sulphide granules were very sparse.

The remaining eleven muscles from four cases (C-61, E-25, S-27, 53) provided no evidence of axonal degeneration by Gross-Bielschowsky, Holmes, or Gross-Schultz silver methods; in Sudan IV stained sections no neutral fat was observed in nerve trunks. In cholinesterase preparations the appearance of the end plates in these muscles was normal except in those scattered muscle fibres in which the fibre segment immediately under the end plate showed evidence of degeneration or nuclear proliferation. In these areas the end plates showed the finely granular loss of form described above, (Fig.64) but because no areas of extensive fibre destruction coincided with the end plate zones in these muscles, most of the motor endings appeared normal. Gross-Bielschowsky and Holmes preparations of end plates of one case (C-27) revealed completely normal axon structure (Fig.65) despite the fact that in the same sections many of these muscle fibres showed severe degenerative changes and fragmentation. In fibres where the muscle immediately below the end plates showed degenerative changes, the sole plate nuclei appeared to be clumped around the structurally intact terminal axon (Fig.66). In several of these end plates teliodendria were not readily visible.

A type V lesion from one animal (S-58) was investigated by means of the Gross-Schultz method for axons and by the Sudan IV method for
neutral fat. Although many intact nerve processes were observed among the muscle fibres and a few apparently ended in fatty tissue, the small nerve trunks that were visible contained both intact and fragmented axons. Suitably stained preparations of these small trunks revealed rows of neutral fat droplets lying between apparently normal myelin sheaths. Larger nerve trunks showed neither fragmentation of axons nor neutral fat.

Frozen sections of two lesions classified as infarcts (case C-75) were examined by means of Gross-Schultz silver and Sudan IV fat stains. Within the zones of infarction there was complete fragmentation of all axons and the small terminal axons could seldom be identified. Beyond this zone axons appeared normal, but in the intervening cellular area one small trunk was seen in which some axons were fragmented and some intact. Fat stains on one of these muscles failed to show any neutral fat; in the other, which had a more pronounced nuclear zone, neutral fat was observed in a small trunk in this area but not in the necrotic or normal areas.
3. **Extrinsic Ocular Muscles**

The normal structure of the eye muscles was studied in preparations from five clinically normal sheep; an additional ten animals suffering from a variety of chronic diseases other than scrapie provided ocular muscles for comparison. Three of these latter cases showed evidence of type IV degeneration in some skeletal muscles. To complete the series fifteen animals with natural scrapie and fifteen from the experimentally scrapied group were also sampled.

**Macroscopic Examination**

Macroscopic examination of all forty-five animals revealed a great variation in volume of the extrinsic ocular muscles, however, none of these contained macroscopically visible lesions such as were observed in the skeletal muscles of several animals included in this survey. The condition of the peri-orbital fat reflected the condition of the carcass fat and in a few cases the peri-orbital fat was reduced to a translucent, wet, gelatinous mass.

**Microscopic Examination**

Histological examination showed that the sheep's extrinsic ocular muscles were composed of a central core of polygonal fibres of variable but relatively large diameter; these were surrounded by a sheath of
rounded, small fibres in which were dispersed occasional large round fibres. When appropriate stains such as Holmes' or kiton fast red were used (Fig.67) this outer layer could be identified by the more prominent myofibrils. It extended about two thirds of the way around the muscle, leaving the side next to the orbit free from such a cover. The round fibre layer varied in depth from five to fifteen fibres at mid-muscle, but was less easy to identify at the tapered poles of the muscle where all fibres tended to be similarly small and rounded.

Sheep's ocular muscles contained numerous spindles particularly in the proximal one third; they appeared to be most numerous just within the outer, round fibre layer. The capsule of the spindle structures appeared more delicate than those of spindles in other skeletal muscles, and the enclosed intrafusal fibres were rounder, more numerous and less tightly packed than in other spindles. As many as eighteen muscle fibres were found in a single spindle and complex spindles of two units were frequently observed. As many as thirty-six spindles were identified in a single transverse section.

In the remainder of the control group the changes attending emaciation consisted of not only some apparent general reduction in fibre diameter, but more obviously, of a marked reduction in the diameter of scattered fibres, particularly those in the peripheral layer. Where this change occurred in the central core, the fibres lost their polygonal shape and became rounded. Depending on the degree of emaciation, a few or a majority of central fibres were round.

In the forty-five sets of extrinsic ocular muscles examined striated muscle-fibre annuletts were frequently seen although none were observed in three of these sets. Cross sections through the mid-belly of the muscles and in the central core region provided the greatest
numbers of annulets, but they did not seem to be narrowly limited to this zone. The highest number of circling, cross striated annulets counted in a single section (sixty-six) appeared in a rectus oculi muscle from an emaciated, three year old animal of the control group (case C-40), although in this group encircling fibrils were generally more numerous in the older animals than the younger ones. In form, these figures were largely of the simple type (Fig.67), but several of the complex variety were observed in normal and diseased control animals (Fig.68).

Naturally and experimentally scrapied animals showed no differences from the animals in the control group regarding the type and number of striated annulets in the eye muscles.

A feature of seven of the fifteen control animals including two of the normal ones, was the presence of occasional single fibres undergoing cellular replacement or obvious fragmentation. As many as five fibres in a single cross section were observed to be affected thus and they were not limited to any particular region. Another feature of these muscles was the presence of clear vacuoles in the centre of a few scattered muscle fibres (Fig.69). This peculiarity did not appear to be associated with more obvious evidence of muscle degeneration and was observed in nine of the fifteen controls, including two normal controls. The size of the vacuoles varied greatly; in some cases one or two appeared to occupy two thirds of the cross sectional area of a fibre. Although most of these vacuoles appeared in extrafusal fibres, a few occurred in intrafusal fibres as well, and one was observed in an intrafusal fibre of a muscle from one of the five normal controls (Fig.70).

Sarcocysts were numerous in some of these eye muscles, but none appeared reactive and no unusual features were observed.

The naturally and experimentally scrapied groups provided essentially
the same picture as the controls in all respects regarding the appearance of the extrinsic ocular muscles. Degenerated fibres were, on the whole, no more numerous, although muscle from one animal of the experimental group did exhibit more (fifteen) in one muscle cross section. Changes concomitant with emaciation were usual in both groups, but vacuolated fibres and striated muscle-fibre annulets did not appear to be more numerous.

**Innervation**

The innervation of the extrinsic ocular muscles was studied in cholinesterase-silver and Sudan IV preparations of rectus oculi muscles taken from ten scrapie and ten control sheep. The number of visible axons and end plates per unit area was obviously much greater than in other skeletal muscles. A more detailed examination revealed that much of the increase in nervous elements was made up of a very dense population of fairly typical end plates, and that these were most numerous in, but by no means confined to the proximal third of the muscles. A second narrow band of motor plates and axons was found in the distal third. In addition to these cholinesterase-containing motor endings, two other types of endings peculiar to ocular muscles were also identified in the cholinesterase-silver preparations.

The middle third of the muscles contained many long axons running parallel to the muscle fibres, and which ended in an irregular elongated deposit of copper sulphide i.e. cholinesterase. When such an axon could be traced proximally to its nerve trunk, it was found, in some instances, that it was merely a branch of an axon whose main component
supplied a typical motor plate in the proximal third of the muscle. Axons with more than two branches were never seen, and in no instance were two of the usual motor endings observed as terminations of a single fine axon.

The second peculiarity of the innervation of the ocular muscle was the large numbers of axons which terminated on or between muscle fibres without cholinesterase-containing end organs. Such axons were particularly numerous in the distal two thirds of the muscles, but were also observed in the proximal third. Over most of their length they ran parallel to muscle fibres, but they did occasionally cross over. The general pattern was of an elongated network made up of roughly parallel nerve fibres which branched only at their very ends, if at all. Elongated copper sulphide encrustations on muscle fibres were occasionally observed in the same areas as these free nerve fibres. This was particularly noticeable in the peripheral zones of the muscle but a direct association between the encrustations and the free axons could not be established. A few axons terminating in connective tissue could be traced to nerve trunks entering the muscle in company with arteries.

Small nerve trunks serving the neuromuscular spindles in the ocular muscles could be identified by their very broad sensory axons and some of these could be traced into the intrafusal spaces. The single, thick sections used in this survey did not allow a detailed study of spindle innervation, although several typical cholinesterase-containing motor end plates could be identified on intrafusal fibres at some distance from the equatorial region of the spindles. The large sensory axons ended in the nuclear bag region of the intrafusal fibres and occasionally could be traced once around the nuclear bag prior to termination.

Examination of the innervation of ocular muscles from ten scrapied
sheep revealed neither axon fragmentation in silver preparations nor neutral fat in myelin sheaths in Sudan IV preparations. When sections from scrapied and control animals were compared, no differences could be detected in the copper sulphide deposition at motor end plates, nor in the distribution or number of the various types of endings. In emaciated animals of both groups there appeared to be a relative increase in density of nerve elements similar to that seen in other skeletal muscles.

4. *Arteries*

*Macroscopic Examination*

Macroscopic examination of the aortas from the sheep included in this survey was singularly unproductive. Even in animals up to seven years of age no gross lesions could be found, although there was a tendency for older animals to show prominent longitudinal folds in the thoracic aorta. A discovery made late in the course of this work was that if the aorta was stained in its whole fixed state with Sudan IV, small well-defined, strongly sudanophilic areas could be seen adjacent to many arterial branches and in the region of the scarred ductus arteriosus. A less well-defined sudanophilia occurred in other areas, particularly at the mouths of the renal and iliac arteries. Aortas from twelve sheep one to seven years of age were stained in this manner and all showed areas of Sudan IV retention. The younger animals, under two years of age, showed this staining only in the region of the renal
branches; older sheep showed a diffuse reddening in many areas and well-defined bright red rings around each of the many branches of the aorta and at the ductus arteriosus. There appeared to be no difference between scrapied and non-scrapied animals.

**Microscopic Examination**

The normal picture of the ovine visceral arteries showed a thin endothelial layer one or two cells thick, within a heavy single inner elastic membrane easily seen in sections stained to show elastic tissue. In older animals some fraying or duplication of this membrane was visible without any other observable change. In arteries with an internal diameter, in fixed tissue, of less than ten to fifteen microns, the elastic membrane was much thinner and in many small arteries could not be identified. The tunica media was made up of circular and longitudinal smooth muscle fibres supported by a network of reticulin fibres. Elastic fibres were not present in this layer, except in the major visceral artery trunks leading directly from the aorta. The outer tunica adventitia of elastic and collagenous fibres was of variable thickness depending on the organ and the proximity of parenchyma.

Intramuscular arteries were similar to visceral arteries, except that a well-defined inner elastic membrane was visible in much smaller vessels.

Abnormalities Observed

It became apparent after examination of a few sets of the arteries
in visceral organs, that in these vessels thickening of the **tunica intima** was far from rare. This increase in intimal thickness was especially evident when an elastic tissue stain was used to demonstrate the inner elastic membrane. An opportunity to study what appeared to be a progression with age of intimal plaque size was provided by the arterial changes seen in the various age groups.

In cross sections of the arteries, the extent of the lesions of intimal sclerosis was seen to vary from small plaques occupying a fraction of the luminal circumference to a complete, thick circular band within the inner elastic membrane. Usually a large plaque or several plaques occupied varying proportions of the luminal wall. Along the longitudinal axis of the vessels, single plaques extended, as a rule, for distances several times their circumferential width. In older animals these lesions became confluent (Fig. 71) but in younger animals normal, unthickened areas of the intima were present between the plaques (Fig. 72). In sections of the liver of five cases, complete occlusion by proliferated intima was observed in one or more arteries. In these cases dilated vasa appeared to have taken over the function of the occluded vessels and in some cases had coalesced to form new vessels (Figs. 73 to 75).

The proportion of elastic tissue in the plaques varied considerably. The small plaques seen in young animals appeared to be rich in fine elastic fibres, which were particularly dense as the inner elastic membrane was approached (Fig. 78). Between the elastic fibrils were poorly differentiated nuclei and an amorphous substance which stained as collagen with van Gieson and lissamine yellow stains, and which gave a metachromatic reaction with toluidine blue. Except in occasional small splenic arteries, the amorphous substance was P.A.S. negative.

In sections stained by Gordon and Sweet's method, argentophilic
reticulin fibres were uniformly abundant in the intimal plaques, and their distribution resembled that of the reticulin fibres in the media. The smaller thickenings seldom contained smooth muscle fibres, and only rarely was the inner elastic membrane altered from normal. Prominent dark endothelial nuclei were seen clustered on the luminal surface of many small and large plaques. Frequently a few red blood cells and wisps resembling fibrin were observed in the same regions (Fig. 76).

In older animals elastic fibrils were abundant at the base of the larger plaques but sparse or absent as the lumen of the vessel was approached. Otherwise the bulk of these sclerotic thickenings appeared similar in composition to the smaller lesions, except that smooth muscle fibres were frequently observed penetrating an interrupted inner elastic membrane. This latter structure was frayed, split and, in many cases, disappeared completely for short distances (Fig. 77).

In plaques the transition from elastic-rich areas to elastic-poor areas was generally gradual, but one example of a distinctly laminated plaque was found in which the inner layer, rich in elastic fibres was sharply divided from the outer (luminal) layer (Fig. 78). Both laminae were equally rich in nuclei and reticulin fibres. Vasa opening into the lumen were rarely observed and then only in large plaques.

In the majority of arteries examined, the media did not appear to be altered from normal. In a few vessels, the muscle fibres adjacent to gaps in the inner elastic membrane were disoriented, the fibres being continuous with those in the thickened intima. The muscular media of the occluded hepatic arteries had lost its affinity for the kiton fast red dye but did not stain with lissamine, a connective tissue dye. A few of these hepatic vessels also showed a focally increased cellularity, but only one example of medial necrosis and calcification was found.
This was in an intramuscular artery of a sheep suffering from scrapie and was associated with a moderate intimal sclerosis (Fig. 79). In a second scrapied animal many small intramuscular arteries contained small scars in the medial zone only.

Adventitial changes were rare and were seen only in hepatic arteries adjacent to hyperplastic, inflamed bile ducts; this latter reaction was assumed to be due to fluke infestation. In the arteries the collagenous elements were increased and formed a net for lymphocytes and neutrophils.

All the arteries observed were negative for hyaline medial change when stained for alcoholic hyaline by Mallory's phloxine; and despite a suggestive hyaline appearance in haematoxylin and eosin preparations, they were also negative for steroid when tested by the Schultz reaction. Negative reactions for amyloid were recorded with Highman's modification of Benhold's stain and, except for one recent thrombus in a hepatic artery, reactions for fibrin with Picro-Mallory Dundee IV and P.T.A.H. were also negative.

Frozen sections of splenic arteries stained with Sudan IV were negative for fat except for specimens from one six year old ewe. In this case small isolated flecks of sudanophilic material were seen in or just under the endothelium. However, sudanophilic material was much more abundant in frozen preparations of the aortas. These sections were taken from regions of the artery which had been selected by staining the whole, fixed structure by the Sudan IV method. Intracellular and extracellular droplets of several sizes were scattered throughout intimal plaques. Frequently, the droplets were concentrated on the intimal side of the inner elastic membrane or just under the endothelium (Fig. 80). Not all aortic plaques contained this material. On the other hand, not all sudanophilic material was confined to plaques, occasionally it was seen in the inner
media adjacent to a heavily deposited plaque. Very occasionally a single foam cell was observed in the inner intimal region.

