

A HISTOLOGICAL STUDY OF THE SPINAL CORD AND PERIPHERAL
NERVOUS SYSTEM IN SCRAPIE DISEASE OF SHEEP.

A thesis submitted for the degree of Doctor of
Philosophy of the University of Edinburgh

by

P.A.L. Wight.

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INTRODUCTION.

Scrapie is a disease of sheep characterised by intense pruritus, progressive incoordination and asthenia. Under natural conditions it usually occurs in animals over 18 months of age, the course frequently extends over many months, and the termination is fatal.

The disease, sometimes known by the colloquial names of the rubbers, the goggles, cuddy trot and yeuky pine, appears to have been present in Great Britain for over 200 years, for according to M'Gowan (1914) it was recorded in 1732 and became a serious economic problem in the latter half of the eighteenth century. Stockman (1913) has suggested that it was introduced here by imported Merino sheep, although Greig (1940¹) thinks this unlikely. However, since the eighteenth century, although its seriousness has fluctuated from time to time, it has been constantly present in this country. More detailed accounts of the history of the disease in Great Britain are given by Stockman (1913), M'Gowan (1914), Gaiger (1924), Greig (1940), and Palmer (1959).

Scrapie has also been present in France (*la tremblante*), and in Germany (*Grubberkrankheit* or *Traberkrankheit*), for many years. Its history on the continent has been well reviewed by Bertrand, Carré and Lucam (1937) who say that the earliest record of its occurrence in France was in 1810, and in Germany in 1750, although at present it is apparently of little importance in the latter country (Behrens, 1957). It has recently been reported in Iceland (Palsson and Sigurdsson, 1958).

Despite its long history scrapie was thought to be confined to Europe until 1938 when it was identified in Canada (Scheffield, 1938). According to Marsh (1958) it first appeared in the United States in 1947, since when it has been observed in a small number of animals in seventeen states, in Australia in 1952, and in New Zealand in 1952. Marsh also

says that these outbreaks have been related to imported sheep.

The cause of scrapie is unknown. In 1936, Guillé and Chelle claimed to have experimentally transmitted the condition in sheep, and this was later confirmed by Gordon (1946), Wilson, Anderson and Smith (1950), and Stamp, Brotherston, Zlotnik, MacKay and Smith (1959). Pattison (1957), Gordon and Pattison (1957), and Pattison, Gordon, and Millson (1959) have recently been able to transfer the disease to goats and to passage the agent in series through goats and back to sheep. However, although experimentally transmissible, there is no definite knowledge regarding the natural spread of the disease; Greig (1940²) has claimed that contamination of pasture may be involved. Wilson et al. (1950) concluded that the causal agent was a virus, but continued research has shown that it has many unusual properties. Thus it is very resistant to chemical and thermal insults (Gordon, 1946; Greig, 1950; Wilson, 1954; and Stamp et al., 1959), it fails to produce antibodies recognisable by the usual serological methods (Chandler, 1959), and it has not been demonstrated by fluorescent antibody methods (Moulton and Palmer, 1959). Although there seems little doubt that the cause of scrapie is associated with a particulate multiplying agent (Stamp et al. 1959), the essential nature of this agent is, despite intensive investigation, still obscure.

Macroscopically there are no characteristic lesions in scrapie, if one excepts secondary changes due to cachexia and the intense pruritus (Besnoit and Morel, 1898; M'Gowan, 1914; Stockman, 1926; Bertrand et al. 1937; Brownlee, 1940). Bosanquet, Daniel and Parry (1956) and Parry (1957), and subsequently Delez, Gustafson and Luttrell (1957), claimed that myopathic lesions similar to polymyositis were constantly present in scrapie, but these lesions had not been seen by any observers in the past, of whom M'Gowan (1914) was particularly notable for his studies of the musculature. Hulland (1958) made a detailed survey of the skeletal muscles of scrapie sheep, but was completely unable to confirm the findings of Bosanquet et al.

Positive knowledge of microscopic changes in nervous tissue dates from the work of Besnoit and Morel (1898), who observed vacuolar alterations, disintegration of the Nissl substance, and in some instances, eccentricity of the nucleus, in a few discretely situated neurones in the medulla oblongata and spinal cord. They also claimed that an intense peripheral neuritis affected the smaller nerves and was of greater importance than the lesions in the central nervous system, but subsequent investigations have not confirmed that a peripheral neuritis is in fact present (Stockman, 1926; Bertrand *et al.* 1937; Brownlee, 1940).

M'Gowan (1914) examined the spinal cord and dorsal root ganglia together with many of the organs. The only finding which he considered significant was the constant presence of sarcosporidia in the muscles, to which he attributed the aetiology of the disease. This theory has been completely discredited by McFadyean (1918¹ & 2), Brownlee (1940), and Hulland (1958).

Stockman (1926) observed vacuolated nerve cells in the medulla, cord, and spinal ganglia. He also saw eosinophilic bodies in the cytoplasm of the neurones, and, although he had seen similar bodies in a case of swayback, he thought these might prove of significance in scrapie. Occasional degenerating fibres were seen in the white matter of the spinal cord, but a peripheral neuritis was not present in Stockman's cases.

Bertrand, Carre and Lucam (1937) examined the medullas of 20 sheep in the terminal stages of the disease and observed a ballooning vacuolation of neurones, as well as chromatolysis, acute swelling, atrophy, severe cell change and neurofibrillary alterations. An astrocytic gliosis was present in the most affected parts of the grey matter. Meningeal reactions were present and also rare perivascular cuffings. These changes were also observed in the spinal cord, but a peripheral neuritis was not seen. They describe the disease process as being a degree less severe than an encephalitis, and refer to it as a subacute polio-myelo-encephalitis.

Schofield (1938) failed to find vacuoles in a case of

clinical scrapie, but he noted that there were many eosinophilic bodies in the cytoplasm of the neurones.

Brownlee (1940) concluded that vacuolation of the otherwise unaltered neurone of the medulla and / or cord was a constant and specific lesion. Apart from scrapie cases he only found a few small vacuoles in necrotic neurones in Louping ill. Using the Marchi method, blackening of a few fibres in the white matter of the cord was seen, but he thought it unwise to draw conclusions from this. He saw vacuoles in the spinal ganglia of scrapie sheep and in a few control sheep, but could not come to any conclusions as to their significance. A few sympathetic ganglia were examined and appeared normal.

Holman and Pattison (1943) found vacuoles in the medullas of all cases of clinical scrapie, very occasionally in sheep suffering from other conditions, and in 1 out of 59 apparently healthy sheep. They recorded the numbers of vacuoles seen in the different nuclei of the medulla.

Plummer (1946), saw vacuoles and eosinophilic inclusions in the medulla and cord in scrapie, and vacuoles and neurone degeneration were also recorded in the medulla and cord by Lucam, Béchade, and Saurat (1950), and by Wagner, Goldstein, Doran and Hay, (1954).

For many years there has thus been a considerable measure of agreement that important changes were present in the central nervous system in scrapie, but recently, Bosanquet et al. (1956) and Parry (1957) and subsequently Delez, Gustafson and Luttrell (1957), have claimed that scrapie was a primary myopathy. Hullahd (1958) made an extensive microscopic study of the musculature of scrapie sheep, but could not confirm these findings. As regards the central nervous system, Bosanquet et al. concluded there was no obvious loss of anterior horn cells in the spinal cord, no inclusion bodies, no foci of nerve cell degeneration or perivascular cuffing. Occasional vacuoles in the large cells of the medulla, and rarely in the anterior horn cells of the cord, were thought to be of little significance because they can be found in nerve cells of normal

sheep and in humans affected by various diseases. They claim that the lesions of the nervous system previously described do not explain the symptoms.