Histological examination of arteries fixed in distention provided evidence that intimal plaques were capable of considerable distention and only rarely did they appear to limit arterial expansion. A few examples were found in which sclerosis of the intima prevented post-mortem contraction in arteries not fixed in distention.

Distribution of Lesions

Intimal sclerosis was by far the most frequent change and was found in one or more organs from all but three mature animals included in this survey. The distribution, based on a single section from each sample, is outlined in Tables X and XI.

The naturally scrapied group consisted of twenty animals; sclerosis was observed in the splenic arteries of all of them, in the renal arteries of eighteen, in the hepatic arteries of sixteen, in the coronary arteries of ten and in the pancreatic arteries of nine. In the experimentally scrapied group of fifteen sheep intimal sclerosis was visible in the splenic arteries of twelve animals, in the renal arteries of five, in the hepatic arteries of four, in the coronary arteries of five and in the pancreatic arteries of four. In the non-scrapied control group of fifteen animals intimal thickenings were seen in the splenic arteries of all fifteen, in the renal arteries of eleven, in the hepatic arteries of eight, in the coronary arteries of eight and in the pancreatic arteries of six. Examination of spleens from twenty-three sheep killed for human consumption revealed an incidence similar to that found in the control group. Fifteen foetuses were sampled in the same way; two had discrete fibro-elastic
plaques within the internal elastic membranes at the mouths of the splenic artery in one case and of the hepatic artery in the other.

In the group of five mature animals from which more extensive samples were taken, the incidence of intimal plaques in areas other than the viscera was considerably lower than it was in the visceral organs. Three sheep had plaques in aortic samples, two had lesions in iliac arteries, two in pectoral arteries, one in the gastric mesentery and one in the intestinal mesentery. No samples from the carotid or subcutaneous arteries showed intimal thickenings. Several small organized thrombi were observed in intramuscular arteries in a fibrotic muscle lesion from a control animal (Fig. 81).

Serial sections of three splenic arteries which were initially classified as negative, revealed that when several levels were sampled, none were exempt from intimal sclerosis.

Veins were apparently free from intimal change, although the absence of a distinct inner elastic membrane made it difficult to detect slight intimal proliferation. Thrombi were observed in two small intramuscular veins from one animal (S-14).

When findings from scraped and non-scraped groups of sheep were compared, it was found that the experimentally scraped group had the lowest incidence of arterial changes and the natural group had the highest (Table X). When age only was considered as a criterion for grouping, the incidence of lesions showed a definite increase with advancing age (Table XI). For example, lesions in the spleen increased from 87% in the 0-1\(\frac{1}{2}\) year old group to 100% in the group over three years. In other organs the comparable figures were: kidney - 47% and 94%, in the heart - 53% and 78% and in the pancreas - 27% and 56%. In the liver, a slightly higher incidence of arterial lesions was recorded in the 1\(\frac{1}{2}-3\) year old
group than in the older group but this did not detract seriously from
the overall progression with age (Fig.82). It might be noted that the
majority of the experimentally scrapied sheep were one and two years old,
while only a few of the naturally scrapied animals were two and most
were three and four.
GUINEA PIGS

Changes Secondary To Denervation

Guinea pigs were the animals used in this experiment.

A. Clinical Examination

Following recovery from the anaesthetic, the guinea pigs carried the denerved leg in a normal position, but the retraction reflex was considerably weaker than in the unoperated leg. This reflex was unaltered throughout the course of the experiment. The thigh muscle was palpated at frequent intervals; at eight days there was a detectable reduction in the volume of the operated leg. By the twentieth day the reduction was marked, however, after twenty-eight days the rate of volume loss appeared to be considerably slowed.

B. Post-Mortem Examination

Macroscopic Appearance

Post-mortem examination revealed that the muscles became progressively paler after nine days; from twenty days onward they lacked resilience and elasticity.
**Microscopic Appearance**

Cholinesterase-silver preparations of normal guinea pig thigh muscles demonstrated that their motor end plates were essentially similar to the end plates in sheep muscle. The terminal rings were usually more clearly defined but of approximately the same size and configuration (Figs. 83 and 84). Terminal axons were comparable.

Following denervation, the changes which occurred were progressive with the exception of fat droplet accumulation in the muscle fibres. In all preparations sensory fibres associated with arteries remained normal and acted as controls of the adequacy of silver impregnation.

**Three Days**

Three days after the operation, end plates appeared to be normal when examined by the cholinesterase method. In the smaller trunks and in fine terminal branches the axons showed distinct fragmentation (Fig. 85), while in larger trunks the breaks in continuity of the axons were apparent only on close examination. No neutral fat could be demonstrated in Sudan IV preparations but the myelin sheaths appeared to be fragmented into small globules.

**Six Days**

Six days after denervation the end plates were not apparently different from those in the contralateral normal muscles. Axonal fragments were considerably more sparse than at three days but were still identifiable in the terminal axons. In larger trunks the nerves were marked by fragments and gaps of approximately equal size. When stained for fat, the smaller
trunks and terminal axons in the muscle were marked by rows of strongly
sudanophilic droplets. Neutral fat was absent from the larger trunks
but at six days the myelin was even more fragmented than it was at three
days. A feature of the six day muscle was the presence of occasional
muscle fibres which were loaded with fat droplets. These fibres were a
bright pink in contrast to the adjacent blue fibres.

Nine Days

At nine days the end plates still showed no change in size or
shape but were, perhaps, a little more dense than those seen in preparations
from the opposite leg. The finer terminal axons were no longer identifiable
as argentophilic fragments, and the small trunks were barely so. Large
trunks were still quite visible, but the axon fragments were very fine.
In nerve trunks neutral fat droplets were still abundant, although they
were beginning to become sparse in the finer terminals. Droplets of all
sizes filled the vacuoles in large trunks. Fat was not observed in muscle
fibres.

Twenty Days

Twenty days after the operation the end plates were both much more
numerous per unit area, and also much more irregular in size than in the
control muscle. A few end plates had lost their ring form and appeared
as irregular, coarse lines. Only the large trunks of axons were still
visible and they were marked by vague lines of dust-like particles (Fig.86).
In Sudan IV preparations, neutral fat droplets were abundant in large and
medium-sized nerve trunks (Fig.87) but were rarely present in smaller
branches. No fat-laden muscle fibres were observed.
Thirty Days

At thirty days the changes in the end plates were more exaggerated than at twenty days. A few of these structures were so small that one could scarcely recognize them as end plates, and the proportion of small plates had increased. Axons were no longer identifiable by the silver method, and in Sudan IV preparations neutral fat droplets were less numerous in the large and medium-sized trunks. At this stage the deposition of interstitial fat throughout muscle bundles was evident for the first time.

Forty Days

At forty days all end plates were obviously reduced in diameter and were frequently elongated in form. The density of the end plates was remarkable (Fig.88). At this stage at least a quarter of the plates were reduced to a pale dot or streak. In Sudan IV preparations the only signs of the larger nerve trunks were occasional neutral fat droplets, although the vacuoles which had previously contained fat were still visible in small branches. Interstitial fat was abundant (Fig.88). Cross striations were easily visible in all muscles up to and including the forty day specimens.

Paraffin cross sections of the denerved muscles showed merely a gradual and uniform reduction in fibre diameter. The increase in muscle nuclei per unit area was obvious (Fig.89) but there was, if anything, a reduction in the number of nuclei per fibre. Interstitial connective tissue appeared condensed but not absolutely increased. After thirty days adjacent fibres were separated by increasing amounts of fat but in no samples was a degenerating fibre observed. An analysis of the changes
occurring in denervated muscles has been made in Table XII.

End plates of a normal muscle were examined by the cholinesterase method both before fixation, and also after six hours fixation in formal saline at four degrees centigrade. No differences could be detected in the amount or form of copper sulphide deposition. However, after twenty-four hours of fixation at room temperature no copper sulphide was deposited in end plates.
DISCUSSION

SHEEP

Clinical

The clinical symptoms of scrapie have been described frequently, and, on occasions, in great detail (Cassirer 1896, Besnoit 1899, Stockman 1913, 1926, McGowan 1914, McFadyean 1918, Gaiger 1924, Bertrand and Carre 1937, Greig 1940, Lucam, Bechaude and Saurat 1950, Stamp 1956, Bosanquet, Daniel and Parry 1956, Parry 1957). The terminology used often varied, but there was general agreement that the name scrapie, or its many synonyms, described a more or less constant syndrome the detailed characteristics of which varied from sheep to sheep. In this respect the present report can add only the apparent variation in trends between breeds with naturally-contracted scrapie, and the apparent variation between natural and experimental scrapie. Although some genuine breed differences appeared to exist, particularly regarding the preponderance of nervous symptoms, it seemed reasonable to attribute some of the breed variation to differences in relative body weight and fat distribution. This may explain in part why an obese, large bodied Suffolk succumbed to the disease while still in relatively good flesh while the thinner Cheviots, and particularly the Dale breeds did not die until they had reached an advanced state of emaciation.
Perhaps the most valid conclusion which might be drawn from a clinical study of the sheep in this survey is that, in scrapie, the symptoms must be considered as a whole. As Stamp (1956) has pointed out, in establishing a diagnosis experience is very necessary and it is doubtful if description alone would enable one to make an accurate evaluation of symptoms. The presence of somewhat similar symptoms in some of the non-scrapied animals described here demonstrates that both clinical and pathological criteria should be considered before making a positive diagnosis of scrapie (Zlotnik 1958a, b, Zlotnik and Rennie 1958).
Post-mortem

Skeletal Muscle

Atrophy

Emaciation and atrophy have long been recognized as part of the scrapie syndrome. However, in the past the significance of this observation in relation to the symptoms of the disease has been the subject of diverse opinions. May (1868), Cassirer (1898) and McGowan (1914) described the muscles of most animals dying of scrapie as pale and soft, while the surrounding fat was undergoing gelatinous change. Cassirer (1898) and later McPadyean (1918) and Gaiger (1924) attributed symptoms of weakness and ataxia to the extreme loss of condition and exhaustion. Cassirer, however, suggested that muscle was not the primary site of the disease since he could find no evidence of local atrophy.

It was obvious from a consideration of the data presented here that the gross atrophy associated with scrapie was extremely variable. The consensus of opinion over recent years has been that the muscular atrophy was probably a purely secondary change, although until 1956 this opinion was apparently based on macroscopic examination only. Bosanquet, Daniel and Parry (1956) and Parry (1957), while showing that muscular atrophy was a frequent feature of scrapie, also reported that some diseased animals supposedly suffering from scrapie gained in weight and condition. No support for this latter view could be found
in the present survey.

The minute histological changes of muscle atrophy occurring under a variety of conditions have often been described (see Adams, Denny-Brown and Pearson 1954) but little attention has been directed towards analysis of fibre diameter of either normal or diseased muscle. This lack of exact information on the subject of fibre diameter is no doubt largely due to the difficulty encountered in expressing fibre size in terms of a single measurement. Because muscle fibres are seldom regular in shape, measurements of cross sectional areas would appear to be the only accurate method of evaluating changes in fibre size (Greenfield, Shy, Alvord and Berg 1957). This of course, would be a very unwieldy method of investigation and previous workers have inevitably resorted to a single measurement of diameter. In the interests of accuracy, one would undoubtedly have to agree with Greenfield et al. that "a consideration of geometric variations of muscle fibres in disease forces one to use only the least diameter of the largest and the greatest diameter of the smallest fibres", but this method does not allow interpretive analysis of muscle fibre diameter by means of a frequency curve. The uniform least diameter measurement used here was undoubtedly inadequate as an absolute value of fibre diameter and as an evaluation of unusually-shaped, small fibres. As a basis for group comparisons, however, the error was approximately equal in all groups, although in the measurement of atrophic muscles there was undoubtedly a "loading" of the smaller diameters as compared to normal muscle, i.e. the least cross sectional measurement of a flat, broad fibre would place it in the same group as a small round one.

A consideration of the plexiform trophic unit distribution (as opposed to muscle unit distribution) would lead one to expect to find,
in a neurogenic atrophy, very small groups of normal fibres or single normal fibres remaining as representatives of healthy neurons (Garven 1925, Wohlfart 1949, Fiendel, Hinshaw and Weddell 1952, Adams et al. 1954). This picture, as Wohlfart points out, might be complicated by the fact that fibres in a single trophic unit may start the process of atrophy with quite variable fibre diameters and undergo atrophy at different rates. Thus, no sharp distinction between the two populations would necessarily exist and there might be no distinction at all in early neurogenic atrophy.

Several of the camera lucida drawings made here from emaciated animals might, by definition, be interpreted as evidence of a neurogenic atrophy i.e. as grouped large fibres among many small ones (Wohlfart 1949) or as large groups of small fibres (Greenfield et al. 1957). Also, some cross sections presented here were very similar to the drawings made by Wohlfart representing neurogenic atrophy.

Turning to the analysis of muscle patterns, the fact that the population of a normal muscle could be expressed as a normal frequency curve was not surprising. The appearance of a double curve in emaciated scraped animals was interesting but lost any significance in view of the less well-marked, but nonetheless evident, double curve in emaciated control animals. The ratios of 1:6 to 1:8 derived by extracting the fibre frequencies at the peaks of the two curves obtained in extreme cases suggested that the large diameter population may have represented the more atrophy-resistant b-fibres of Wohlfart (1949). The 1:8 ration (vs. the normal ratio of 1:6) might be interpreted as indicating that some b-fibres were also beginning to fall into the first curve. At any rate, the fibre diameter differences due to b-fibres appeared to account for many of the irregularities shown here in camera lucida drawings. Thus, more complex explanations for the patterns, including a neurogenic origin, were not
required.

In view of the appearance of the frequency curves, an abnormal population could only be ascertained if a third curve appeared on the graph. This was not found in the muscles subjected to frequency analysis in this survey. The fact that a second curve was not discernable in some atrophic muscles i.e. that all fibres appeared uniformly small, might be interpreted as a later stage of atrophy since it has been found that muscle fibres undergo atrophy rapidly until they have reached 15 microns then regress much more slowly (Wohlfart 1949). In theory at least then, time would tend to equalize all fibres. If this were in fact so, one would be forced to conclude that the rate of atrophy in some muscles in a given animal is much more rapid than in others, or that this is true of b-fibres at least. Adams et al. (1954) have reported similar observations for human muscles in cachexia, but their further assertion that the variable distribution in the body was without plan would seem not to be entirely true of the sheep examined here. Quite consistently some muscles revealed a mixed population while others showed a population of small fibres only. Jewsbury and Topley (1912) related this phenomenon to different diseases but this view has not received general support (Adams et al. 1954).

Cachectic atrophy has been shown to produce fibre size differences between adjacent secondary bundles which probably have no overlapping trophic units (Adams et al. 1954). This type of atrophy appeared to account for the picture seen in one camera lucida drawing presented here in which the two halves of a single field contain fibres of different diameter.

In view of the above facts, a critical evaluation of the muscle patterns described here would inevitably lead one to the conclusion that, with one possible exception, the muscle patterns found in scrapied sheep
could not be attributed to motor neuron degeneration. The one exception may represent an example of a local neurogenic atrophy, but it is more likely that it represents a small focus of inexplicably hypertrophic fibres, since a frequency curve of this muscle revealed only the usual double curve with an elongated tail to the right; in other words, the population of large fibres was not sufficiently large to produce a visible third curve and among the smaller fibres there were two curves which suggested cachexia rather than neurogenic atrophy.

By direct comparison, no differences in the type or degree of atrophy existed between scrapied and non-scrapied animals. The earlier postulate that generalized muscle atrophy was the cause of some of the symptoms of scrapie leaves unexplained the cause of symptoms in sheep affected with advanced scrapie but which showed no detectable atrophy. Neither does it explain the absence of symptoms of scrapie in emaciated control animals. In conclusion, the evidence presented here indicates that the usual muscular atrophy of scrapie does not provide an explanation for the symptomatology of the disease.

**Hypertrophy**

In chronic human diseases in which the muscle fibres are largely atrophic, a relative hypertrophy of some fibres has been suspected by several authors (Durante 1902, Jewsbury and Topley 1912, Wohlfart 1949). In such muscles the presence of fibres as large as the largest in comparable normal muscle has led to speculation about selective physiological hypertrophy. Unless such fibres were considerably larger than normal however, hypertrophy was difficult to prove and the matter remained
subject to variable interpretation. Such was the case here in emaciated scrapied and control animals; attempts to clarify the status of such fibres were barren because the methods of fixation used obscured individual myofibrils, and it was through counts of these that hypertrophy might have been proved (Adams et al. 1954).

True hypertrophy was found to occur here only in association with other changes and will be discussed under the heading of type V degeneration.

Degenerative Changes in Muscle and the Status of the Relevant Nerves

Before discussing the types of muscle degeneration described in the body of this report, it seems advisable to reiterate a precautionary observation which applies particularly to muscle studies. This is that any comparative discussion of histological changes must be tempered by the fact that muscle has a somewhat limited range of potential reactions and consequently that a muscle lesion is seldom diagnostic (Adams et al. 1954, Blaxter and McGill 1955, Walton and Adams 1956). Abundant evidence of this fact was found in this survey but, since histological examination is often the only available method for differential diagnosis of muscle lesions, an attempt has been made to group similar degenerations on the basis of histological features.

Type I

Type I changes of single, scattered muscle fibre degeneration
appeared in their proper perspective when considered as changes to be excluded when considering significant muscle lesions. The changes which might be expected in a random series of muscle samples from normal individuals have not as far as is known, previously been subjected to analysis; it does not seem unreasonable to assume that a certain level of fibre degeneration in otherwise normal muscles is frequently ignored as insignificant. The tabulation of type I changes according to age revealed a distinct increase in the percentage of muscle samples showing single fibre degeneration as age progressed. Admittedly, the number of animals four years old and older was inadequate for complete analysis but in the first three years the increase from 29% to 53% was strongly indicative of an age relationship. This progressive increase with age was in agreement with the general impression that type I changes constituted isolated, random events, quite probably with several etiologies, which tended to be cumulative. These changes must be considered as a sub-strata unrelated to and sometimes obscured by significant muscle changes.

Type II

Type II lesions of focal reduction of fibre diameter and increase in muscle nuclei without extensive fibre fragmentation presented a distinctive appearance. Type II changes within the carcasses were distributed in the superficial muscles where scrapied animals have frequently been seen to rub, namely the sternocephalicus, semimembranosus, semitendinosus and longissimus dorsi muscles. Such an anatomic distribution made it tempting to consider them directly or indirectly traumatic in
origin. Skin and subcutaneous fat occasionally showed evidence of traumatic damage and it is conceivable that muscle could also be involved. On the other hand, the focal reduction in fibre diameter and the increase in muscle nuclei without evidence of primary muscle fragmentation suggested an interruption of nerve impulses at some point in the motor chain. The similarity of type II changes to the appearance of muscle in early poliomyelitis (Adams et al. 1954) was remarkable, and the inverse relationship between nerve continuity and the number of muscle nuclei observed by Tower (1937) lent further support to a neurogenic etiology. An hypothesis relating such muscular changes in scrapie to nerve degeneration would find support from Besnoit's (1899) report of peripheral neuritis, and also circumstantial support from the fact that there is often considerable neuronal damage in the medulla of scrapied sheep (Zlotnik 1958). Against such an hypothesis is the evidence presented in this work that peripheral motor axons and end plates appeared normal even in advanced cases of scrapie, and the observations of Wight (1958) that the ventral motor horn cells of the cord appear not to be involved in any degenerative processes.

However, the possibility that occasional nerves were undergoing degenerative changes could not be entirely ruled out and it would seem possible in theory at least, that direct trauma from rubbing might have produced a local neuritis. Despite this convenient explanation, however, the precise etiology of type II lesions must remain conjectural.

Type III

The type III lesions were distinguished by fibrosis and the
absence of extensive degenerative changes in the muscle fibres. Although the changes present lacked inflammatory cells or other distinguishing features of myositis, it was not possible to determine with any certainty that they did not represent the scarred recovery stage of an inflammatory lesion. Type III fibrotic changes were distinguished from long-standing infarcted lesions by the fact that the muscle architecture appeared to be infiltrated with fibrous tissue rather than replaced by it, and by the presence within the fibrous tissue zones of viable spindles, nerves and large arteries. The proximity to four type III lesions out of seven of otherwise rare intramuscular arterial changes suggested a relationship, but the arterial changes may not have been primary. In any event the resulting reduction in blood flow could not have been complete. In connection with vascular changes and fibrosis, Adams et al. (1954) cite reports that venous, but probably not arterial, occlusion can produce fibrous hyperplasia in muscles. Tower (1937) has further observed that fibrosis around nerves and vessels in disuse atrophy results in secondary muscular degeneration. The association in type III lesions of striated muscle-fibre annulents with arterial thrombosis finds a parallel in the report of Perry, Smith and Wren (1956) in which such figures were associated with chronic, constrictive arterial changes.

Within three out of four examples of type III lesions with arterial changes, there was also evidence of nerve degeneration. In two examples, coarse, circular motor rings and subterminal branches of peripheral nerves were strongly suggestive of regeneration after a prolonged period of degeneration (Tower 1935, Gutman and Young 1944), and a third example contained a degenerating nerve trunk. It seemed reasonable to assume that the arterial and nerve changes were related and that of the two, the arterial changes were probably primary; it would be difficult to explain
arterial thrombosis on the basis of nerve degeneration. A second plausible explanation of type III lesions lies in a focal incident of myositis, arteritis and, perhaps simultaneously, neuritis.

**Type IV**

By virtue of their macroscopically visible size and their common occurrence in this survey type IV lesions require more comprehensive consideration that the previous types. The many similarities between them and previously reported lesions in sheep muscles also deserve discussion, and this aspect has been incorporated into the discussion of type IV features.

A rather surprising finding in reference to type IV changes was the complete absence of symptoms directly referable to the muscle lesions. However, in five ewes attention was directed to an indirect sign of muscle degeneration, that of abdominal rupture. On post-mortem examination each of the ruptures was found to be associated with type IV changes and in each muscle additional macroscopic foci were observed some distance from the site of rupture. Conversely, no ruptures were seen in otherwise normal muscles. Additional circumstantial evidence linking type IV lesions and rupture was that abdominal muscles were involved in fifteen out of the twenty-one sheep showing type IV lesions. These abdominal changes were all seen in ewes which had lambed within three months prior to death or sacrifice.

In three of these animals abdominal rupture was associated with protracted dystocia, yet the lambs did not appear unduly large and were presented normally. In an additional five ewes with a similar dystocia,
Type IV lesions were found in the abdominal muscles. This correlation between dystocia and lesions and/or rupture of the abdominal musculature appeared at first to provide an explanation for an abnormally high number of dystocias in ewes which lambed and survived. On the other side of the picture, however, were two ewes which suffered from dystocia yet had no lesions in the abdominal muscles, and four ewes with abdominal lesions, including two with ruptures, which had no apparent dystocia. Nevertheless, the correlation between dystocia, abdominal muscle lesions and rupture certainly appeared to be more than coincidental, and one might plausibly attribute the three features to additional stress placed on the abdominal musculature during pregnancy and particularly during the later stages of parturition. This supposition gains further support from the fact that these ewes were part of a larger outbreak of a disease characterised by muscular lesions, and one might reasonably hypothesise that the muscles at that time were more susceptible to degeneration. Of interest in this respect and in connection with weak lambs born at the same time, were the observations of Pappenheimer (1941, 1948) that in laboratory animals adequate vitamin E levels appeared to be essential for maintenance of pregnancy even though the females showed no muscle lesions at that stage. He also stated that occasionally young died in utero or were born dead or weak.

Histologically, the type IV lesions reported here bore many similarities to those of experimental vitamin E deficiency in lambs and to the many reports of dystrophy attributed to a deficiency of, or defects in the metabolism of that vitamin. Undoubted similarities to the myopathy reported by Bosanquet et al. (1956) and Parry (1957) and to blue-tongue in sheep also exist (Thomas and Neitz 1947). It seemed advisable therefore
to explore the differences in an attempt to determine the status of type IV lesions.

**Age**

The presence of type IV lesions in the muscles of ewes of all ages and in young lambs from the same groups was strongly suggestive of a direct relationship. Many reports exist of similar dystrophic lesions in lambs (see Blaxter and McGill 1955) but little or no attention has been directed to the condition of the muscles of ewes producing such lambs. On the other hand, a number of authors have described such lesions in lambs of six months to a year old (Willman, Loesli, Asdell, Morrison and Olafson 1946, Gotchin 1947, Anderson 1953, Shanks, Stamp and Wilson 1954, Marr, Sharman and Blaxter 1956, Bosanquet *et al.* 1956, Hartley and Dodd 1957), and in fully mature sheep (Marston and Pierce 1942, Watt 1954, Hartley 1954, Dodd 1954, Bosanquet *et al.* 1956, Parry 1957, Hartley and Dodd 1957). Of the latter group of authors only Bosanquet *et al.* and Parry specifically observed that young lambs did not appear to be affected though lambs of five months of age were affected. The only unusual feature of type IV lesions encountered in this survey then, would appear to be the fact that they were found together in young lambs and ewes. Undoubtedly, however, the present outbreak might well have been recorded in lambs only if the lesions in the ewes had not been discovered incidentally during a routine and thorough examination of muscle.

**Incidence**

The incidence of dystrophic lesions does not appear to be a differentially identifying feature. In a single outbreak in animals of several
ages the incidence can vary from one per cent to one hundred per cent (Blaxter and McGill 1955, Hartley and Dodd 1957), depending particularly on conditions of exercise. In this survey the incidence of type IV lesions was quite low (12.3%) when all sheep examined over the twenty-one month period were considered. However, during the last five month period from Feb. 1 to July 1, over 40% of the forty-six sheep examined in detail were affected. This increased incidence was not associated with increased exercise.

The term "enzootic muscular dystrophy" has been generally used to describe seasonal outbreaks which occur from Jan. to June (Blaxter and McGill 1955). The outbreak of type IV lesions appeared to differ slightly from these in that two animals did not fit into what was otherwise a late winter - early spring epizootic. The importance of this observation was that it appeared to represent the link between the strictly seasonal outbreaks and the myopathy of Bosanquet et al. and Parry in which most cases did not fit the seasonal pattern.

**Diet**

Natural outbreaks of dystrophy have occurred on both good and poor diets, however, the important factor under experimental and some natural conditions has been proved to be the vitamin E content of the ration (Willman et al. 1945, 1946, Swahn, Obel and Wanntorp 1949, Whiting, Willman and Locsli 1949). Clearly the problem is not always quite so simple, since Blaxter (1955) has indicated that the unsaturated fatty acid content of the diet is important, and that similar lesions can be precipitated by conditions of circulatory trauma, experimental anoxia, or exposure to cold or to virus infection. Type IV lesions in this survey appeared in four mature animals which had been supplemented for eighteen months with
over 100 mgs. of alpha tocopherol per day. The diet contained no added fat, and was adequately supplemented to supply the known requirements of minerals and vitamins; in fact, the dietary regimen was specifically designed not to interfere with vitamin E metabolism since it was instituted after a previous appearance of muscle lesions (Mibet, Butler and Macintyre 1959). One of the dystrophic lambs born of ewes in this group also received alpha tocopherol supplementation shortly after birth. It was difficult to understand how the lesions in these cases could have been related to vitamin E metabolism, or for that matter, why any of the animals in this survey should have been suffering from a vitamin E deficiency, particularly the two ewes on good, late-summer pasture. The lack of correlation between lamb blood and maternal colostrum tocopherol levels in lamb dystrophy and the futility of preventative supplementation reported by Safford et al. (1956) is of interest in this respect.

Despite the above observations on the adequacy of diets used here however, nutritional factors could not be completely ruled out in the production of type IV lesions, even though there was a diversity of diets involved which lacked even one common constituent. A similar observation has been made by Walton (1956) of the myopathy reported by Bosanquet et al. (1956).

**Exercise**

Unusually strenuous or unaccustomed exercise has been incriminated as the precipitating factor in some cases of muscular dystrophy in both lambs and sheep (Schofield 1952, Hartley 1953, Marr et al. 1956, Hartley and Dodd 1957). This factor did not appear to play a part in the production of type IV lesions since most of the sheep were very much confined and none
were driven for any distance. Absence of unusual exercise was also observed in the myopathy of Bosanquet et al. and Parry.

**Symptoms**

In previous reports on cases occurring under both natural and experimental conditions, attention was first directed to an animal with muscular dystrophy because of the clinical symptoms of trembling, stiffness, ataxia or weakness (Culik, Bacigalupo, Thorp, Leuke, and Nelson 1951, Bacigalupo, Culik, Leuke, Thorp and Johnston 1952, Hartley 1953, Marr et al. 1956, Hartley and Dodd 1957). With the possible exception of trembling, the mature animals showing type IV lesions observed here showed no clinical symptoms which could be considered referable to the muscle damage. Admittedly, five animals did show symptoms of scrapie, including some degree of ataxia of the hind quarters, but the lesions in these five cases were almost completely confined to the forequarters and the abdomen. Bosanquet et al. (1956) and Parry (1957) related similar muscular lesions to symptoms which were undoubtedly those of scrapie. Since it is a finding of this survey that most scrapied sheep show no significant muscle changes, it is difficult to avoid the conclusion that their myopathy was often as asymptomatic as the type IV lesions described here.

The fact that no symptoms referable to muscle lesions were observed indicated that mature sheep are probably capable of tolerating a moderate degree of muscle destruction. The evidence presented suggests that, in this respect, lambs and mature sheep differ, since three of the four dystrophic lambs showed stiffness and reluctance to move. This difference might well account for the fact that previous reports of dystrophy in lambs contain no observations on the condition of muscles in the dams.
Distribution

The distribution of the lesions in the sheep carcass does not appear to be a distinguishing feature of any single type of myopathy. In the experimental and natural muscular dystrophies the lesions were widespread and usually, but not always bilateral (Metzger and Hagan 1927, Shanks et al. 1954, Marr et al. 1956). This description of distribution appears also to fit "myopathy" (Bosanquet et al. 1956, Parry 1957), as well as the lesions of blue-tongue (Thomas and Neitz 1947) and the type IV lesions reported here.