Recent studies have convincingly shown however, that the lesion in the medulla cannot be dismissed as of no importance. Thus Zlotnik and Rennie (1957, 1958) found vacuolated neurones in the medullas of a high proportion of normal sheep of several breeds, but the incidence of vacuolation in both naturally occurring and experimentally induced scrapie was very significantly greater than in the normal animals, (Zlotnik, 1957², 1958^{1 & 2}). Furthermore, Zlotnik reported a series of degenerative changes and an astrocytic gliosis in the brain stem of scrapie sheep. The studies of Palmer (1957²) have also provided further evidence of the involvement of the medulla, although the intravacuolar material which Palmer (1957¹) considered significant in scrapie has been shown by Zlotnik (1957¹) to occur in vacuoles in healthy sheep. The transmission of the disease to goats and the demonstration of vacuoles in the medullas of these, (Pattison, Gordon and Millson, 1959) has provided further evidence of involvement of the central nervous system.

This review of the history and literature on scrapie indicates that further research is necessary. There are two main reasons for this. Firstly, its long history and the consequent widespread occurrence of scrapie in Europe meant that any eradication scheme would be expensive, inconvenient to the sheep industry, and unlikely to give the desired results (Stamp, 1956). However, the recent appearance of the disease in American and Australasia has resulted in further economic disadvantages to exporting breeders in Europe, and made its diagnosis, prevention and eradication of great importance. These factors have resulted in increased interest in the disease for purely economic reasons. Secondly, the unusual nature of the disease itself, and the sometimes contradictory information concerning it, present intriguing problems from the viewpoint

of fundamental pathology.

Although several investigators have noted abnormalities in the spinal cord, its histology in scrapie has never been investigated in detail. The incidence, distribution, and significance of the lesions when adequately compared with control animals, is quite unknown, as is the relationship between lesions and clinical signs. It was therefore decided to undertake a detailed study of the histology of the spinal cord in scrapie.

Those parts of the peripheral nervous system which are anatomically and functionally closely related to the cord were also studied. It would seem essential in a disturbance of skin sensation to eliminate involvement of the sensory ganglia, and yet recorded examinations of these structures in scrapie are relatively few and brief. Besnoit and Morel (1898) say that they had become engorged and infiltrated secondary to the peripheral neuritis which they considered so important. M'Gowan (1914) found no abnormalities at all in the ganglia, while Stockman (1926) found eosinophilic intracytoplasmic inclusions, and Brownlee (1940) found vacuoles but could not decide on their significance. Although Bertrand et al. (1937) have suggested that the pruritus seen in scrapie might be due to autonomic involvement, only the briefest examination of sympathetic ganglia has been attempted in the past (Brownlee, 1940)

The following histological study of the spinal cord, spinal sensory ganglia, sympathetic ganglia and the Gasserian ganglia was undertaken in the hope that the information obtained would add to the basic knowledge upon which further progress depends.

(10), Charvát (11), Meyer (12), and others (13), of which the Meyer (12) / Charvát (11) was the commonest; 20 were females and 4 males. They were kept under observation from 1 to 120 days depending on the severity of the symptoms; the average period was 56 days.

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MATERIALS AND METHODS.MATERIAL.

The material used in this study was obtained from 169 sheep of which 75 were affected with naturally occurring scrapie, 40 with experimentally induced scrapie, and 54 were used as controls. It consisted of spinal cords from 68 natural cases of scrapie, 30 experimental cases of scrapie, and 36 clinically healthy controls, and 7 sheep affected with neurological diseases other than scrapie. Spinal ganglia were examined from 30 natural cases of the disease, 10 experimental cases, and 10 healthy controls. Gasserian ganglia were examined from 5 animals from each group, and sympathetic ganglia from 30 natural cases of scrapie, 15 experimental cases of scrapie and 11 healthy controls. This data is recorded in Table I.

In addition, an examination was made of neurones in the adrenal glands from clinically healthy sheep and sheep affected with scrapie disease.

Natural cases of scrapie.

The majority of the natural cases of scrapie were obtained from farms in the South of Scotland and the North of England, (Lothians 20, Fife 11, Lanarkshire, Stirling and Angus 5, Berwickshire 3, Cumberland 13, Yorkshire 11, Northumberland 9, and Westmorland 3). Their average age at death was 2.7 years (range 2 to 5 years). 73% were between 2 and 3 years old at death. The breeds included Suffolks (29), Swaledales (22), Cheviots (11), Border Leicesters (4), Dalesbred (1), and crossbreds (8), of which the Border Leicester / Cheviot cross (6) was the commonest; 69 were females and 6 males. They were kept under observation from 1 to 330 days depending on the severity of the symptoms; the average period was 56 days.

As the purpose of one aspect of the study was to

determine whether a relationship existed between the severity of the symptoms and the histological changes in the spinal cord, the cases were broadly classified as "advanced" and "non-advanced" on clinical grounds only; the criteria for this classification were mainly symptomatic, as the history prior to arrival at the Moredun Institute was often unknown or unreliable. The non-advanced cases were kept under observation only long enough to establish a diagnosis of scrapie; when they were killed they were affected with little more than slight pruritus and in some cases mild incoordination or ataxia. The advanced cases were all affected with ataxia and incoordination and had at some time shown varying degrees of pruritus.

However, as at the present time the diagnosis of scrapie in the living animal is entirely dependant on clinical signs, it is worthwhile describing in more detail than as above the more important symptoms seen in the scrapie sheep. Furthermore, it was considered important to know whether subsequent histological findings could be correlated with the clinical signs.

The most constant early sign of the disease was pruritus. Initially mild and manifested only by a roughening of the wool, the pruritus often progressed until, in severe cases, the skin became denuded of wool, excoriated through continual rubbing and biting, and focally infected. Irritation seemed to affect any part of the integument, but was often most noticeable on the head, base of tail, lumbo-sacral region, shoulders and lower limbs. Pruritus usually persisted throughout the disease; in a few cases however, it regressed until just before death it was negligible. All the animals used in this study had exhibited some degree of pruritus.

In several cases it was possible to elicit the "nibbling response" - rubbing woolled areas with deep or light pressure caused the animal to make nibbling movements with its lips or to attempt to rub or bite the area stimulated. Probably because of the timidity of sheep, extraneous factors often inhibited the nibbling response, but in six cases of a total of 75 it was a prominent symptom.

Hyperaesthesia attracted attention in several cases. It was manifested by restlessness, the head being held high with the ears cocked and often twitching. A slight noise was liable to excite the animal and in severe cases brought about violent trembling. In some cases (12) there was a continuous tremor or fasciculation of certain muscle groups either in the head alone or in the head, shoulders and forelimbs.

Abnormalities of gait were seen in 69, that is in all but the mildest, cases of scrapie. They usually began as slight inability to coordinate the front or hind legs, sometimes with a shortening of the length of step or dragging one or more limbs at the fetlock. The "Cuddy trot" was sometimes prominent - the forelimbs were lifted exceptionally high showing a rather stiff extension followed by sudden exaggerated jerky flexion at each step. There was occasionally marked jerking of the head. In many cases, particularly in the Suffolks, the hind limbs splayed at the hock at each step and the hind quarters swayed from side to side so that the animal was unable to maintain its balance. In one case ataxia was so severe that the animal was unable to stand but on being lifted rolled over several times on its long axis. Incoordination and ataxia frequently progressed until the animal was unable to rise without assistance or to stand when lifted. This was described as progressive asthenia and ultimate paresis. True paralysis was never seen; in all cases deep and superficial reflexes could be elicited.

In some sheep a dullness or lethargy which might almost be described as hypoaesthesia was a prominent sign. It usually developed late in the disease and was particularly noted in 14 cases, many of which were Swaledales. A terminal loss of condition was often prominent, but the Suffolks as a group showed this less than most breeds.

There were other less characteristic signs which were sufficiently noticeable to merit recording in one or two cases -

a fixed and glassy stare, amaurosis, exophthalmos, salivation, and regurgitation of ruminal contents.

The natural course of the disease was invariably fatal but it did not always progress through pruritus, hyperaesthesia, incoordination, asthenia, paresis and death. Death often occurred suddenly when symptoms were not unduly severe. In some of these cases parturition seemed to exacerbate the symptoms and hasten the fatal termination, and in many cases a sudden inclement change in the weather, particularly cold rain and frost in winter or early spring, was followed by the death of an animal which had shown mild clinical symptoms.

The breed, age, sex and predominant symptoms in the individual cases of naturally occurring scrapie are recorded in Table II.

Experimental cases of scrapie.

The experimentally induced cases of scrapie originated as clinically healthy lambs bought at auction in Scotland (Inverness-shire 9, Roxburgshire 8, Peeblesshire 4, Lanarkshire 4), or born from healthy parents at the Moredun Institute (15). They were all Cheviots. 21 were females, 6 were males, and 13 were castrated males.

Scrapie was induced by inoculation with a suspension of pooled brains obtained from the serial passage of scrapie brains originating from the successful transmission experiments of Wilson, Anderson and Smith (1950). After the appearance of symptoms of scrapie the animals were kept under observation until they were killed, the individual periods of observation varying from 12 to 120 days with an average of 53 days.

The experimental cases fell into three main age groups depending on the age at which they were inoculated and subsequently killed.

1. 11 sheep were inoculated intracerebrally within 12 hours of birth, and they were between 5 and 12 months old (average 8.3 months) when killed.

2. 26 sheep were between 1 and $2\frac{1}{2}$ years old (average 1.9 years) at death. 24 of these had been inoculated between 4 and 15 months old, and 2 were inoculated at 9 months and again at 20 months. The inoculations were subcutaneous or intracerebral.
3. 3 sheep were about 7 years old and had been inoculated subcutaneously 9 months previously.

The symptoms in the sheep of the experimental group differed only in degree from the natural cases. There was pruritus in every case which, in the group as a whole, was more intense than in the natural cases. Hyperaesthesia was noted in a few cases, Incoordination was usually marked, and splaying of the hind legs and dragging of the hind fetlocks were particularly common features. The back often appeared weak and the hind quarters swayed uncontrollably from side to side. Ataxia was seen. Asthenia and paresis usually followed, and pruritus was often not then so obvious, perhaps because the animal was unable to rub itself. There was often terminal lethargy and condition had usually become poor before death.

All the experimental cases were killed in the terminal stages of the disease.

The breed, age, sex, data relating to the inoculation, and predominant symptoms in individual cases of experimental scrapie are recorded in Table III.

Control sheep.

The majority of sheep used as healthy controls were obtained from similar sources to the experimental sheep. Many were obtained from other departments of this Institute, having, for instance, been used as experimental stock in parasitological experiments but showing no clinical signs of helminthiasis. In no case were there neurological symptoms, nor was there any reason to suspect that the nervous system was not normal.

The ages of the control sheep covered the range of

ages of the natural and experimental scrapies - the average age was 2.6 years; the range 8 months to 7 years. The breeds available were Cheviots (23), Blackfaces (14), Merino (1), Blackface x Cheviot (8), Suffolk x Cheviot (1). 34 were female, 4 males and 9 castrates.

In addition, 7 sheep were available for comparative study which were affected with various abnormalities of the nervous system - Louping ill (3), Russian Spring-Summer encephalitis (2), Coenurus cyst in the mesencephalon (1), intra spinal abcess (1).

The breed, age, sex and symptoms if any, of the control group are given in Table IV.

METHODS USED IN THE PREPARATION AND EXAMINATION OF THE SPINAL CORD.

Methods of killing and postmortem procedure.

The majority of the animals were killed by venisection and the cord removed within one hour of death. 13 cases of natural scrapie died.

After removal of the skin and musculature of the dorsum, the cord was exposed by cutting the vertebral arches with bone forceps. The cord was most likely to be damaged between the 1st and 8th thoracic vertebrae. It was usually necessary to break away the dorsal part of the intervertebral foramina to expose the spinal ganglia. The cord with the meninges intact, was then carefully lifted out of the canal by cutting the nerve roots distal to the sensory ganglia. By holding the tough dura of the upper cervical region with rat-tooth locking forceps, the entire cord was gradually lifted out with the minimum of handling.

The meninges were then opened along the dorsal and ventral surfaces and the cord divided into a cervical portion (C_1 to C_8), a thoracic portion (T_1 to T_{13}), and the remaining lumbo-sacral coxygeal portion.

Histological methods.

Fixation. Preliminary investigations to find the best method of fixing the sheep's spinal cord included the use of the following 12 fixatives for various trial periods of time.

1. 10% formol - saline.
2. 10% formol - saline post fixed in corrosive sublimate.
3. Formalin ammonium bromide.
4. Bouin's fluid.
5. Carnoy's fluid.
6. Susa.
7. Zenker's fluid.
8. Helly's fluid.
9. Regaud's fluid.
10. Equal parts of 96% alcohol and 10% formol - saline.
11. 96% alcohol containing 5% of acetic acid.
12. 9 parts of absolute alcohol and 1 part of 40% formalin.

Fixatives numbers 3 to 9 were made up according to Mallory's (1938) instructions, while fixatives numbers 10 and 11 were suggested by Hopkins (1924) as giving improved Nissl pictures, and fixative number 12 is recommended by Bensley (1939) for glycogen studies.

The fixative ultimately found to give constantly reliable results was 10% formol - saline with corrosive sublimate post - Fixation.

The three lengths of cord (vide supra) were suspended in 10% formol - saline for one month by threads through the dura. Transverse or longitudinal pieces about 4m.m. thick were then cut from the cord and post - fixed in saturated mercuric chloride solution for 48 hours. A fortnight was found to be the minimum satisfactory period of fixation in 10% formol - saline, although a month gave better results. Longer periods were not adverse, and indeed, the spinal cords of Russian Spring-Summer encephalitis had been kept in formol-saline for 20 years. This method gave good nuclear and cytoplasm pictures and minimal perineuronal shrinkage. The

Nissl substance was perhaps coagulated into finer granules than by alcoholic fixatives, but this was not disadvantageous for studying the Nissl substance in the walls of vacuoles. This method was used as a routine.

Prior to placing the cord in formol-saline, transverse pieces about 2 m.m. thick were fixed in formol ammonium bromide for 5 days for subsequent staining of astrocytes.

Frozen sections for fat and myelin staining methods and for certain silver impregnations for nerve fibres, were cut from the material which had been fixed in 10% formol-saline for a minimum of 6 days.

Of the other fixatives, Bouin, Carnoy, Zenkers and Helly, in this order, were considered next in usefulness. With Bouin, Carnoy, and 96% alcohol containing 5% acetic, best results were obtained by fixing 4 m.m. thick pieces in these fluids already chilled, and leaving in the +4°C. refrigerator overnight. These three methods were used when a rapid examination was required, as additional methods of study particularly of the nucleus and Nissl substance, and for certain staining procedures, for example, Bouin for the aldehyde fuchsin method and Carnoy or 96% alcohol containing 5% acetic for methyl-green-pyronin.

2 to 4 m.m. thick pieces were fixed in an already chilled solution of 9 parts of absolute alcohol to 1 part of 40% formalin (Bensley, 1939) in the +4°C. refrigerator overnight, for subsequent staining for glycogen.

Quite good results, particularly as regards the Nissl picture, were obtained by fixing for 3 days in equal parts of 96% alcohol and 10% formol-saline.

All these different fixatives were also useful as control methods for the elimination of fixation artefacts.

Further processing and preparation and staining of sections. As a routine, transverse pieces of tissue were processed from the mid-cervical, cervical intumescencia, mid-thoracic, upper lumbar and lumbo-sacral intumescencia. In 55 animals sections from 10

segments of each cord were examined and in the remaining 85 animals, 5 segments were examined. From 10 to 30 serial sections per segment were used. Longitudinal sections from the two intumescencia and the mid-thoracic region were also examined in many cases.