Macroscopic Appearance

It has been indicated in the body of this report that the macroscopic appearance of the muscular lesions was not a reliable indication of the histological changes, and this has been observed of muscle lesions generally by Jewsbury and Topley (1912). Still, excluding those lesions obscured by haemorrhage or fat, the description of type IV lesions varied but little from the descriptions of the nutritional muscular dystrophies and "myopathy".

Microscopic Appearance

Histologically, type IV changes showed considerable variation depending on the degree of predominance of secondary changes. The common factor in all, however, was the presence of fragmented fibres and the distribution of these indicated a high degree of selectivity. In most mature animals the appearance of the fragments generally suggested hyalinization (Zenker's degeneration) while in others, and in lambs, granular degeneration appeared to predominate. Both types, however, appeared in virtually all lesions, and the intensity with which the sarcolemmal nuclei proliferated
seemed dependent on the predominant type of primary degeneration. The predominance of hyaline degeneration in older sheep has been previously observed by Marston and Pierce (1942) and Marr et al. (1956). A relationship also appeared to exist between the predominant type of primary degeneration, i.e. hyaline or granular, and calcification. In lamb muscle, and some mature muscles, granular degeneration was accompanied by abundant mineralization, whereas the more usual hyaline fragments seen in many mature animals were free of calcification. In this regard, type IV lesions again appeared to provide the link between the myopathy of Bosanquet et al., which has been reported to lack mineralization, and the natural and experimental muscular dystrophies which usually contain abundant calcification.

The variable degree of regeneration and the amount of fibrous and fat replacement in type IV degeneration has been observed also in nutritional dystrophies and the myopathy of Bosanquet et al. A reciprocal relationship between regeneration and fibrous or fatty replacement in dystrophic rabbits and guinea pigs has been observed by Pappenheimer (1941, 1948) i.e. in the more chronic lesions there was less regeneration and more fibrous tissue and/or fat replacement. Adams et al. (1954) have observed that the terminal stage of nutritional muscular dystrophy in mature guinea pigs is fat replacement, while in young animals regeneration was often rapid (Goettsch and Pappenheimer 1931, Pappenheimer 1941).

Two features of some type IV lesions apparently not encountered in the experimental muscular dystrophies nor in natural outbreaks of comparable lesions in sheep, were haemorrhages and complete necrosis of areas of muscle. Necrosis was seen always in conjunction with haemorrhage, usually in muscles showing macro- or microscopic rupture of muscle fibres. Circumstantial evidence suggested that primary rupture of muscle fibres
was sufficiently violent to cause capillary rupture, haemorrhage and a local stasis of blood. Suggestive evidence of partial or transient haemostasis, not sufficiently severe to cause acute necrosis, was provided by one haemorrhagic muscle showing discoid degeneration (see Harman 1947, 1948). Similar necrotic and haemorrhagic foci have been reported in experimental blue-tongue in sheep (Thomas and Neitz 1947) and in a probable nutritional dystrophy of rabbits (Innes and Yevich 1954).

One histological feature common to all variations of type IV lesions which seemed to be of some importance as a possible differential diagnostic feature was the granular and fragmentary degeneration of the intrafusal muscle fibres in neuromuscular spindles. Intrafusal fibres generally are remarkably resistant to degenerative muscular changes affecting the extrafusal fibres (Adams et al. 1954, Greenfield et al. 1957). The two exceptions to this generalization reported in animals are those of Chor and Dalkart (1949) working on experimental vitamin E deficiency in guinea pigs and of Bosanquet et al. (1956) in their report on myopathy in sheep. The obvious conclusion that the three conditions may have a similar aetiology does not necessarily follow, however, especially when one considers how little is known of spindles in animal myopathies generally. These structures will be discussed more fully later though it might be added here that in none of the other types of muscle degeneration encountered in this survey was intrafusal fibre degeneration observed.

**Motor Innervation**

One final aspect of type IV changes which requires discussion is that of the intramuscular nerves. The innervation of muscle was studied in fourteen muscles and in three of these abundant evidence of axon and myelin degeneration as well as end plate changes was found. In eleven
other type IV muscle lesions the axons and myelin appeared normal, but in some end plates there was an alteration in the distribution and amount of copper sulphide granule deposition. The presence of a considerable fibrous tissue reaction in the three muscles mentioned above but not in the other eleven did not appear to be merely coincidental, and one might suppose that fibrosis had caused nerve degeneration, or, more likely, that a partial haemostasis had stimulated fibrosis and at the same time caused nerve degeneration. This combination of changes has been reported as occurring in the viable areas at the borders of muscle infarcts (Lyons and Woodhall 1949; see also the infarcts described in this survey). The fact that these muscles also contained the most extensive muscular damage seen suggested that a local toxic degenerative product may have been responsible for axon degeneration. In contrast in experimental nutritional dystrophy, the muscle damage, however extensive, apparently has no effect on peripheral axons or myelin sheaths (Rogers, Pappenheimer and Goetsch 1931, Pappenheimer 1941, 1942, Chor and Dolkart 1949).

Whatever may be the precise aetiology of the axon degeneration seen by us in these muscles, it would appear to have been a retrograde degeneration rather than a spinal one since both spindle and motor nerves were involved.

In the remaining eleven type IV lesions terminal axons and myelin sheaths appeared normal, and in silver impregnated sections the only change was a clumping of the sole-plate nuclei and an apparent loss of teliodendria. This conformed to the expected appearance and similar findings have been previously reported in muscular dystrophies now considered due to vitamin E deficiency (Rogers et al. 1931, Pappenheimer 1941, 1942, 1948, Chor and Dolkart 1949). Bosanquet et al. (1956) reported no abnormalities in their myopathy. In cholinesterase preparations, the loss of form and fine granularity of the subneural apparatus was an
unexpected change in motor end plates. In many there was a great loss of affinity to the terminal neural structure for copper sulphide indicating a probable reduction in cholinesterase. This change appeared highly selective and was found only in those end plates under which the muscle fibre showed evidence of degeneration. This was interpreted as indicating a specific relationship between muscle fibre degeneration and cholinesterase loss. Further evidence that this change was not an artifact was provided by the fact that similarly processed preparations from fifty-eight sheep muscles in various stages of cachectic atrophy and twenty-one muscles from guinea pigs in various stages of denervation atrophy showed no such change.

The primary implication arising from these considerations is that the cholinesterase which was responsible for the localization of copper sulphide was no longer sharply localized within certain areas of the end plate, and secondarily, that it was then quite rapidly removed from, or altered at, the site of the subneural apparatus. The rapidity with which this second change occurred was confirmed by the fact that the muscle fibres were not atrophic, yet there was, proportionately, much less copper sulphide deposited in some of these end plates than there was in guinea pig muscles after forty days denervation. The degenerative muscle changes appeared to be of a recent nature.

The observations of Bloch (1942) and Hess and Bicollier (1948) that in vitamin E deficient (dystrophic) muscle there was a significant reduction in cholinesterase as determined by chemical evaluation lends substantial support to the assumption that cholinesterase is lost from the end plates in muscular dystrophy. The observations noted in this survey appear to be the first visual demonstration of this chemical determination. In view of this relationship between muscular dystrophy
and cholinesterase loss, it would be tempting to hypothesize that the loss of cholinesterase was primary and that the muscle lesions might have been a result of acetyl choline excess. It would not be unreasonable to assume that the resulting prolonged contraction of muscle might produce a local acidosis and myofibril fragmentation (see Thomas and Neitz 1947). On the other hand, however, reasonable such a chain of events might appear, three pieces of evidence presented here dictate against formulation of such a convenient explanation. These are: first, that at least some cholinesterase was present in some end plates located directly over degenerate muscle; secondly, that degeneration of muscle fibres was often sharply segmental immediately below the end plate rather than throughout a single fibre; and thirdly, that no apparent reduction of end plate cholinesterase was seen on normal muscle fibres which might have represented the "pre-dystrophic" stage.

The only conclusion that we can draw is that in sheep with type IV lesions there was usually no anatomic interruption of muscle innervation but that there was a loss of cholinesterase, which was probably secondary. In brief, there was a biochemical lesion but only in conjunction with an anatomic muscular lesion. Such a biochemical alteration has been postulated in other diseases, (Peters 1949) but the idea has fallen into disrepute because many investigators could find no evidence of later anatomic nerve changes to support it (Denz 1951, Harris 1954, Snell and McIntyre 1956); it has been generally concluded that any chemical loss of cholinesterase was only relative and a result of atrophy of muscle alone.

From the foregoing comparison of type IV changes with similar reports of muscular lesions in sheep and laboratory animals, it is evident that a great many similarities and very few differences exist. It must therefore
be concluded that on the basis of the evidence presented, type IV lesions cannot be distinguished from other types of myopathy some of which are known to have a nutritional origin nor from the myopathy of Bosanquet et al. (1956).

Type V

In many respects the type V lesions appeared to represent a histologically distinct picture, quite easily separable from the other degenerative lesions described. Histologically, the changes presented many remarkable similarities to the progressive muscular dystrophies and progressive spinal atrophies in humans (see Hassin and Dublin 1945, Adams et al. 1954). The hypertrophic muscle fibres haphazardly distributed throughout groups of atrophic fibres of many sizes, and the massive increase in fatty tissue to the point of severe architectural distortion, produced a picture quite unlike the usual animal myopathies. Longitudinal division of the large fibres, many central nuclei, and occasional hyaline fibres completed a picture virtually inseparable from human progressive muscular dystrophy. The fact that spindle structure and innervation appeared normal lent support to this impression, and the myelin and axon degeneration found could have been interpreted as evidence of a progressive spinal atrophy.

In the literature on animal myopathies, reports exist of similar fatty lesions occurring in several species (Ostertag 1904, Nieberle and Cohrs 1949, Innes 1951, Hupka 1952, Whitney 1958). In dogs, cattle and pigs these lesions have been identified as progressive muscular dystrophy or atrophy (Nieberle and Cohrs 1949, Innes 1951, Whitney 1958). However,
since they have apparently been identified on the basis of histological examination of muscle alone, it appears somewhat premature to call them the animal counterpart of a human disease which can only be identified after an extensive clinico-pathological examination. The same reasoning dictated against calling type V lesions progressive muscular dystrophy or atrophy. The first authentic case in animals will have to be very well documented, since there is little doubt that in known animal myopathies of purely nutritional origin the end result could be very similar (Pappenheimer 1948, Adams et al. 1954). It would not take a great deal of imagination for example, to visualize a long standing type IV lesion with many similarities to a type V lesion. If primary degeneration in a mature animal were extensive the replacement fat cells could be abundant and coincidental or secondary changes in motor innervation could conceivably produce a similar pattern of atrophy and compensatory hypertrophy.

No convincing visual evidence was obtained in this survey with regard to the very large fibres observed here to indicate that hypertrophy and the type of fibre division described under type V could occur as a purely compensatory response to neuro-atrophy in adjacent fibres. The fact that similar changes occur in progressive muscular dystrophy in man has led to a great deal of caution in interpretation of such changes elsewhere (Adams et al. 1954). On the other hand, true, absolute hypertrophy without longitudinal division of fibres occurs so rarely and under such specialized conditions of heredity (Adams et al.) that it could hardly be called a normal physiological response. It did not appear unreasonable, therefore, to consider the hypertrophy and hyperplasia of the muscle fibres in type V within the realm of physiological change.

The inevitable conclusion arrived at regarding type V lesions was
that, although many histological similarities to human myopathies existed, sufficient evidence was lacking to establish a comparable entity in sheep.

**Inflammatory Changes**

The observations on the myositis lesions require little discussion, since in most cases the source of the inflammatory reaction was easily found. Parasitic myositis due to tapeworm larvae has been observed in many species (Nieberle and Cohrs 1949, Smith and Jones 1957) and presents a problem only when heavy infestations occur. The effect on sheep of only occasional muscular cysts is probably negligible (Ross and Gordon 1936, Newson 1952).

The disseminated mycotic myositis seen in an otherwise healthy animal was a pathological curiosity since such actinomycoses, although occasionally found in muscle, are generally confined to areas adjacent to ulcerations of skin or mucous membrane (Shahan and Davis 1942, Smith and Jones 1957). No primary ulcerative lesions could be found and it seemed not unlikely that the organism invaded the body by way of the digestive tract to be disseminated in the blood stream.

**Infarcts**

The necrotic lesions occurring in the final category of muscle changes found in this survey were of interest principally because of their
probable aetiologies. In two animals in which the changes were a result of antibiotic injection, it appeared likely that local pressure resulted in haemostasis and necrosis of muscle fibres. Toxic degeneration due to the injected substances seemed an unlikely explanation since many of the animals in this survey had received similar injections prior to death yet the muscles contained no lesions. In the third animal the multiple necrotic lesions were undoubtedly infarcts. Since a source of emboli was not found, and since the animal suffered from a long-standing paraplegia and bilateral femoral luxation one could suppose that the muscle lesions were due to haemostasis resulting from abnormal positioning of the hind limbs with periodic occlusion of the femoral veins or arteries. Assuming that pressure occlusion was in fact the cause of the muscle infarction in this case, it appeared much more likely that the low pressure, thin-walled veins were involved than the arteries. Both, however, may have been occluded, but the fact that organized thrombi were observed only in veins would suggest a primary venous occlusion. Adams et al. (1954) and Harman (1947) have remarked on the difficulty of producing muscle infarcts by occluding intramuscular arteries.

Other Changes

Round Fibres

Observations made here on normal muscles indicated that at least some muscle fibres in any normal muscle might be round in cross section. Adams et al. (1954) point out that on fixation connective tissue contracts
more than muscle and that this lateral pressure compresses fibres together
to produce the angular appearance so often seen in paraffin sections.
Atrophic muscles, and the fatty ones such as the vastas intermedius and
pectineus muscles, contained a much higher percentage of rounded fibres
than other normal muscles. Two explanations of this phenomenon might be
presented.

The first explanation is that when a muscle undergoes atrophy,
the lateral pressure of skin, connective tissue and adjacent muscle fibres
is reduced. During fixation the connective tissue contraction exerts
less pressure on the smaller fibres and many retain their rounded form.
This appeared a reasonable explanation for the many round fibres in
muscles from scrapied animals. In the fatty muscles a similar explanation
might hold since the muscle fibres could retain their rounded form at
the expense of the more malleable fat which virtually surrounded every
muscle fibre. The staining reaction of these round fibres would,
understandably, be little altered from normal.

A second explanation of rounded fibres and in this case of the
strongly eosinophilic ones as well, has been pointed out by Adams et al.
(1954) and Greenfield et al. (1957). When a muscle is fixed too soon
after death, dark contraction bands are frequently seen. In cross section
these bands appear as strongly eosinophilic fibres which retain their rounded
form at the expense of adjacent non-contracted fibres. Under these
circumstances, striations and Coenherm's fields would be lost or distorted.
In view of the four to twenty-four hour time lapse between death and
fixation utilised on the material in this survey, it is doubtful if such
artifacts could have been common but the possibility cannot be excluded
since after death adjacent muscle fibres relax at greatly different rates
Undoubtedly, the many round fibres in type IV lesions were similar to contraction bands. The contracted, strongly eosinophilic fragments could retain a rounded cross section because of both the internal contraction pressure in the absence of tension and the reduced lateral pressure of adjacent, empty sarcolemmal tubes.

Bosanquet et al. (1956) in their examination of muscle from scrapied sheep appeared to give considerable weight to rounded fibres as evidence of an early stage of fibre degeneration. Since it is evident that several sets of circumstances can produce round fibres in sheep muscle, the precautions of Adams et al. seem pertinent i.e. that changes visible in normal muscle must be disregarded even in definitely pathological muscle lesions. Delez et al. (1957), working on scrapie, also suggested that rounded fibres, particularly those in the vastas intermediius, pectineus and ocular muscles, indicated early myopathy. Examination of normal and emaciated muscles from non-scrapied animals would surely have convinced them otherwise.

Cross Striations

Adams et al. (1954) have observed that one of the most difficult tasks confronting a pathologist working on muscle is to evaluate the subtle changes of the sarcoplasmic constituents. The finer details of striation were investigated here in an attempt to find an early change which appeared to introduce the more obvious, irreversible changes used in this report as criteria of degeneration.

The loss of Qh and Z lines has been reported by Aumonier (1952) as the earliest change in virus myositis. Miller (1933) observed that
this loss of lines might occur as the first sign of degenerative change, but also that post-mortem autolysis could produce virtually identical changes. Miller also pointed out that contracted or relaxed muscle lacks both lines, consequently the evaluation of the presence or absence of lines must be made only on semi-contrasted muscle fibres.

The observations on degenerative muscles reported indicated that, in some fibres, a loss of lines was probably a premonitory sign of more severe degeneration since a zone lacking lines separated normal and degenerate regions of a fibre. Absence of this intermediate zone in some damaged fibres and the presence of both lines in some obviously degenerate fragments indicated, however, that it was not an invariable early change. On the other hand there were two pieces of evidence which indicated that this change by itself could not be utilized as an indication of degeneration; firstly, a loss of lines could be a staining artifact and secondly, some fibres in otherwise normal control muscles also showed a focal loss of lines. The correct explanation of this change is probably that given by Miller (1933) regarding autolysis. It was concluded that a loss of the Qh and Z lines alone did not appear to be a sufficiently consistent change to be regarded as a first irreversible sign of degeneration. The change did appear useful, however, in defining the true extent of otherwise confirmed lesions.

The observation that the absence of cross stria in formol or formol-sublimate fixed muscle sections was of no significance in itself was in agreement with many previous authors (see Adams et al. 1954). Harman (1947) went so far as to conclude that the presence of prominent cross striations in his rabbit muscle was the first irreversible stage of ischaemic necrosis. Harman also indicated that the type of fixative used had no bearing on the appearance of cross striations. In the light
of the facts presented here, it was pertinent to conclude that the presence or absence of cross striations alone had little bearing on the state of muscle fibre health.

Sarcocysts

Sheep have long been known to harbour particularly heavy infestations of sarcocysts and the relationship between this parasite and scrapie has been the subject of several investigations (McGowan and Rettie 1913, McGowan 1914, 1918, Stockman 1918, McFadyean 1918, Walker 1918, Brownlee 1940). The great variation in severity of infestation in all groups of sheep examined here bears out the generally accepted conclusion that no relationship between scrapie and sarcocyst infection exists. Undoubtedly a very severe infestation would have an additional debilitating effect in a chronic, fatal disease such as scrapie but the significance was impossible to evaluate here.

Cellular reaction around infested sheep muscle fibres has been reported by Scott (1930). Eisenstein and Innes (1956) agreed that this did occur, but made the qualifying statement that such reactions were observed only during the stage of parasite degeneration when Rainey's corpuscles were no longer clearly defined. Evidence presented here indicates that such parasite degeneration is not a prerequisite for cellular reaction and that some type of systemic sensitivity appeared to be present in some individual animals. These observations cast some doubt on the conclusion that the effect on the host, if any, is mediated exclusively through an endotoxin.
Striated Muscle-Fibre Annulets

The striated muscle-fibre annulets observed in a naturally-occurring skeletal muscle lesion in a sheep included in this survey were of doubtful pathological significance but were of considerable academic interest. In man these annulets or "Ringbinden" in limb muscles, when occurring in conjunction with other muscular changes, have long been associated with a group of myopathies which includes dystrophia myotonica, progressive muscular dystrophy and such endocrine disturbances as acromegaly, myxoedema and exophthalmic goiter (Bergstrand 1938). This specific relationship has recently been questioned (Perry, Smith and Wrenn 1956), since annulets were found to be abundant in muscle changes associated with arteriosclerosis, neuritis and even osteomyelitis.

In animals, annulets are known to occur in increasing numbers with advancing age in the small voluntary muscles of the eye and ear of many species including sheep (Malan 1934, Bosanquet et al. 1956). They have also been found singly in other normal skeletal muscles from man, rabbit, frog and rat (Wohlfart 1932, Bergstrand 1938, Gunther 1952). Despite the many reports describing annulets as pathological entities, Adams et al. (1954) and Perry et al. (1956) concluded that they were artifacts though perhaps a manifestation of an abnormal irritability of some fibres. It was difficult to reconcile this conception with the observations reported here regarding some of the extremely complex forms or with the fact that in the ring fibrils the nuclei were oriented parallel to the circling myofibrils. This last observation has previously been cited by Greenfield et al. (1957) as evidence supporting the theory that
striated muscle-fibre annulets as such exist in vivo. If in fact the rings were an artifact of the outer myofibrils twisted into a subsarcolemmal spiral as Perry et al. suggest, one would expect to find no nuclei in the circling fibrils.

Whatever the precise aetiology of these rings their presence in lesions in sheep skeletal muscles lends considerable support to the belief of Perry et al. that they are not pathognomonic of a particular group of diseases, but rather that they are indicative of a change in muscle which might be produced in a number of ways. The conditions which appeared to lead to their production in our animals were thrombosis of some arteries in the area, and fibrosis and nerve regeneration after a period of degeneration. These appear to be very similar to the conditions in some of the cases described by Perry et al.

One must conclude from the foregoing observations that striated muscle-fibre annulets were absent in normal sheep body skeletal muscles and rare in pathological muscle. Their presence provided further evidence of their lack of specificity for certain human diseases.

Sarcoplasmic Masses

Sarcoplasmic masses, attributed to ante-mortem destruction of myofibrils, have been described in dystrophia myotonica (Wohlfart 1951, Greenfield et al. 1957). Wohlfart thought that these figures might be specific for the disease.

The structures reported here in two fibrotic muscular lesions, and designated as sarcoplasmic masses, appear to be identical in cross section to the masses described in the above reports. Greenfield pointed out, however, that positive identification could be made only if the
masses were seen in longitudinal sections as well as cross sections since they might be confused with contraction bands. The fact that muscles in this survey were left on the carcasses for at least four hours prior to fixation should have excluded any tendency to form contraction bands (Adams et al. 1954). The absence of these figures in all but two muscles examined also suggested that they were not artifacts.

These sarcoplasmic masses then, appeared to be ante-mortem structures. If this was so, it indicates a lack of specificity of these structures for dystrophia myotónica. Their significance in sheep muscle is uncertain.

Neuro-Muscular Spindles

The precautions taken during measurement of intrafusal fibres of neuro-muscular spindles in this survey served to a large extent to eliminate the tapered ends of the fibres. In spite of this, because of the variable diameter of a single fibre at different levels and the great variation between adjacent fibres (Barker 1948), the measurements understandably covered wide ranges. The values obtained for spindle diameters could not be considered completely accurate as absolute values since they failed to take into account lateral compression. Since, however, all three groups being compared were measured in the same manner, these errors did not seriously impede comparison.

The normal dimensions of mammalian spindles reported by Sherrington (1894) and Barker (1948) of 30 to 200 microns for spindle diameter were somewhat higher than the normal range found here. This perhaps reflects the error incurred in measuring the least rather than the greatest diameter.
In the number of intrafusal fibres (2 to 10) and in the diameter of fibres (6 to 28 microns), the figures of the above authors were comparable, although the slightly lower limits of the range of our fibre diameters (4 to 25 microns) reflected again the least diameter techniques used.

In the scrapied group the slightly smaller fibre size could not be justifiably interpreted as fibre atrophy in view of the wide range of normal diameter. It has been shown, however, that intrafusal fibres undergo atrophy just as extrafusal fibres do (Adams et al. 1954), and it would not have been unreasonable to suppose that some atrophy of intrafusal fibres had occurred. The larger range of spindle diameter for the scrapied group, and the proteinaceous material in the intrafusal spaces, might be accounted for by the oedema of cachexia (Elwyn 1930) since Sherrington (1894) has demonstrated that the intrafusal space is continuous with lymphatic spaces and the animals concerned had very oedematous muscles.

In the spindles of the third group, i.e. those muscles showing type IV lesions, the secondary fibrosis on the outside of the spindles appeared to have no effect on the internal structures. The most remarkable feature of this group was the apparent reduction in the number of fibres per spindle and the evidence of degeneration observed in existing intrafusal fibres. Spindles are remarkably resistant to muscular degenerations and atrophies generally (Tower 1939, Wohlfart 1949, Greenfield et al. 1957) and in man degeneration of these organs is usually expressed as intrafusal fibrosis. In animals, change in muscle spindles appears to be a neglected topic, however Chor and Dolkart (1949) have described acute degeneration of intrafusal fibres in experimental vitamin E deficiency in guinea pigs and Bosanquet et al. (1956) have described somewhat similar changes in a myopathy of sheep. The implications of these reports
regarding type IV change have already been discussed.

The internally fibrotic spindles reported here in three cases and associated with type III lesions, presented an interesting similarity to those seen in dermatomyositis and anterior compartment syndrome (Greenfield et al. 1957) and in polynéruritis (Wohlfart 1949). As in these diseases of man the spindle muscle fibres appeared to be quite normal. Another similarity was the heavy perimyseal and perineural bands of connective tissue, which in the cases reported, appeared to be the primary lesion. It would seem unrealistic however, to suppose that these fibrotic spindles were indications of a more generalized disease. In all probability they resulted from a very localized, undetermined stimulation of connective tissue.

Motor Innervation of Skeletal Muscle

An examination in search of changes in motor nerves and end plates such as the one conducted here is subject from the outset to two limitations. The methods of examination that are available dictate that, at best, the sample must be a very small proportion of the total number of end plates in the body. The second limitation is that imposed by the fact that not all functional changes in neuromuscular transmission are detectable in histological preparations. Drugs toxic to the myoneural junctions leave no changes that can be detected by methylene blue, gold or silver methods (Denz 1951). The cholinesterase method shows promise in this line of investigation (Woolf, Bagnall, Bauwens and Bickerstaff 1956), but the changes revealed by this method have not yet been clearly
defined. It is also evident that, because of cord or brain damage, the peripheral nerves may be functionally useless yet the myoneural junctions remain anatomically intact (Tower 1937). On the other hand, many diseases do show selective or widespread changes which would be detectable by silver, gold and Sudan IV stains (Adams et al. 1954).

In scrapie peripheral neuritis has been reported by Besnoit (1899). Although this finding could not be confirmed by Cassirer (1899) nor by Bertrand, Carre and Lucam (1937), the possibility that some peripheral nerve change might be present was revived by the inconclusive findings of Brownlee (1940). Bosanquet et al. (1956) and Palmer (1957) have recently stated that in scrapied sheep nerves in muscle appeared normal, but these surveys were undoubtedly very limited.

Within the limits defined, the results reported here of examination of end plates and terminal axons indicate convincingly that in scrapied animals there is no detectable degenerative changes in these structures. Since these terminal structures are usually the most sensitive part of the motor neurons, it must be concluded that the motor neurons are probably not involved in mediating the symptoms of scrapie. One of the assumptions on which this conclusion rests is that the neurodegenerative changes which might explain the ataxic symptoms of scrapie, are progressive. The progressive clinical symptoms and the chronological increase of neuron degeneration in the medulla (Zlotnik 1958a, b) suggest that this assumption is a reasonable one. It must be pointed out, however, that the methods of peripheral nerve examination used here could not be expected to demonstrate a generalized reduction in motor horn cell function due to degeneration of nerve elements other than the ventral horn cells of the cord.

The changes associated with myodegeneration have been discussed previously.
Extrinsic Ocular Muscles

The findings reported here concerning the extrinsic ocular muscles of the sheep agreed essentially with those of previous authors regarding the pattern and structure of the muscle fibres, and the distribution of the neuromuscular spindles (Cilimbaris 1910, Cooper and Daniel 1949, Cooper, Daniel and Whitteridge 1955). The occurrence of sarcosporidia in these muscles requires no discussion here, since their presence appeared merely to reflect generalized infestation in skeletal muscles. In normal ocular muscles striated muscle-fibre annuletts were often numerous. Large numbers were not always associated only with older sheep; the greatest number observed was in a cross section of a rectus oculi muscle from a three year old ewe. Another point at variance with Bergstrand's (1938) observations on the effect of age on these muscles was that some of these so-called normal annuletts in eye muscle were extremely complex, while Bergstrand reported that complex annuletts were generally confined to pathological lesions in skeletal muscle.

Muscle fibre degeneration in extrinsic ocular muscles has been cited as a feature of scrapie (Bosanquet et al. 1956). Delez et al. (1957), in support of the first authors, reported atrophy and rounding of fibres as evidence of myopathy in this disease. Neither group of workers defined the extent of the changes, but Bosanquet et al. described vacuolation of intrafusal fibres along with lack of cross striation, granular degeneration and minute nuclear changes as evidence of degeneration in these muscles.

In this survey the criteria used to judge muscular degeneration generally i.e. cellular replacement or definite fragmentation of fibres
were not observed in intrafusal fibres of ocular muscles. The presence of vacuoles in intrafusal fibres of normal controls indicated that this was of little significance in scrapie; Adams et al. (1954) has also reported them as artifacts in normal muscles. The absence of cross striations in routine stains has been discussed previously as being of questionable significance; atrophy and rounding of fibres were undoubtedly characteristics of emaciation and not just of scrapie.

In all three groups the occasional occurrence of degenerating extrafusal fibres similar to type I degeneration of skeletal muscle generally, suggested again that such an underlying level of fibre destruction might be found in any animal, and that no significance should be attached to it. The one scrapied animal showing more than a normal number of such fibres deserves passing mention, but does not appear to detract from the general lack of significant changes.

Innervation of Extrinsic Ocular Muscles

The distribution of motor end plates described in this report agreed essentially with previous reports on the subject of extrinsic ocular muscles of sheep (Cooper et al. 1955), and indicated many similarities to other species.

Typical motor end plates on short terminal axons presented no problems of identification, though their number relative to the number of muscle fibres was impressive. Accessory motor end plates and "terminaisons en grappe" endings (Hines 1931) could be identified only tentatively as the copper sulphide marked plates on longer axons distal to the main motor band. The methods of examination used did not allow
a more detailed comparison of finer endings with the data obtained by other methods; previous morphological evidence (Hines) suggested that these endings might contain cholinesterase, though there is still some doubt about the "terminaisons en grappe" endings (Daniel 1948).

The method and type of function of the nerve endings in the peripheral third of extrinsic ocular muscles have been the subject of many reports (Daniel 1946, Cooper and Daniel 1949, Cooper et al. 1955, Cooper and Fillenz 1955), yet some perplexing questions remain unanswered. Examination of this region in the present survey revealed no elucidating features, and the presence of some copper sulphide crystals diffusely distributed could conceivably be interpreted as non-specific deposit rather than as evidence of motor activity.