Dehydration was through graded alcohols (50%, 70%, 96% and 2 changes of 100%), clearing in equal parts of absolute alcohol and benzene, followed by benzene, and impregnation in soft wax (melting point 50°C.) for 2 hours, followed by hard wax (melting point 56°C.) for half an hour. It was most important to keep the time in the ovens as short as possible to avoid shrinkage and other artefacts.

Transverse and longitudinal sections were cut at 7 to 8 microns and attached to the slide with egg albumen (Mayer, P., 1883, quoted by Lee, 1950). Only very occasionally was it necessary to celloidinise the slides, even with methods requiring prolonged staining at above-room temperatures.

More than 15,000 sections of transverse areas of the cords were stained routinely with haematoxylin and eosin, as well as a varying number of longitudinal sections. Sections from nearly every case, and in interesting cases from every segment of the cord were stained with cresyl fast violet for the Nissl substance, and by the Holmes method for neurofibrils. Slides of 4 or 5 serial sections were always used so that the structure of the larger neurones could be adequately examined.

Apart from these routine staining methods, a number of other stains were used on selected slides. The number of cases in which these were used varied. For instance, Palmgren's and Romanes' silver techniques were used with Holmes' method only until it was established that, in the author's hands, the latter was preferable, whereas Einarsen's gallocyenin was used throughout the study as an additional Nissl method. Phloxine-tartrazine was used in a survey of slides from a considerable number of cases. Other methods, such as Best's carmine and Gomori's aldehyde fuchsin, were used for specific purposes as required.

Transverse pieces of spinal cord from 2 natural and 1 experimental cases of scrapie and 1 control were embedded in celloidin and sections cut at 15 microns. They were stained with haematoxylin and eosin and with toluidine blue.

Frozen sections of transverse pieces from the 7th to 8th cervical intersegment, the 6th to 7th thoracic intersegments, and the 5th to 6th lumbar intersegments, which had been fixed in F.A.B. for 5 days, were cut at 20 microns thickness and stained by the Cajal gold chloride sublimate method for astrocytes.

Frozen sections of formol fixed material were cut at 25 microns and stained with Sudan IV counterstained with haematoxylin and by Spielmeyer's method for neutral fats and degenerating myelin, and by the Bielschowsky-Glees method for degenerating axons. For these methods transverse sections were routinely taken from C₄, C₈, T₅, and L₆. Longitudinal sections from just caudal to these segments were cut at three depths in the cord so as to include the ventral, lateral and dorsal funiculi. 17 natural scrapie, 12 experimental and 17 control sheep were examined thus. In addition, transverse sections from C₈, T₅, and L₆ of a further 26 natural cases of scrapie were stained by Sudan IV / haematoxylin.

Paraffin and celloidin embedded sections and the frozen sections stained by Bielschowsky-Glees method were dehydrated, cleared and mounted in D.P.X. (Kirkpatrick and Lendrum, 1941). Cajal's gold chloride sublimate and Spielmeyer stained sections were mounted in Apathy's mountant (Lillie and Ashburn, 1943). Frozen sections stained with Sudan IV / haematoxylin were mounted in glycerine; this was never acid and gave a clearer picture than Apathy's mountant.

All the staining methods used and the purpose for which they were particularly used in examining the spinal cord, described as routine and additional methods, are given below. Any variations in technique from that indicated by the reference are briefly described.

Staining methods - routine.

1. Mayer's haemalum and eosin as a general oversight stain, (Mayer, P., 1903, quoted by Lee, A.B., 1950). 50 gm. of chloral hydrate and 1 gm. of citric acid were added to each litre of haemalum to improve stability.
2. Cresyl fast violet for Nissl substance, (Weil, A., 1946).
3. Holmes' method for neurofibrils and axons, (Holmes, W., 1947). Although Holmes recommends Bouin, Carnoy, and similar mixtures, excellent neurofibril preparations were obtained after formol-saline-corrosive sublimate fixation.

The proportions of substances in the impregnating solution were also slightly modified, the following being used.

1. 24% Boric acid	5.5 ml.
1. 9% Borax	4.5 ml.
0. 1% silver nitrate, aqueous	1 ml.
1. 0% Pyridine, aqueous	5 ml.
Distilled water	ad 50 ml.

This 50 ml. filled a coplin jar and satisfactorily impregnated as many as 10 slides at one operation, when it was discarded. Holmes recommended at least 20 ml. of solution per slide.

4. Sudan IV for fat, (Kay, W.W., and Whitehead, R., 1935).

Triethyl phosphate was usually used as a dye solvent in preference to alcohol. The sections were placed in 30% triethyl phosphate for 5 minutes followed by 30 seconds in 60% triethyl phosphate, and then 20 to 25 minutes at 37°C. in a saturated solution of Sudan IV in 60% triethyl phosphate, prepared exactly as the Sudan IV-ethyl alcohol solution. After staining and brief passage through 60% and 30% triethyl phosphate solutions, the sections must be well washed in 3 changes of dist. aqua., leaving for at least one hour in the last change, or patchy staining with haemalum results. The sections

were much more pliable with triethyl phosphate than alcohol, and consequently flatter mountings could be easily prepared.

5. Spielmeyer's method for myelin, (Spielmeyer, W., 1930, quoted by Mallory, F.B., 1938).
6. Bielschowsky-Glees' method for axons, (Nauta, W.J.H., and Gyax, P.A., 1951).
7. Cajal's gold chloride sublimate for astrocytes, (Cajal, S. Ramon, y., 1913 and 1916, quoted by Lee, A.B., 1950).

Staining methods - additional.

1. Eosin-methylene blue as a general oversight stain and for Nissl substance, (Mallory, F.B., 1900, quoted by Mallory, F.B., 1938).
2. Giemsa, (overnight), differentiated in 0.25% celephonium resin in 95% alcohol for Nissl substance and for inclusion bodies, (Mallory, F.B., 1938).
3. Toluidine blue for Nissl substance in celloidin sections, (Mallory, F.B., 1938).
4. Galloeyanin-chromalum for Nissl substance, (Einarson, L., 1932).
5. Phloxine-tartrazine for inclusion bodies, (Lendrum, A.C., 1947).
6. Chrome alum-haematoxylin-phloxine for inclusion bodies, (Gomeri, G., 1941, and Smith, S.W., 1951).
7. Safranin O-erioeyanin A for the differentiation of necrotic neurones, (Lillie, R.D., 1954).
8. Heidenhain's iron alum-haematoxylin for inclusion bodies, (Heidenhain, M., 1892, quoted by Lee, A.B., 1950).
9. Palmgren's silver method for axons, (Palmgren, A., 1955).
10. Romanes' silver method for axons, (Romanes, G.J., 1946).
11. Weigert-Pal.-Ehrhart. for myelin, (quoted by Gurr, G.T., 1957).
12. Weil's method for myelin, (Weil, A., 1946). Instead of differentiating in iron alum followed by borax-potassium ferricyanide as recommended by Weil, frozen sections

were taken from the iron-alum-haematoxylin stain, rinsed in 2% iron-alum, then differentiated in 10% iron-alum at 37°C. for 5 to 10 minutes and finally differentiated in 2% iron alum.

13. Cresyl fast violet on 100 μ thick frozen sections, (Rexed, B., 1952).
14. Sudan black for inclusions, (Pearse, A.G.E., 1954).
15. Phosphotungstic acid haematoxylin for glial fibres, (Mallory, F.B., 1900, quoted by Mallory, F.B., 1938).
16. Best's carmine for glycogen, (Best, F., 1901, quoted by Bensley, C.H., 1939).
17. Bauer-Feulgen for glycogen, (Bauer, 1933, quoted by Bensley, CH., 1939).
18. Feulgen reaction for desoxypentose nucleic acid, (Feulgen, R., and Rossenbeck, H., 1924, quoted by Lillie, R.D., 1954).
19. Periodic acid-Schiff reaction for polysaccharides, mucopolysaccharides, glycoproteins, or glycolipids, (Lillie, R.D., 1954).
20. Methyl-green-pyronin Y., for nucleic acids, (Kurnik, N.B., 1955). The routine formal-saline corrosive sublimate fixation gave very inferior results. Good results were obtained with Carnoy or alcoholic fixatives.
21. Perl's reaction for ferric salts, (Perl, M., 1867, quoted by Russel, D.S., 1939).
22. Stein's method for bile pigments, (Stein, J., 1935, quoted by Cowdry, E.V., 1952).
23. Papez-Henschel.-cresyl violet for inclusion bodies, (Papez, J.W., 1944).
24. Gomori's aldehyde-fuchsin for inclusion bodies, (Scott, 1952). Two minutes in aldehyde fuchsin was found to be adequate to stain lipochrome granules. Phloxine was not used, but after rinsing in 3 changes of 96% alcohol, sections were brought to water and stained for 30 seconds in 0.1% light green, dehydrated, cleared, and mounted in D.P.X.
25. Picro-Mallory for inclusion bodies, (Zlotnik, I., 1953).
26. Carbol fuchsin-haematoxylin-tartrazine, (Wolf, A., and Pappenheimer, A.H., 1945).

27. Congo-red for amyloid, (Benhold, H., 1922, quoted by Mallory, F.B., 1938).
28. Iodine reaction for amyloid, (Mallory, F.B., 1938).
29. Lepehne-Pickworth method for brain capillaries, (Campbell, A.C.P., and Alexander, L., 1938, quoted by Mallory, F.B., 1938).
30. Argentaffin reaction for inclusion bodies, (Gomori, G., 1952²).
31. Methyl green for inclusion bodies, (Popper, H., Gyorgy, P., and Goldblatt, H., 1944).

Methods used to estimate the significance of vacuoles and of certain inclusions.

One of the main objects of the present study was to find the incidence, distribution, and significance of vacuolated neurones in scrapie. To this end, vacuoles were carefully counted in 13,450 sections of spinal cords, and many more sections were examined, without counting vacuoles, in order to thoroughly confirm these findings.

Vacuolated neurones were counted with the 4 m.m. objective in 7 - 8 μ transverse sections stained with haematoxylin and eosin. Specific nuclei cannot easily be identified in thin paraffin sections and vacuolated neurones were too distorted to put them in cytologically similar groups. Accordingly the grey matter of the cord was arbitrarily divided into three major zones which could be easily demarcated in any section. Zone A comprised all the neurones ventral to the substantia gelatinosa Rolandi and dorsal to the large neurones which lay in relatively well defined nuclei in the ventro-lateral and ventro-medial aspect of the ventral horn. The majority of the latter are motor neurones and they were designated Zone B. Zone C consisted of the nucleus intermediolateralis where it formed a well defined horn (T_6 and L_3). Zone B was divided into medial and lateral groups of neurones. The number of vacuolated neurones counted in each zone was recorded. If vacuoles occurred amongst a well defined group

of cells which could fairly certainly be presumed to be a specific nuclei, this was also noted. The occurrence of vacuoles outside the zones was also noted.

100 μ thick frozen sections were cut from each segment of the cords of 3 control sheep and stained by cresyl fast violet after the method recommended by Rexed (1952). From a study of these it was possible to adequately identify, by comparison with data available for other animals, the more important nuclei in each zone.

In this manner vacuoles were counted in 200 sections from each of the following cases:-

1. 10 sheep with clinically advanced natural scrapie.
2. 10 sheep with clinically non-advanced natural scrapie.
3. 20 sheep with experimental scrapie, of which 12 were $1\frac{1}{4}$ to $2\frac{1}{4}$ years old when destroyed, 5 were 6 to 9 months old, and 3 were cast ewes of about 7 years old when destroyed.
4. 15 control sheep of ages ranging from 8 months to 7 years.

The 200 sections were made up by 20 serial sections from each of 10 segments of the spinal cord from the mid-cervical region (C_4), the cervical intumescentia (C_6 , C_7 , C_8 , and T_1), the mid-thoracic and upper lumbar region (T_6 and L_3), and the lumbo-sacral intumescentia (L_5 , L_6 and S_1).

In order to confirm the above findings, counts of the same zones were undertaken on 50 sections from a further 30 cases of natural scrapie and 19 control sheep. The 50 sections were made up by 10 serial sections from 5 segments which again represented the regions of the cord examined in more detail above. (C_4 , C_7 , T_6 , L_3 , and L_6).

A further 18 natural cases of scrapie and 10 experimental cases were examined, but detailed counts were not attempted, and finally in 3 cases, 5 serial sections were examined from every segment of the cord from the 2nd. cervical to the 1st. coxygeal inclusive.

The total numbers of nucleoli visible in Zones A, B, and C of the grey matter was counted in 5 serial sections

from each of the 10 segments of the cord in 10 of the control sheep. From this and the foregoing count of vacuoles, it was hoped to find out if vacuoles were more or less numerous in those areas of the cord where there was a normal large or small population of neurones.

A small number of frozen sections 240μ thick from the cervical intumescencia and lower thoracic region of 5 control sheep were stained by the Lepehne-Pickworth method in order to show the capillary network of the various zones.

To determine the significance of phloxinophilic intracytoplasmic inclusion bodies, the number of neurones containing these bodies were counted in sections stained by the phloxine-tartrazine method, (Lendrum, 1947), from 4 serial sections from each of 5 segments of the cord (C_4 , C_8 , T_6 , L_3 and L_6) in 15 natural scrapies, 10 experimental scrapies, and 15 control animals. The positive cells were recorded as containing small (less than 0.5μ in diameter), medium (between 0.5μ and 2.5μ) and large bodies (more than 2.5μ). The same number of sections from a further 15 control animals was examined but only those cells containing large bodies were recorded. The ocular micrometer was used to measure the bodies.

METHODS USED IN THE PREPARATION AND EXAMINATION OF THE PERIPHERAL NERVOUS SYSTEM.

Methods of killing and postmortem procedure.

The specimens were obtained from animals which had died or were killed as previously recorded.

Spinal Ganglia.

The cord was exposed as before and the intervertebral foramina opened with bone forceps. By cutting the nerve roots distal to the ganglia the cord and attached ganglia could be easily removed.

Gasserian ganglia.

The skull was opened and the brain removed, when the large trigeminal nerves were visible on the floor of the cranial cavity. Part of the nerves with the attached Gasserian ganglia were then dissected away from the fibrous tissue in the foramen lacerum basis cranii.

Sympathetic ganglia.

The posterior cervical and the 1st. thoracic ganglia (the stellates), the thoracic sympathetic trunks with the thoracic sympathetic ganglia, and the coeliaco-mesenteric ganglia were removed from each side in one piece. To do this the abdomen was opened and the stomachs, intestines, and major abdominal organs, except the adrenal glands, were removed. The ventral and lateral walls of the thoracic cavity were removed by cutting through the ribs an inch from their heads, and both entire chains of sympathetic ganglia dissected away from the over-lying pleura by cutting the rami communicantes. The chains were left attached to the vagus nerves anterior to the stellates while dissection continued caudally through the diaphragm to the coeliaco-mesenteric ganglia, which lay close to the adrenals in relation to the origin of the coeliac and anterior mesenteric arteries. When the coeliaco-mesenteric ganglia had been removed, the anterior attachments at the vagi were severed and the whole chains laid on a piece of cardboard.

The single posterior mesenteric ganglion was dissected from the origin of the posterior mesenteric artery, and also laid on cardboard.

The two anterior cervical sympathetic ganglia were exposed by cutting away the parotid glands and overlying mandibular muscles, and breaking off the paramastoid processes with bone forceps.

In order to avoid artefacts, particularly stretch artefact of axons, these dissections were carried out as gently as

possible. The sympathetic trunk or ganglia were never nipped with the forceps, but held lightly in the fingers, and related nerves were severed with a sharp scalpel.

Histological methods.

Fixation. Two methods of fixation were used for the majority of specimens.