A finding more significant than the anatomical detail described in this report was the complete absence of visible axon or myelin sheath degeneration in both scrapied and control animals. It has been indicated earlier that the muscle changes in extrinsic ocular muscles of scrapied sheep were negligible. In view of the observations discussed here it must also be concluded that the peripheral nerves of these muscles were equally free of degeneration. These two observations tend to implicate the central nervous system as the origin of the ocular deviation frequently seen clinically in scrapie. In this respect ocular muscles appear to be similar to other skeletal muscles.
The Relationship of Muscular Lesions to Scrapie

The most significant piece of evidence against a positive relationship between scrapie and muscular lesions was the complete absence of any macroscopic lesions, other than generalized atrophy, in the muscles of eighty-three sheep suffering from advanced scrapie. The wide variety of muscular lesions in the remaining eleven sheep, and the presence of similar macroscopic lesions in non-scrapied control animals, added further support to the observation that lesions other than atrophy are not a characteristic of scrapie. This observation was in general agreement with that of many previous investigators of the disease.

Early works on scrapie invariably included observations on muscular atrophy, but none described macroscopic lesions (May 1868, Cassirer 1898, McFadyean 1918, Stockman 1913, Gaiger 1924). McGowan (1914) attributed the loss of condition and pruritis to a severe infestation of sarcocysts in the muscle, since he could find no other muscular changes to account for the disease. Bertrand et al. (1937), Greig (1940) and Lucam, Bechade and Saurat (1950) also remarked on the absence of significant muscular changes, and Bertrand et al. further commented on the probability of the central nervous system being the primary site of the disease.

In contrast with these reports Bosanquet et al. (1956) and Farry (1957) reported finding sufficient muscle change in fifty-two cases to support their hypothesis that scrapie was a primary degeneration of skeletal muscle. According to these authors the symptoms of scrapie could be related directly to the muscular lesions and were dependent on the location, age of development and extent of the myopathic process. Lesions were found by naked eye examination, and appeared in the form either
of intense pallor (focal or diffuse), wasting of muscle, "firm lesion" or as an excessive deposition of fat. Palmer (1957) found macroscopic myopathic lesions in three out of ten sheep and goats suffering from scrapie. Delez et al. (1957), in support of Bosanquet et al., reported finding muscle lesions in three out of three sheep.

Turning to a consideration of the microscopic degenerative lesions reported here, the changes other than atrophy (which has already been considered), fell into two categories as far as their relationship to scrapie was concerned. One group consisted of those changes observed in both scrapied and control animals, and included the lesions of types I, III and IV and myositis and infarction. The other group was made up of type II and type V lesions which were seen only in scrapied animals.

Of the first group, type I changes of single fibre destruction were broadly though sparsely distributed through muscles of sheep in all categories. Any difference in the incidence between the two groups (36% for scrapied and 46% for non-scrapied) appeared to be accounted for by the average age difference between them (2.4 vs. 2.9 years). In view of the absence of symptoms referable to mobility, even in animals showing the more extensive type IV lesions, it was inconceivable that type I changes could be considered in any way responsible for the symptoms of scrapie. The similarities between type I changes and the myopathy described by Bosanquet et al. (1956) as "destruction of one fibre per field" were noteworthy.

Type III and IV lesions were found in eight out of ninety-four scrapied animals, and also in seventeen out of seventy-six controls. This distribution alone made it extremely unlikely that the symptoms of scrapie could be related to this type of muscle degeneration, or even
that the muscle changes could be considered secondary. Additional evidence supporting this contention was the lack of correlation between lesions and symptoms previously mentioned and the features of the type IV changes which suggested a dietary origin.

The lesions of myositis and necrosis, which in most cases had a clearly explicable origin, for this reason fell outside the range of those lesions which must be considered in discussion of the relationship of muscle changes to scrapie.

The second group of lesions, those observed only in scrapied animals, could not be related to symptoms and were seen in far too few animals (six out of ninety-four) to be considered of any significance in the pathogenesis of the disease generally. Although the type II lesions showed some evidence of neurodegeneration, this was inconclusive; it did however suggest that they might be secondary to changes in the cord or brain, or secondary to the rubbing trauma. From the examination of muscle nerves and muscle fibre patterns it seemed quite clear that degeneration of peripheral nerves was not a characteristic of scrapie. It is conceivable of course that in any animal isolated nerves could be damaged, for example by wounding. Scrapied sheep would undoubtedly be more prone to such small wounds in the severe pruritic stages of the disease. The circumstantial evidence linking scrapie and type II lesions could be neither refuted nor supported by evidence presented.

Type V lesions also showed evidence of neurodegeneration, and from the data available it could not be decided whether it was the nerve changes or the muscular changes that were primary. The possibility of type V lesions being a later or complicated stage of type IV has been discussed previously; it does present an alternative which seems at least
as likely as that of a neurogenic aetiology. Whatever the origin of these lesions however, the changes, particularly those of hypertrophy and fatty replacement, suggested a duration much longer than one would expect of the symptoms of scrapie. This would indicate that type V lesions probably have no relationship whatever to scrapie.

From a consideration of the data presented in this survey the inevitable conclusion that must be reached is that muscle changes play no part in producing the symptoms of scrapie, and that probably most if not all the lesions present were coincidental. This view is clearly at variance with those held by Bosanquet et al. (1956) and Parry (1957), and probably others. Perhaps however the elimination from Parry's survey of such incidental changes as atrophy, and the changes reported here as type I, would leave a residue of significant muscular lesions not too different from those described here. It appears superfluous to postulate a large series of myopathic syndromes, of which scrapie is supposedly but one, in order to account for lesions found in scrapied and non-scrapied animals. It does seem quite certain however that myopathic changes, of which the cause or causes are as yet uncertain, do occur in a proportion of mature sheep in Britain.
Arteries

A discussion of pathological changes in arteries would be incomplete without preliminary consideration of the normal physiological changes which accompany aging. There is, however, very little agreement on the point at which physiological changes in the arteries can be said to become pathological, and opinions on the subject are as diverse as they are numerous.

The finding of intimal fibro-elastic thickenings at the mouths of branch arteries in sheep foetuses concurs with similar findings in infants (Stryker 1946, Prior and Jones 1952) and in a variety of animals (Wolkof 1924). Studies of the developmental changes occurring with age indicates that relatively early in postnatal life there is a sharp increase in the number and size of plaques and that this change is subject to wide individual variation. After puberty there is a more gradual increase, which is chiefly in size (Movat, More and Haust 1958). The end result of complete intimal sclerosis is undoubtedly pathological.

The fact that McLetchie (1952) could produce fibro-elastic intimal thickenings at the mouths of pulmonary artery branches in rats by the systemic injection of emboli would suggest that any such thickening might be considered pathological. Also, the suggestion by Duguid (1926) and Pareira, Handler and Blumenthal (1952) that some structural quirk might also produce similar changes as a compensation to stress, or as a result of turbulence, could be interpreted as indicating that intimal plaques are pathological. Lyding (1907), Wolkof (1924) and Fox (1939), working with several species of animals, considered thickening of the intima
with age as physiological, and classified a thickening as pathological only when it was adjacent to a degenerating elastic membrane. The fact that several old animals in this survey did not have thickened intimas in all areas, and that several very large plaques were not accompanied by elastic degeneration would seem to make this division untenable in regard to sheep. Krause (1927) went even further and suggested that calcification of the media might also be normal, yet he found this change in only three out of one hundred goats, and Zinserling and Krinitzky (1926) found none in seventy-five young sheep. Lignières (1912) and Sivori (1907) on the other hand, found extensive calcification in sheep arteries and considered all medial and intimal calcification to be pathological. In this survey calcification was extremely rare and it was concluded therefore that such mineralization must be considered to be pathological.

Perhaps the most realistic limits of changes due to aging are those presented by Aschoff (1933); he defined them as changes in the relative proportion of elements, and changes in the size and tortuosity of the vessels. He concluded that any sclerotic changes were pathological.

Since the definitive point between physiological and pathological changes in sheep arteries in this series could not be determined, any fibrous thickening of the intima, or fibrous metaplasia or calcification of the media has been considered as pathological. While this arbitrary distinction is not completely realistic, precedents are provided by recent reports of work in other species (Lindsay, Chaikoff and Gilmore 1952, Gotlieb and Lalich 1954). Fraying and splitting of the internal elastic membrane without other evidence of pathological processes has been considered a physiological change of aging as Wolkof (1924) and Aschoff (1933) observed.
Changes similar or identical to the fibro-elastic plaques reported here have been reported in many species; indeed, in many other surveys they appeared to be the change most frequently found. The interpretation of the significance of the variable amounts of elastic and fibrous tissue in the plaques and their positioning in the arteries has, however, given rise to differences of opinion among workers on the subject of atherosclerosis. One group concluded that following a breakdown of the internal elastic membrane or secondary to fatty deposition intimal sclerosis was reparative, (Zinserling 1931, Wilens 1942, Blumenthal, Lansing and Wheeler 1944, Fareira et al. 1952, Prior and Jones 1952, Lindsay and Chaikoff 1955). This group suggested that the fibrous production was a function of the cells at the base of such a plaque and adjacent to the elastic membrane. A second group (Duguid 1948, Lindsay et al. 1952, McLetchie 1952) believed that the plaques increased on the luminal side by the process of encrustation and absorption by the endothelium of fibrin and, probably of red blood cells. According to this conception any increase in fibroblasts occurred just under the endothelium, and Altschul (1950) went further to suggest that plaques were made up of de-differentiated endothelial cells, not of fibroblasts.

The material examined here contained several features which supported the luminal encrustation theory as proposed by Duguid (1948) and Crawford and Levine (1952). These were, firstly, that no thrombi as such were seen, which might have suggested that the plaques were a result of organized mural thrombi (Duguid 1948); also, the cellular reaction which accompanies thrombus organization (Harrison 1948, Barnard 1953, Prior and Hutter 1955, Williams 1955) was never seen. Secondly, on the surface of many plaques the endothelial cells were more prominent and more numerous than normal;
in these areas red blood cells were observed adhering to the endothelium and strands of fibrin were occasionally present. Thirdly, evidence of luminal vascularization of plaques was rare although trans-medial capillaries were frequently observed; this suggested a slow production, probably by encrustation (Geringer 1951). Fourthly, the elastic-rich base of the plaques indicated that these areas were of earlier evolution than the rest of the plaque since the proportion of elastic components in such plaques is known to be reduced in older individuals (Movat et al. 1958). The single laminated plaque observed might be attributed to two incidents of arteriosclerotic activity separated by a static period, as suggested by Altschul (1950).

It is an often-repeated dogma that domestic animals do not suffer naturally from true atherosclerosis (Fox 1939, Nieberle and Cohrs 1949, and many others). According to the strictest terms of definition i.e. athero=mush, this appears to be true in sheep, despite the sometimes numerous deposits of sudanophilic material that have been observed, particularly in the aorta. Similar observations have been made in other animals (Zinserling and Krinitzky 1924, Krause 1927, 1932, Zinserling 1931, Gottlieb and Lalich 1954), and the most that might be said of such changes is that they are similar to the "pre-atherosclerotic" lesions in man (Winternitz, Thomas and Le Compte 1938). If, in fact, accumulation of fat represents a comparable preliminary stage the paucity of reports of the atherosclerotic end result in animals becomes rather difficult to explain. One can only surmise that a basic difference between animals and man exists. The present report can provide nothing further, except to note that the complete absence of necrosis in sheep arterial plaques might point to a basic difference in vascularization by vasa; this may
provide a network of neocapillaries that is less fragile or less susceptible than it is in man. In this respect Winternitz et al. (1938) have observed that an embryological basis exists for species differences in the origins of vasa from large vessels.

An interesting and in animals previously unreported phenomenon in connection with the vasa is the extent to which they apparently can replace the function of occluded vessels. In this respect at least they resemble their counterparts in the human, though the presence of a replaced lumen in a two year old sheep suggested that in these animals the transition is much earlier and more rapid.

Between the several reports of arteriosclerosis in ungulates generally and the data described here two points of difference exist. The first concerns calcium deposition and the second is the absence of hyaline arterioles.

Calcification of the media and usually also of the intima has been considered a salient feature of arterial thickening in hoofed animals (Lyding 1907, Sivori 1907, Lignières 1912, Zinserling and Krinitzky 1924, Krause 1927, Fox 1933, 1939, Creech 1941, Hutyra, Marek and Manning 1946, Nieberle and Cohrs 1949) other than pigs (Gottlieb and Lalich 1954). In the sheep in this survey calcification was found in only a single artery of one animal; this low incidence was similar to that found in pigs. Perhaps however, the emphasis placed here on the examination of visceral arteries rather than just on the aorta might partly explain the low incidence of calcification found since many authors report calcification in the aorta only. The emphasis by many other workers on the more accessible aorta and its major branches might also account for the relatively low and variable incidence of all arterial lesions reported
in ungulates, since, as Cadiot, Lesbouyries and Ries (1925) have pointed out, in animals arteriosclerosis most frequently appears in the smaller visceral arteries. This is borne out by the much higher incidence reported by Krause (1927) who examined splenic arteries, and by the data presented here. The routine use here of an elastic tissue stain may have led to the detection of more lesions, particularly the early ones.

The second major difference between this report and previous ones was the absence of detectable hyaline change in the small arteries and arterioles of the spleen and kidneys in sheep. This change as demonstrated in van Gieson preparations has been reported in a high incidence in horses, cattle, pigs and goats (Krause 1927) and in non-ungulate species. An explanation for this disparity could not be found.

The increase with age in the size and number of arterial lesions requires no discussion since this agrees with all previous reports on spontaneous animal arteriosclerosis in which this aspect was considered. It was of interest to note that the report here of the rarity of plaques in foetal arteries and their relatively high incidence in the one and one half year old group was in agreement with similar observations in humans (Movat et al. 1958). This would indicate that the period between birth and puberty is the one in which the most extensive changes take place. Since this occurs in apparently normal animals, such observation should preclude a tendency often noted in veterinary literature to postulate unusual toxic or infectious diseases or abnormal dietary factors as a cause of intimal sclerosis.

Although several workers on the subject of scrapie have presumably included some examination of systemic arteries, only Brownlee (1940) specifically mentions examining these structures; in a very limited survey he reported no visible changes.
The examination in the course of this work of intramuscular arteries revealed rare examples of a variety of arterial changes. These were found in three scrapie sheep and in only one control animal, but the incidence was so low that it was of no significance when the two groups were compared. This lack of significant difference was also apparent when the groups were compared with regard to visceral vascular changes. When the incidences were corrected according to the average age of the animals in the respective groups, there appeared to be no differences between them.

It was concluded that neither the muscular atrophy nor the generalized cachexia of scrapie could be attributed to anatomic vascular changes.
GUINEA PIGS

Changes Secondary to Denervation

The results of experimental denervation in guinea pigs reported here were in general agreement with the reports of Snell and McIntyre (1956) and Bergner (1957) who used the cholinesterase method only. Regarding axonal changes, although different staining methods were used, they were also in broad agreement with many other reports on denervation atrophy (see Adams et al. 1954). These results were at variance with those of Snell and McIntyre in that in the work reported here forty days after denervation many small but essentially normal terminal rings were still visible. In this respect this report agrees with Bergner's (1957) results, although the current findings were at variance with those of Bergner regarding the effect of formol fixation on the subneural apparatus. She reported a fifty per cent reduction of cholinesterase after fixation, while here fixation at four degrees centigrade appeared to have no appreciable effect. Both of these differences were probably attributable to the improved cholinesterase method of Coupland and Holmes (1957) used here.