In the first method the spinal ganglia and sympathetic trunks with associated ganglia laid on cardboard were placed in formol-saline for a month. Selected ganglia or pieces were then post fixed in saturated mercuric chloride solution, while the remaining pieces were left in formol-saline for gelatin embedding. The following were fixed by this method:-

1. Spinal ganglia from 30 natural cases of scrapie, 10 experimental cases, and 10 healthy controls. The ganglia in each case were divided into 3 groups comprising cervical and cervical intumescencia (C_4 , C_7 and C_8), mid-thoracic and upper lumbar ganglia (T_7 , T_8 , L_2 and L_3), and the lumbo-sacral group (L_6 and S_1).
2. Stellate ganglia from 26 natural, 6 experimental, and 7 healthy controls. After removing the ganglia from formol-saline, they were cut into pieces about 2 m.m. thick, one or two pieces from each side being retained in formol-saline as indicated above. Pieces of the sympathetic trunk both cranial and caudal to the ganglia were included with pieces for post fixation and for retention in formol-saline.
3. Thoracic sympathetic ganglia from 26 natural cases, 6 experimental cases and 7 healthy controls. The ganglia were cut out of the trunk and, as with the stellate ganglia, several pieces of sympathetic trunk were included with both fixatives.
4. Coeliaco-mesenteric ganglia from 26 natural, 6 experimental cases and 7 healthy controls. The ganglia were treated similarly to the stellates (2).

The second method of fixation was in 96% alcohol containing 5% acetic for 24 hours. This method was recommended by Hopkins (1924), as giving a superior Nissl picture with ganglia. The ganglia, including those of the thoracic sympathetic trunk, were cut with scissors into small pieces, about 2 - 3 m.m. cubes, immediately after post mortem and selected pieces dropped straight into the fixative. The following were fixed by this method:-

1. Thoracic sympathetic trunk ganglia, stellates and coeliaco-mesenterics from 4 natural cases of scrapie, 9 experimental cases, and 4 healthy control sheep.
2. Posterior mesenteric ganglia from 5 natural cases of scrapie, 5 experimental cases, and 5 healthy control sheep.
3. Anterior cervical sympathetic ganglia from 5 natural cases of scrapie, 5 experimental cases and 5 healthy control sheep.
4. Gasserian ganglia from 5 natural cases of scrapie, 5 experimental cases and 5 healthy control sheep.

In addition individual spinal or thoracic sympathetic trunk ganglia and small pieces of the other ganglia was occasionally fixed in Bouin, Carnoy, equal parts of 96% alcohol and 10% formol-saline, and 10% formol-saline alone.

Further processing and preparation and staining of sections.

The ganglia or small pieces of corrosive fixed material were dehydrated with graded alcohols and cleared in cedar wood oil. (50% - 8 hours, 70% - 16 hours, 96% - 2 hours, 100% - 2 changes of 1 hour each and over night in cedar wood oil). The excess cedar wood oil was washed out by 15 minutes immersion in benzene, and they were then impregnated with paraffin (1 hour in soft wax, $\frac{1}{2}$ hour in hard wax). Embedding was done in solid watch glasses.

Material fixed in alcohol-acetic was transferred straight to 96% alcohol, but otherwise treated as above.

Routine sectioning and staining of the paraffin embedded

tissues was the same as for the spinal cord, except that Einarson's galloxyanin was used as a Nissl stain instead of cresyl fast violet. A minimum of 16 serial sections from each block were stained with Mayer's haematoxylin and eosin, and, as each block contained several ganglia or several pieces of ganglia, a total of more than 15,000 sections of individual ganglia were examined. To determine the significance of vacuolation in the spinal ganglia, the stellates, the thoracic sympathetic trunk and the coeliac-mesenteric ganglia, the numbers of vacuoles in 16 sections of ganglia from each block were counted. In all the number of vacuoles in 5,136 sections were counted.

Many of the stains listed under additional methods for the spinal cord were also used on selected sections of ganglia and are mentioned if the results merit it. In addition, the following methods were used for specific purposes on selected sections:-

1. Masson's trichrome for connective tissue, (Masson, P., 1928).
2. Van Gieson's picric acid-acid fuchsin for collagen, (Van Gieson, I., 1889, quoted by Mallory, F.B., 1938).
3. Chromaffin reaction of Lissou, (Cowdry, E.V., 1952).
4. Ferric ferricyanide reduction method of Schmorl for chromaffin, (Lillie, R.D., 1954).
5. Cajal's formula 3 for spinal ganglia, (Lee, A.B., 1950).

The material which remained in formol-saline, including spinal ganglia from the intumescentia and mid-thoracic region, was embedded in 10% gelatine in the usual manner, - 24 hours washing in running water, 24 hours in 5% gelatine at 37°C., and 6 hours in 10% gelatine at 37°C. By allowing a layer of gelatine almost to set in the embedding dish, the tissues were orientated so that transverse as well as longitudinal sections of nerve trunks were cut in the same block. The blocks were hardened and stored in Baker's (1944) fluid., (formal-calcium cadmium).

ADDITIONAL MATERIAL - NEURONES OF THE ADRENAL MEDULLA.

As adrenal glands from a considerable number of sheep were being studied while the present work was in progress, some histological observations on the neurones of the adrenal medulla will also be included.

Both adrenal glands from 112 control sheep, 57 cases of spontaneous scrapie, and 13 cases of experimentally induced scrapie were available. The control sheep were obtained from the local abattoir or from similar sources to those used in the major part of this study, and varied in age from 6 months to more than 3 years. The age and sources of the natural and experimental cases of scrapie were approximately similar to those previously reported.

The glands were fixed in 10% formal-saline prior to processing, embedded in paraffin, and 7μ thick sections stained by Mayer's haematoxylin and eosin.

The scrapie sheep were selected with changes of white vacuolization was perhaps the most striking, but not the sole change. With a few exceptions, when vacuolated neurones were numerous, other neuronal changes were also present (Tables V and VI). The variation between individual cases was, on the whole, one of degree, although the less advanced types of change predominated in the experimentally induced disease (Tables VII and VIII). Vacuolated neurones are described below.

Characteristics. Neurones showed the following degrees of vacuolization:-

1. Vacuolization of these cells was confined to the nucleus; elsewhere in the cell the fluid substance was of normal appearance. The cell was slightly swollen; the nucleus and nucleolus were unchanged in appearance and position.
2. The fluid granules throughout the cytoplasm were pushed against close to the cell membrane, where they were several or several times usual. The cell was moderately swollen. The nucleus lay essentially, and was only slightly swollen.
3. The centre of the cell was devoid of fluid granules, but

RESULTS.

THE SPINAL CORD.

Section I.

Macroscopic observations.

A comparison of the spinal cords and spinal meninges from 68 natural and 30 experimental cases of scrapie with those from 36 clinically healthy control sheep, revealed no macroscopic difference between the three groups.

Microscopic observations.

The Neurone. Histological examination of the spinal cords showed that a varying number of neurones in the scrapie sheep were affected with changes of which vacuolation was perhaps the most striking, but not the sole change. With a few exceptions, when vacuolated neurones were numerous, other neuronal changes were also frequent (Tables V and VI). The variation between individual cases was, on the whole, one of degree, although the less advanced types of change predominated in the experimentally induced disease (Tables VII and VIII). These neuronal changes are described below.

Chromatolysis. Neurones showed the following degrees of chromatolysis:-

1. Powderiness of those Nissl bodies close to the nucleus; elsewhere in the cell the Nissl substance was of normal appearance. The cell was slightly swollen; the nucleus and nucleolus were unchanged in appearance and position.
2. The Nissl granules throughout the cytoplasm were powdery except close to the cell membrane, where they were normal or coarser than usual. The cell was moderately swollen. The nucleus lay eccentrically, and was often slightly swollen.
3. The centre of the cell was devoid of Nissl granules, but

coarse flakes were present at the periphery. An accumulation of basophilic material was also sometimes present on the nuclear membrane, forming a "nuclear cap". The cell was moderately swollen. The nucleus lay close against the cell membrane, even bulging it in some cases, and the nucleolus was sometimes eccentric.

4. Only a few coarse flakes of Nissl substance were present close to the cell membrane, and the cytoplasm stained acidophilically in H & E preparations, or with a ground glass-like appearance with cresyl fast violet, Giemsa, and Einarson's gallocyanin. The nucleus was often distorted, rather elongated, and had a more basophilic appearance. The nuclear membrane was often indented and irregularly thickened, producing the so-called "folded" appearance.
5. The Nissl granules were very finely fractionated throughout all the cytoplasm. The cell was slightly swollen. The nucleus was of normal appearance and was centrally placed or occasionally was eccentric.

Changes numbers 1 to 4 were classed as central chromatolysis (Figs. 1 and 2). They seemed to represent a sequence of changes of which 1 was the earliest, and 4 the last and most severe stage, probably being followed by acidophilic necrosis, severe cell change, or vacuolar degeneration.

Change number 5 was classed as diffuse chromatolysis (Fig. 3).

Central chromatolysis was the predominant type of Nissl change observed in the cord. Diffuse chromatolysis was comparatively rare, and peripheral chromatolysis was never seen.

Chromatolysis was observed in all cases of scrapie. In the natural cases if many cells were vacuolated then chromatolytic cells were generally also numerous. Usually one or two cells per section were chromatolytic in advanced cases, and less than this in the non-advanced, but in some cases, usually those showing severe neuronal vacuolation and degeneration, up to 20 chromatolytic cells were seen in

each section. Although the number of chromatolytic cells observed was generally about the same as the number of vacuolated cells, there were exceptions to this, as shown in Tables V to VIII. In some cases (S3, S10, S56 and S30) chromatolysis throughout the cord seemed more common than vacuolation, whereas other cases were severely vacuolated but showed few chromatolytic cells (S2). Again chromatolysis was sometimes unusually severe in a particular nucleus, such as in cases S40, S57 and S58 where, although vacuoles were rare, the neurones of the intermedio-lateral column were markedly chromatolytic. Most chromatolytic neurones occurred in the intermediate zone, particularly the cellulae disseminatae posteriores, intermediae and anteriores, the nucleus cornu-commissuralis anteriores and posteriores, the substantia grisea centralis, the nucleus sensibilis proprius, the nucleus reticularis spinalis, and in the intermedio-lateral column. It was often noticed that the same nucleus, sometimes apparently corresponding individual cells; were affected on both sides of the cord; the degeneration was bilaterally symmetrical. The large motor neurones of the ventral horn were very rarely chromatolytic.

In the experimental cases, central chromatolysis was more common than other degenerative processes, but it was nevertheless infrequent (Table VII). Generally only one chromatolytic cell was seen in three or four sections, although in one case as many as five chromatolytic cells were seen in a single section. Usually the cells were in the primary stages of change (stages 1 and 2), with slight swelling of the cell rather noticeable. Stage 4 was infrequent. The same nuclei as in the natural cases were involved.

Acute swelling. Pronounced swelling of some neurones was seen, and these swollen cells could be divided into two groups.

1. The cell body and processes were very swollen, the former having a "rounded off" appearance, and the Nissl substance was finely powdered except for a few coarse flakes at the periphery of the cell and in the base of the processes.

The nucleus was lightly stained and centrally situated. (Fig.4).

2. The cell and processes were swollen as above, but no Nissl substance was present. The nucleus was pyknotic and eccentric. Cavitations, or foamy vacuolation, usually seen as pale circular areas, were present in the hyoplasm. (Fig.5).

These forms of cell change were classed as acute swelling. The second group was considered to represent a more advanced degenerative phase. It might be classed as either advanced acute swelling or early severe cell change depending on the degree of degeneration apparent in the nucleus, the eosinophilia of the cytoplasm, and the extent of foamy vacuolation.

Acute swelling was relatively uncommon and was only seen in 5 advanced cases. In these, occasional acutely swollen neurones were present in the intermediate zones, the anterior cornu commissuralis nucleus, and the nucleus reticularis spinalis.

Severe cell change. Neurones were seen in which all the Nissl granules, or all but a few coarse flakes close to the cell membrane, had dissappeared. The nucleus was usually shrunken, distorted and eccentric, and stained very darkly. The cytoplasm stained eosinophilically, or like pale blue ground glass after Giemsa, cresyl fast violet, or Einarson's gallocyanin.

Cavitations or fenestrations of the cytoplasm and irregular indentations of the edge of the cell wall were present (Fig. 6). These areas of dissolution affected the cell body and the processes. The cell was nearly always slightly, and occasionally considerably, swollen. In sections prepared by the Holmes' method the neurofibrils were either dissolved into fine dust or were visible as aggregated argentophilic masses (Fig. 8).

This form of neurone degeneration was classed as severe cell change. It was seen in all the natural cases of scrapie and roughly the same number of cells were affected

and in the same nuclei, as those showing chromatolysis. Bilateral symmetry was often noted. It was uncommon in experimental scrapie.

Sclerosis. Neurones were seen which had a shrunken appearance. Their nucleus was pyknotic and eccentric, the pyrenophore was dark staining, and the processes often tortuous and deeply and prominently stained. When the Nissl substance was distinguishable, it appeared coagulated into large darkly stained clumps. Several small vacuoles were sometimes present on the concave borders of the neurone (Fig. 17). This type of change could be seen in scrapie cords processed by different methods, and in serial sections after different stains.

This change was classed as sclerosis or Nissl's chronic neuronal disease (Fig. 9). Such cells comprised a fairly numerous percentage of diseased cells in all cases of scrapie, and they affected the same groups of nuclei as did chromatolysis. Generally, as with chromatolytic neurones, the more severely vacuolated the case, the more numerous were sclerotic neurones. In some experimental cases however, sclerotic, or cells which because their cytoplasm was very little shrunken, might be better described as chromophil cells, were more numerous than vacuolated neurones. Such sclerotic or chromophil neurones were very rarely vacuolated.

Sclerotic cells were occasionally seen in the control animals, particularly in the nucleus reticularis spinalis and amongst the posterior marginal cells, but they were not common in well processed healthy sheep spinal cords. Prolonging the time in the 57°C. ovens during embedding in paraffin wax was found to bring about a pyknotic shrunken appearance very similar to sclerosis. Usually in such cases the majority of the nerve cells showed the change, whereas in the genuine pathological picture sclerotic neurones were discretely scattered amongst healthy or otherwise diseased nerve cells. In any cases of doubt, fresh pieces of tissue were taken from formol storage and reprocessed.

Necrosis. Probably necrosis was the most commonly observed change after vacuolation in the naturally occurring cases. In some natural cases, particularly Sl2, necrosis was more common change than vacuolation. The same nuclei as noted under chromatolysis were affected, and the degeneration was often bilaterally symmetrical. In experimental cases necrosis was rare, and only two healthy control animals showed necrotic nerve cells (4 cells in one case, 2 cells in another).

The appearance of the necrotic cell was typically a round or tadpole-shaped structure showing intense eosinophilia (Fig. 12). After the Nissl stains the cytoplasm appeared pale blue like ground glass. They also stained blue with safranin O-eriocyanine A. A nucleus was rarely present at this stage, but sometimes the remains of the nucleolus could be seen. Occasionally one or two small vacuoles or cavitations were present. The eosinophilic "tail"-like structure present on some necrotic neurones probably represented the degenerated axon. In some cases this was seen as a short chain of eosinophilic granules.

Cells were often seen in the last stages of chromatolysis in which the cytoplasm was intensely eosinophilic, the nucleus was dark and eccentric, and only a remnant of peripheral Nissl remained. Some cells were similar but no Nissl was present, while in others only the remnant of a nucleus was distinguishable. In cases in which the frankly necrotic cells described above were numerous, these latter types of cells were also common. This suggests that these various morphological pictures represented a series of stages in the degeneration of neurones which may start as central chromatolysis and terminate in acidophilic necrosis (Figs. 10, 11, and 12).