The combined cholinesterase and silver method, and the use of parallel Sudan IV stains, allowed broad limits to be set on a series of detectable degenerative changes. These were:

(1) Axon degeneration was detectable from the third to at least the twentieth day after denervation.
(2) Neutral fat in myelin sheaths was visible from the sixth day to at least the fortieth day.

(3) Cholinesterase was present in end plates for at least forty days.

(4) Interstitial fat deposition was evident from the thirtieth day onward.

These observations differed from previous reports in that axon degeneration was detectable for a longer period (17 vs. 10 days - Adams et al. 1954) and neutral fat was detectable earlier (at 6 vs. 16 days in the denervation of poliomyelitis - Denst and Neubuerger 1950). The species differences may have had some bearing on the appearance of some of these degenerative signs, but axon fragmentation appears to occur on the third day in many species (Chor 1933, Denst and Neubuerger 1950) suggesting that species differences may not be great. One difference between guinea pigs and humans was the absence in guinea pigs of a prolonged stage following denervation during which the muscle fibres were loaded with fat droplets (Denst and Neubuerger). This change was present in some fibres at six days only and may have represented a very transient change or was more likely due to individual idiosyncrasy.

Perhaps the most important contribution of the guinea pig series to the investigation of the peripheral nerve status in scrapie was the confirmation of the efficacy of the methods used to detect the degenerative changes occurring over a considerable period of time. The denervation experiments also provided a series of changes of known duration with which to compare the atrophic muscle changes of scrapie. A study of the atrophic changes which followed denervation made it evident that the cachectic muscle changes found in scrapie were quite different.
CONCLUSIONS

As a result of the foregoing discussion the conclusions arrived at fell into two major groups. The first contained those conclusions relating to the morphological changes that were observed, and in the second group were the conclusions relating to scrapie.

A. Conclusions Relating to Morphological Changes

Sheep Muscle

(1) The modification of Lendrum's erythrocyte stain that was used has proved to be a versatile and very useful stain in the examination of striated muscle.

(2) A frequency curve of normal sheep muscle based on the diameter measurements of fibres produced a "normal" curve.

(3) In cachectic atrophy of muscle a frequency curve of fibre diameters exhibited a double curve. The second curve was dependent on the resistance to atrophy of a population of fibres which were probably b-fibres. The atrophy resistance of the b-fibres varied from muscle to muscle, but tended to be a function of a given muscle rather than of the individual animal.

(4) Absolute hypertrophy of muscle fibres was rare in sheep muscle, but was observed. Relative hypertrophy of muscle fibres in
atrophic muscles appeared to occur frequently.

(5) Several types of histologically distinct muscle degeneration were observed in mature sheep.

(6) Degeneration of scattered single fibres was observed in normal sheep muscles and was considered of no significance in the evaluation of muscle lesions. This type of degeneration tended to be cumulative and increased with age.

(7) In a few samples of sheep muscle, focal atrophy and a secondary increase in sarcolemmal nuclei may have been a result of local degeneration of motor nerves.

(8) Focal fibrosis in sheep muscle appeared to have been a result of local arterial insufficiency.

(9) A myopathy histologically indistinguishable from "white muscle disease" in lambs occurred in outbreak proportions in mature animals. This type of myodegeneration probably had a nutritional aetiology but did not appear to be related to an absolute dietary deficiency of vitamin E. When this type of degeneration occurred in ewes the lesions were frequently in abdominal muscles and referable to late pregnancy or parturition. The lesions appeared to be responsible for dystocias and weak or dead lambs, and in some cases myodegeneration was responsible for partial or complete rupture of these abdominal muscles. This myopathy was associated with intrafusal muscle fibre fragmentation in neuromuscular spindles, and with a loss of cholinesterase at motor end plates usually in the absence of any evidence of axonal degeneration. Occasionally retrograde axonal degeneration did occur.

(10) A myopathy characterized by a mixture of absolute hypertrophy and
atrophy, and with fatty and fibrous replacement of muscle fibres has been observed in sheep muscle. This had many histological similarities to progressive muscular dystrophy and progressive spinal atrophy in man.

(11) Parasitic myositis due to tapeworm larvae was very limited while the parasite was viable, but more extensive when the parasite degenerated. The more extensive cellular reaction was predominantly eosinophilic.

(12) An unidentified member of the Actinomyces group of bacteria was the cause of a focal, disseminated, eosinophilic myositis in one sheep.

(13) Infarction of the thigh muscles of one sheep occurred, probably as a result of the transient occlusion of femoral veins or arteries.

(14) Muscle fibres appeared round in cross section under a wide variety of circumstances and this roundness could be accorded no significance.

(15) The changes in the minute structure of sheep skeletal muscle striations were not a reliable indication of degenerative changes, but were useful in establishing the true extent of lesions confirmed by other means. The simple presence or absence of cross striations in routine H and E stains was not a reliable indication of the health of a muscle fibre.

(16) Sarcocysts in sheep muscle were capable of stimulating a reaction, even though the Rainey's corpuscles showed no evidence of degeneration.

(17) The number of striated muscle-fibre annules in the eye muscles of sheep was not necessarily related only to age, nor were complex annules related only to particular muscle diseases. Striated muscle-fibre annules in a sheep's limb muscle appeared under
conditions of hypoxia and motor nerve regeneration after a prolonged period of degeneration. They could not therefore be considered specifically related to certain myopathies of man.

(18) Structures resembling sarcoplasmic masses occurred in fibrotic lesions in sheep muscle, consequently these figures could not be considered pathognomonic of dystrophia myotonica as seen in man.

(19) Vacuolation of intrafusal and extrafusal muscle fibres in extrinsic ocular muscles was not associated with disease but may have been an artifact of fixation.

(20) Fibrosis of the intrafusal space of neuromuscular spindles was probably a localized phenomenon and not a manifestation of generalized muscular or spinal disease.

(21) The described cholinesterase - silver method of demonstrating motor nerves in muscle has proved superior to the existing methods for demonstrating degenerative changes.

(22) Secondary to cachectic atrophy of sheep muscle, the motor end plates were reduced in size only to the degree that corresponding muscle fibres were reduced in cross sectional area. The number of septae and projecting processes in motor end plates was reduced and terminal axons were crenated.

Sheep Arteries

(1) The described combination of aldehyde fuchsin and Lendrum's stain proved useful and versatile in demonstrating arterial changes.
In sheep intimal plaques occurred very frequently in the visceral arteries, less frequently in the aorta and other major arteries, and even less frequently in intramuscular arteries. Intimal plaques increased in size and number with age.

Fatty infiltration occurred in a proportion of aortic plaques, but very rarely in plaques in smaller arteries.

Medial degeneration and mineralization changes were very rare in sheep arteries.

**Guinea Pig Muscles**

Secondary to motor denervation of guinea pig muscles, fragmentation of axons could be observed from the third to the twentieth day, and neutral fat in myelin sheaths could be seen from the sixth to at least the fortieth day. Cholinesterase at the end plates persisted for at least forty days and interstitial fat could be observed from the thirtieth day onwards.

Formol fixation of muscle in itself did not apparently reduce the amount of cholinesterase at end plates provided it was carried out at $4^\circ$C. Prolonged fixation at room temperature resulted in complete loss of cholinesterase.

**B. Conclusions Relating to Scrapie**

Pruritis, locomotor difficulties and loss of condition were consistent clinical signs of scrapie. The symptoms exhibited
by scrapied sheep were necessarily considered as a whole in establishing a diagnosis, and it was found desirable to confirm the diagnosis by examination of the medulla. The so-called scratch reflex by itself was not a reliable indication of scrapie.

(2) Scrapied sheep exhibited a generalized muscular atrophy of the cachectic variety. Very occasional foci of neurodegeneration may have been a result of trauma incidental to rubbing.

(3) Significant myodegenerative changes were not a characteristic of scrapie. Degenerative changes observed in the muscles from a few scrapied sheep were coincidental with the disease. These changes could not be related to the clinical symptoms exhibited.

(4) Muscles from scrapied sheep exhibited no differences from muscles from non-scrapied sheep with regard to round fibres, sarcocysts, cross striations, striated muscle-fibre annules, vacuolated fibres or sarcoplasmic masses.

(5) Neuromuscular spindles and motor end plates were not significantly altered in scrapie.

(6) Arterial abnormalities in cases of scrapie were not significantly different from those in non-scrapied sheep.
This work reports the results of the detailed examination of muscles from ninety-four sheep suffering from scrapie and seventy-six sheep which were normal or were suffering from diseases other than scrapie. The macroscopic and microscopic abnormalities encountered have been described and, where possible, categorized on the basis of histological appearances. Muscular changes have been discussed in relation to existing knowledge of muscle, and in relation to the clinical symptoms observed prior to death of the animals.

The cross sectional muscle fibre patterns associated with atrophy have been investigated by two methods and the implications of the results discussed. A myopathy, apparently nutritional in origin, appearing in mature sheep has been reported and discussed and its relationship to difficulties at parturition described. The end structures of motor and sensory nerves have been examined in some detail in scrapied and non-scrapied sheep and the results compared with the changes found in the guinea pig following denervation. Intramuscular and visceral arteries have been subjected to analysis in both scrapied and non-scrapied sheep.

Since the muscular atrophy in scrapie has been shown to be of the cachectic type, and since the incidence of muscular change was in fact lower in the scrapied than in the control group, and since the sensory and motor innervation to muscle was not significantly altered from the normal in scrapie it has been concluded that the symptoms of
scrapie cannot be considered referable to muscular changes, and that any abnormalities in muscle must be considered incidental to the disease rather than its primary cause or else as secondary to central nervous system changes. Since arterial changes in the muscles and viscera of sheep with scrapie were not significantly different from those of the non-scrapied group it has also been concluded that arterial changes play no part in the production of the symptoms of scrapie.

In the light of structural detail observed in sheep muscle it has been further concluded that muscles from animals can exhibit changes hitherto reported only in the muscles of man.
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THE SKELETAL MUSCLE OF SHEEP AFFECTED WITH SCRAPIE

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By

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INTRODUCTION

During the past century, a number of workers have investigated scrapie in an attempt to find characteristic microscopic postmortem changes. These attempts have been singularly unproductive. The predominant change reported by all was emaciation and general loss of condition. May (1868), Cassirer (1898) and McGowan (1914) described the muscles of most animals dying of scrapie as pale and soft, while the surrounding fat was undergoing gelatinous change. Cassirer (1898) and later McFadyean (1918) and Gaiger (1924) attributed the symptoms of weakness to extreme loss of condition and exhaustion. Cassirer dismissed muscle as the primary site of the disease since he could find no evidence of local atrophy. McGowan (1914) on the other hand, attributed the loss of condition and pruritis to a severe infestation of sarcocysts in the muscle. Stockman (1926) refuted this belief and was later supported by Brownlee (1940). Stockman (1913), Bertrand, Carré and Lucam (1937), Greig (1940) and Lucam, Béchade and Saurat (1950) remarked on the absence of significant muscular change and Bertrand et al. (1937) further commented on the probability of the central nervous system being the primary site of the disease.

In contrast Bosanquet, Daniel and Parry (1956a) and Parry (1957) reported finding sufficient muscular change in fifty-two cases to support their hypothesis that scrapie was a primary degeneration of skeletal muscle. While they conceded that one of the principal features of the disease was a "slow wasting of body flesh", they stated that in addition to wasted cases, non-wasting forms of the disease were encountered. According to these authors, the symptoms of scrapie can be directly related to muscular lesions and depend on the location, age of development and extent of "myopathic" processes. Lesions were found by naked eye examination and appeared in the form of either intense pallor (focal or diffuse), wasting of muscle, firm lesions or finally as an excessive deposition of fat. These macroscopic lesions showed microscopic changes ranging from destruction of "one fibre per field" to such intense destruction that very few normal fibres could be found in a field. They describe early changes in the muscle fibre as:— rounding, indistinct appearance of striations, increase in sarcolemmal nuclei, fragmentation of the fibres and granular change in the sarcoplasm.

In the opinion of these authors, the irregular distribution of degenerated fibres was quite different from the distribution of degenerated fibres which occurs after interruption of nerve supply. The similarities between the picture presented and that in the human diseases dermatomyositis and progressive muscular dystrophy were remarked upon, but the term "myopathy" was preferred to define a muscle degeneration of unknown cause. The authors presented a series of nine myopathic syndromes which included scrapie.
The purpose of this paper is to present some observations on the skeletal muscle of sheep showing symptoms of natural and experimental scrapie, and to discuss their significance.

MATERIALS AND METHODS

The sheep examined in this survey were from several different areas in Scotland and included Cheviot, Swaledale and Suffolk sheep. The natural cases were selected by shepherds and practising veterinarians and were observed for periods ranging from four days to five months. One animal was destroyed on arrival since it was in extremis.

With the exception of case 48, any animal which failed to show typical symptoms of scrapie while under observation, was discarded, as were animals which on post-mortem examination showed evidence of any disease process which may have complicated the symptoms. Although, case 48 showed only uninterrupted and generalised tremors, it was considered a possible atypical case of scrapie and was included in the present survey as the history of the flock revealed two other probable cases of scrapie and microscopical examination of the medulla showed severe and widespread vacuolation of neurons (Zlotnik, 1957).

The experimental cases, produced by intracerebral or subcutaneous inoculation of brain suspensions of clinically positive sheep, maintained in serial passage, were kept under special observation from the time that first symptoms of pruritis appeared.

The criteria for acceptance of a clinical case were: (a) severe pruritis (with consequent rubbing) and loss of wool in the absence of ectoparasites or other irritants; (b) progressive loss of condition; (c) locomotor difficulty and progressive weakness, in most cases to the point of inability to rise. In addition, many cases showed hyperaesthesia, quivering, severe ataxia and a swaying gait.

This survey includes sixteen natural and fourteen experimental cases of scrapie, and twelve animals which showed no clinical symptoms of the disease. Thirteen of the natural and all fourteen of the experimentally induced cases showed advanced clinical symptoms and most of them were killed when death seemed imminent. Three were killed three to ten weeks after the appearance of symptoms. Evisceration and skinning were carried out within one or two hours and the carcasses left in a cool room or cold-box for four to twenty-four hours to allow the muscle to “set”. During the gross examination, each muscle was separated at origin and insertion and carefully inspected for abnormalities. Next, each was examined after slicing transversely or longitudinally into approximately half-inch slices. The samples of muscle selected for histological examination included any haemorrhagic portions and any areas which contained a streaking. In this manner, each case yielded sixteen to ninety-four samples from different areas, although in some cases more than one sample was taken from a single muscle.

Samples were fixed in formol-saline, imbedded in paraffin and cut at 7 to 10 μ. One section from each sample was stained with haematoxylin and eosin and selected slides were stained by several additional methods, notably phosphotungstic-acid haematoxylin, Giemsa, Kull, van Gieson, von Kossa and Heidenhain’s iron haematoxylin.

The control animals used in this survey included animals within the same age range as the natural and experimental cases, and consisted of
apparently normal animals and animals killed or dying of various chronic diseases. Killing, sampling and processing were carried out in a similar manner to that described previously.

RESULTS

Post-mortem Examination

Of the forty-two sheep examined, only one, a natural case of scrapie, showed gross muscular lesions. The carcasses of the remaining forty-one exhibited only variable emaciation ranging from nearly normal to extreme wasting. An estimate of carcass condition is made in column 2, Tables 1, 2 and 3, and it will be seen that no correlation existed between emaciation and histological groupings.

<table>
<thead>
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<th>Serial number</th>
<th>Loss of condition</th>
<th>Number of muscles examined</th>
<th>Number of muscles containing changes</th>
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<td>*48</td>
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Nos. 7, 32, 40, 44—Suffolk
Nos. 5, 13, 16, 29, 31, 43—Cheviot
Nos. 21, 22, 30, 35, 39—Swaledale
No. 48—Cheviot Crossbred.

*No. 48 showed macroscopic lesions and Type IV microscopic changes in 10 muscles.
No abnormality whatsoever could be detected in the muscles from four scrapie and six non-scrapie animals. Ten scrapie and three non-scrapie controls showed a slight reduction in volume of skeletal muscle and an apparent reduction in fat cover. Nine scrapie and two control animals showed a marked reduction in fat cover and a moderate reduction in muscle volume. Seven scrapie and one control sheep showed a complete absence of body fat and a marked reduction in muscle volume. The muscles were soft and pale and surrounded by oedematosus, gelatinous connective tissue. With regard to wasting, no apparent difference existed between natural and experimentally induced cases of scrapie, and any breed difference was attributed to basic anatomical differences in fat reserves. A feature in four scrapie animals with almost normal carcasses was the presence under the skin of the back of single or multiple necrotic or sero-sanguineous pockets in the subcutaneous fat layer. These lesions varied in extent from small foci 1 cm. in diameter to a large multilocular cavity extending from the sacral

<table>
<thead>
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<th>Serial number</th>
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<th>Microscopic examination</th>
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<td>53</td>
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<td>16</td>
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</table>

All animals are of the Cheviot breed.
to the cervical region. These were considered to be due to traumatic fat necrosis. A characteristic of the very emaciated carcasses was the absence or incompleteness of rigor mortis, even after several hours in a cool room.

**TABLE 3**

**MUSCLE CHANGES IN NON-SCRAPIE ANIMALS**

<table>
<thead>
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All animals are of the Cheviot breed.

The animal showing gross muscular lesions was in good condition and had a moderate fat cover. Several muscles contained numerous, minute creamy-white foci which, in the more extensively involved muscles, were so dense that the entire muscle had a creamy colour. In some areas the lesions involved only the surface fibres, while in others the entire muscle was involved. In general, these muscles did not appear enlarged, but were somewhat firmer than normal. Muscles showing lesions in bilateral symmetry were:— extensor carpi radialis, supraspinatus and gastrocnemius. Those with a unilateral distribution were:— sub-scapularis, latissimus dorsi, semi-tendinosus and sartorius. The other skeletal muscles did not appear to be involved and although several were sampled, none contained further lesions.

**Histology**

Since a detailed description of relatively small changes tends to
give them a false importance, a proper perspective might be established by emphasising the minor nature of most changes found in this survey. With the exception of Type IV to be described, none were sufficiently extensive to produce a grossly visible lesion. Again, with the exception of Type IV and the very minor changes of Type I, they were confined to a very few muscles in a small percentage of scrapie cases.

On the basis of differences in distribution within a sample and differences in morphology, the microscopic changes were classified under four groups as below. The numerical grouping does not necessarily denote a progressive increase in extent or severity of the changes. Variable histological atrophy of muscle fibres visible in many scrapie cases and also in some non-scrapie animals bore no relationship to other changes seen, with possible exception of Type II. Particularly in the more emaciated animals, the general reduction in fibre diameter resulted in a condensation of interstitial tissue.

Type I. Changes of single fibre degeneration or focal increase in muscle nuclei were seen in one or more muscles from all but one case of scrapie and in one or more muscles from all non-scrapie animals. This group includes three somewhat different deviations from normal—hyaline degeneration, replacement of cytoplasm by proliferating muscle nuclei and proliferation of muscle nuclei in the absence of any other change. These three deviations can be considered together because their distributions were similar, none appeared to be more than a very minor alteration and all three were visible in apparently normal control animals. Two of the changes, hyaline degeneration and replacement of cytoplasm by muscle nuclei, were usually confined to two or three fibres, rarely up to ten, in a muscle sample containing from five to twenty thousand fibres. The third variation, proliferation of muscle nuclei, involved more fibres but to a lesser degree. The foci occasionally filled a high power field, though the fibres involved showed no other abnormalities.

Hyaline or granulo-fatty fibres appeared swollen, flat and pale when stained with haematoxylin and eosin. Cross and longitudinal striations were absent or greatly distorted, and muscle nuclei were not visible. In those single, isolated fibres where a nuclear reaction had been stimulated, the nuclei replaced the cytoplasm or merely surrounded normal or degenerating cytoplasm. In these latter fibres, a ring of nuclei, one or two deep, often surrounded a central mass of cytoplasm containing a sarcocyst (Fig. 1). Occasionally, the nuclei were vesicular, but more often they were elongated, small and dark staining (Fig. 2). An occasional macrophage was visible in the centre or at the edge of these fibres.

In longitudinal sections, Type I changes seldom involved more than a short segment of a single fibre, although nuclei of adjacent normal fibres frequently multiplied where damaged and normal
fibres touched. The diameter of fibres which were involved varied greatly: some appeared reduced in size, while others were distended and packed with nuclei. In some cases variations of Type I changes apparently represent stages in a single process, but in view of their sparse, though wide, distribution they can be accorded no real significance.

**Type II.** Degenerative and atrophic changes were seen in one muscle from an animal with natural scrapie and in three muscles from two experimental scrapie cases, but not in muscles from non-scrapie controls. They consisted essentially of a diffuse increase in muscle nuclei over part or all of the sample and, in addition, ten or more fibres showed evidence of cytoplasmic damage. The cellular appearance was due primarily to enlargement and proliferation of nuclei at the borders of the fibres (Fig. 3). Few fibres were completely ringed by nuclei, but some of those that were showed hyaline or granulo-fatty change and a few in each sample were at least partially replaced by macrophages and proliferating muscle nuclei. More than two damaged fibres were seldom seen in a single primary bundle and many primary bundles contained none.

Although there was generally a focal reduction in fibre diameter and a gradual variation down to about ten microns, most fibres in these areas appeared normal in staining reaction and striation. Occasionally, in the connective tissue surrounding primary bundles, aggregations of lymphocytes, macrophages and eosinophils were visible, particularly adjacent to small vessels. Although some condensation of connective tissue was visible, there seemed to be no absolute increase. The distribution and extent of the lesions in this category suggest that they are more than a simple extension of Type I changes, although the focal increase in muscle nuclei of Type I may represent an early stage of a Type II lesion.

**Type III.** Fibrotic changes were seen in three muscles from one natural and in one sample from one experimental case of scrapie. They were not seen in non-scrapie controls. The lesions were focal in distribution and contained ten or more damaged fibres. A single focus contained up to fifty or sixty fibres showing some degree of replacement of cytoplasm by large vesicular muscle nuclei. Not more than one focus was visible in a single muscle sample. As in Type II, occasional single fibres showed hyaline degeneration or fragmentation of the cytoplasm, but no macrophages or lymphocytes were visible. A characteristic of Type III lesions was an absolute increase in connective tissue between individual fibres which seemed to extend from the heavier connective tissue bands around vessels and nerves (Fig. 4).

Two samples from the natural case showing Type III lesions also showed a vascular change which was visible in or adjacent to the muscular lesions. A small thrombus lay in one artery, while in the other the intimal layer showed signs of reduplication and partially
filled the lumen. Quite different arterial changes, mostly consisting of calcification or scarring of the medial layer, were seen in four other scrapie animals in addition to the case mentioned above. In these there was no apparent muscular change related to the vascular lesions.

Type IV. Dystrophic lesions were seen only in the animal showing gross lesions (number 48) and consisted of widespread and complete necrosis of fibres in the muscles involved. In more severely affected areas, very few recognisable fibres remained, but in adjacent areas the fibres appeared normal in every respect. Occasionally, fibres directly adjacent to damaged fibres did show evidence of fragmentation, or, less frequently, hyaline degeneration, but as a rule the limits of the lesions were sharply defined. The very cellular appearance of the lesions was due to a periphery of large, vesicular muscle nuclei which surrounded an angular mass of degenerating cytoplasm or, more frequently, an amorphous, finely granular mass (Fig. 5). On staining by von Kossa’s method, this granular substance proved to be calcareous (Fig. 6). In other fibres, the proliferating nuclei completely filled the space formerly occupied by cytoplasm and these fibres were somewhat larger than normal ones in the same slide. Rarely, macrophages were visible in or adjacent to fibres which still contained fragments of cytoplasm, and in a few areas collections of lymphocytes were seen near small blood vessels.

In two muscles, in addition to the changes described, a single focus, about one mm. in diameter, showed a marked reduction in the diameter of otherwise normal fibres. Peculiar to these areas was an absolute increase in perimyseal connective tissue. There was little evidence to suggest that an inflammatory reaction was involved, and the distribution of destroyed fibres followed no pattern which suggested a neurogenic origin.

DISCUSSION

Previous to 1956, all investigators were unanimous in agreeing that the only significant change in muscle from scrapie sheep was that of emaciation and consequent loss of muscle volume. It is conceivable that some of these workers may not have examined muscle in any great detail, but it seems unlikely that all would have overlooked [the gross lesions reported by Bosanquet et al. (1956a) and Parry (1957).

The present survey supports the view that muscle lesions are not a characteristic of scrapie and therefore scrapie cannot be considered a primary muscular disease. The apparent equal distribution of Type I changes in scrapie and control animals makes them obviously unimportant in scrapie. Type II and III changes borrow a false significance from the fact that they were seen only in scrapie animals. Their distribution and severity warrant little
Significance even as secondary changes in the disease, and they were seen in far too few cases (five out of thirty) to account for any symptoms seen in the disease. Type IV lesions are undoubtedly significant as secondary or incidental changes, but since they were seen in only one animal with scrapie they can in no way explain symptoms or pathology of scrapie generally.

Any comparative discussion of histological changes of skeletal muscle must be tempered by the observations made by Adams, Denny-Brown and Pearson (1954) and Walton and Adams (1956) that muscle has a somewhat limited range of potential reactions, and consequently that a muscle lesion is seldom diagnostic. Although a consideration of the muscle changes reported in this survey must, at this point, be largely speculative, certain features might be pointed out and discussed.

Type I changes involving such widely scattered, single fibres, can scarcely be considered manifestations of a general disease process. The finding of similar changes in all control animals suggests that they are little more than physiologic variations which could be found in any sheep.

Type II changes were found in muscles lying near the surface and in areas where scrapie sheep are frequently seen to rub, namely sternocephalic, semi-tendinosus, semi-membranosus and longissimus dorsi muscles. Such an anatomic distribution makes it tempting to consider them traumatic in origin. Skin and subcutaneous fat often show evidence of traumatic damage, and it is conceivable that muscle could also be involved. On the other hand, the focal reduction in fibre diameter and the increase in muscle nuclei, without evidence of dystrophic change, might suggest an interruption of nerve impulses at some point in the motor chain. The severe ataxia in many scrapie animals, and the variable number of vacuoles found in the brain and cord, point to some deviation from the normal. It would be surprising if the muscle did not occasionally show the effect of these central nervous disturbances. The inverse relationship between nerve continuity and the number of muscle nuclei observed by Tower (1937) is of interest in connection with Type II changes.

The fibrotic Type III lesions contained no features which suggest either a traumatic or a systemic nutritional origin, and the anatomic distribution is of no help here. The proximity of arterial lesions in two examples suggest a relationship, but the assumption that the vascular changes were primary is unwarranted. In connection with the fibrosis, Adams et al. (1954) cite reports that experimental nerve section and venous, but probably not arterial occlusion, can produce a fibrous hyperplasia. Tower has further observed that fibrosis around nerves and vessels in disuse atrophy results in a secondary muscular degeneration. In view of the nonspecificity of muscular changes generally, it is difficult and probably
inadvisable to attempt a definite classification of Type II and III changes.

On the other hand, the dystrophic, Type IV lesions seen in case 48, present a number of features which allow at least a general classification. The distribution of acutely damaged fibres and the massive calcification of necrotic fibres is similar to, if not identical with, changes in the metabolic disease attributable to a deficiency of vitamin E. The histologic picture presents no features to suggest that anything more is involved, and even the atrophic, fibrotic variations seen in two muscles have been described by Marston and Pierce (1942) as a feature of muscular dystrophy attributed to vitamin E deficiency. Although some similarities exist, an essential difference between Type IV lesions and the "more severe" lesions reported by Bosanquet et al. (1956a) and Parry (1957) is the presence of calcium in the damaged areas. Investigation into the history of this sheep revealed nothing unusual about the diet comprising grass and turnips, and no unusual exercise could be traced. It is interesting to note that although the muscle lesions were moderately extensive, the animal clinically showed no indication of stiffness, ataxia or instability. Bosanquet, Daniel and Parry (1956b) point out that vitamin E controlled dystrophy is usually confined to young lambs and calves, but several authors have reported dystrophy in more mature sheep (Marston and Pierce, 1942; Cotchin, 1947; Marr and Sharman, 1956). These last authors also cite a similar report by Dodds (1954), and unpublished reports by Hartley (1954) and Watt (1954). Hadlow (1955) has also reported similar lesions in a wild Virginian deer in the U.S.A.

CONCLUSIONS

Skeletal muscles from thirty cases of scrapie, fourteen experimentally induced and sixteen natural, have been minutely examined and compared with muscles from twelve non-scrapie control sheep. Macroscopic lesions were found in one natural scrapie sheep and minor microscopic changes were found in five more, two natural and three experimental.

It is concluded that scrapie cannot be considered a primary disease of the muscle, and that muscle changes play no apparent part in producing clinical symptoms of that disease.

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**LEGENDS TO ILLUSTRATIONS**

**Fig. 1.** Type I change surrounding a fibre containing a small sarcocyst. A second sarcocyst in the upper part shows no accompanying cellular increase. \( \times 320 \).

**Fig. 2.** Type I change showing proliferating muscle nuclei replacing muscle substance. \( \times 320 \).

**Fig. 3.** Type II change showing a general reduction in fibre diameter and a diffuse increase in muscle nuclei. \( \times 320 \).

**Fig. 4.** Type III fibrotic lesion showing massive increase in perimysial connective tissue. \( \times 130 \).

**Fig. 5.** Type IV lesion demonstrating the severe destruction of muscle fibres and the granular calcification of necrotic fibres. \( \times 320 \).

**Fig. 6.** Type IV change stained to show calcification. \( \times 320 \).

Photomicrographs 1 to 5 have been made from slides stained with haematoxylin and eosin. Number 6 is of a slide stained by von Kossa’s and van Gieson’s methods. All are from advanced cases of scrapie.