Vacuolation. Vacuolated nerve cells were the most characteristic abnormality seen in scrapie, and were constantly present in 68 natural cases and in 29 out of 30 experimental cases. Comparatively insignificant numbers of cells showing certain types of vacuolation were seen in the control sheep.

The vacuoles were present in paraffin and celloidin embedded material and in frozen sections. They could also be demonstrated after the 12 methods of fixation which were investigated in this study;-

1. 10% formol-saline.
2. 10% formol-saline post fixed in corrosive sublimate.
3. Formol-ammonium bromide.
4. Bouin's fluid.
5. Carnoy's fluid.
6. Equal parts of 96% alcohol and 10% formol-saline.
7. 96% alcohol and 5% acetic acid.
8. Susa.
9. Zenker's fluid.
10. Helly's fluid.
11. Regaud's fluid.
12. 9 parts of absolute alcohol and 1 part of 40% formalin.

Their presence after these varied procedures and their relative absence in cords from both healthy and diseased control animals processed by exactly the same methods as the scrapie material, very definitely indicates that they are not an artefact, but represent a significant change.

Vacuolated neurones could be divided into two main groups.

- a. Those in which the cell was otherwise normal by the histological methods used. Nissl substance, nucleus, nucleolus, cytoplasm and neurofibrils appeared quite healthy.
- b. Those in which the cell was abnormal. All the types of degeneration previously described in unvacuolated neurones were seen in vacuolated cells.

Numerous varieties of vacuolated neurones were present so that it was difficult to classify them other than in these two large groups. The following appearances however, cover most vacuolated neurones seen.

- a. The cell was otherwise normal.
 1. A single small "punched-out" vacuole in the cell body, often near the cell membrane, or in one of the processes (Fig. 14).

2. Multiple small vacuoles, but rarely more than two, in the cell body usually near the cell membrane, or in the processes of the cells.
3. Single vacuoles of medium or large size. The largest vacuoles were seen to balloon the cell, but, apart from displacement of the nucleus, Nissl substance, cytoplasm, nucleus and nucleolus were normal. The neurofibrils were rather condensed round the edge of the cavity as if they had been pushed together by the expanding vacuole, but elsewhere in the cell they were quite normal.

b. The cell was abnormal.

1. Single small vacuoles in the cytoplasm or process of cells which were normal apart from powderiness of the Nissl substance round the vacuole (Fig. 15).
2. Single small vacuoles in cells showing severe central chromatolysis, or, less frequently, severe diffuse chromatolysis (Fig. 16).
3. Multiple small vacuoles, usually not more than 2 or 3 in cells which were normal apart from dissolution of Nissl substance round the vacuole (Fig. 18).
4. Multiple small vacuoles in the pyrenophore or processes of cells showing severe central or diffuse chromatolysis, or necrosis (Figs. 19 and 20). Up to 8 vacuoles were counted round the periphery of one chromatolytic cell in a single plain of section.
5. Single large or medium sized vacuoles in cells which were undergoing various forms of degeneration (Fig. 15). The vacuoles were very similar to those seen in healthy cells (group a.3.) and although the vacuole was very large and extensively ballooned the cell, there might be no abnormality other than dissolution of the Nissl substance in the thin walls of the vacuole, whilst elsewhere in the cell Nissl was normal. Alternatively, chromatolysis might be more extensive; frequently only a little Nissl was piled up round the nucleus and present as a few coarse flakes at the periphery of the

cell. Often cells were severely chromatolysed and degenerative changes were beginning to affect the nucleus. Some cells showed acidophilic necrosis, and in others all that remained of the cell was a thin eosinophilic rim round an apparently empty space. The neurofibrils were unaffected in chromatolytic vacuolated cells (Fig. 27), as in similar normal cells (a. 3. above), but when necrosis occurred they were fragmented.

These degenerative changes did not depend entirely on the size of the vacuole which might be of medium size in a severely diseased cell, or very large in a cell which showed only slight chromatolysis.

6. Small vacuoles in sclerotic cells. Usually several small vacuoles were present, often clustered along the edges of the cell or its processes. Occasionally one of the vacuoles was of medium size (Fig. 17).
7. Multilocular vacuoles in degenerating cells. As with the large single vacuoles (b. 5 above), the cell might show slight dissolution of the Nissl round the vacuole (Figs. 21 and 22), severe chromatolysis (Figs. 23 and 24), or necrosis (Figs. 25 and 26). Usually only small vacuoles were present in the earliest forms of chromatolysis, although appreciable quantities of Nissl and a displaced but otherwise normal nucleus were often present with quite extensive vacuolation (Figs. 22 and 23). Neurofibrils appeared normal (Fig. 27) unless severe degenerative changes had affected the cell. Often in quite extensive multilocular vacuolation, fine, normal neurofibrils could be seen in the walls of the loculi. The large multilocular vacuoles consisted of numerous small chambers or a few large ones, and the thin interocular septa in some cells suggested some of the latter may be formed by coalescence of smaller chambers (Fig. 21). Sometimes the vacuoles were so large that only a thin rim of cytoplasm remained,

sometimes the vacuole walls had obviously collapsed into a necrotic remnant, and sometimes only a tattered strip of eosinophilic cytoplasm, which was perhaps all that remained of the cell, was seen lying at the edge of a hole in the cytoplasm. These holes were never so numerous as to constitute "Luckenfeld"(field of holes).

8. Vacuolation of a process of an apparently normal pyrenophore. All the previous types of vacuolation of abnormal cells occurred in the cell body alone, both the cell body and processes, or in the processes alone. In this final type of vacuolation however, the cell body appeared quite normal, but such a large vacuole or chain of vacuoles occurred in one of the processes that it was difficult to believe the parent body had not suffered some effects (Fig. 29). In these cells only vacuolated dendrites were seen.

In healthy control sheep only 4 normal cells containing single small vacuoles (a.1.) and one sclerotic cell containing a medium sized vacuole (b.6.) were seen. The majority of vacuoles seen in experimental sheep were the single small punched-out types in otherwise normal or slightly chromatolytic cells (a.1 or b.1), although all the other types of vacuole listed were seen, and every positive experimental case showed some large single or multilocular vacuoles in abnormal cells (b.5 or b.7). All types of vacuoles were seen in the natural cases. The earliest suggestion of vacuole formation was disintegration of Nissl substance and neurofibrils over a small area in the cytoplasm. Fig. 28 shows that the neurofibrils are broken into fine dust in such an area in the cytoplasm, while at the periphery of the cell 2 small, but distinct, vacuoles are seen. The commonest type of vacuole in the natural cases was perhaps the large and medium sized single vacuole in a degenerating cell (b.5.), although large and medium sized single vacuoles in apparently healthy cells (a.3.) and multilocular vacuoles in degenerating cells (b.7.) were also common. Multilocular vacuoles (b.7.) were most frequent in cases which showed many

vacuolated neurones, but this was not invariable. In one natural case (S2) large numbers of single small or large vacuoles were present in normal cells (a.1 or a.3).

Inside the vacuoles of natural and experimental scrapie cases fine granules and spheroidal bodies up to about 10 microns in diameter were often present. This material usually occupied less than a third of the chamber, lying near the periphery (Fig. 60), but occasionally it nearly filled some of the smaller single and multiple vacuoles (Fig. 61). It had a glassy appearance and stained pink with eosin. The material was not phloxinophilic when differentiated according to Lendrum's original method, was P.A.S. negative, Feulgen negative, Bauer-Feulgen negative after alcohol-formalin fixation, negative by Kurnick's methyl-green pyronin, negative for acid-fastness by Ziehl-Nielson's carbol fuchsin, negative for iron (Perl's), negative for the Schmorl ferric ferricyanide reduction method, did not stain with aldehyde fuchsin and stained only the faintest blue by Einarson's gallocyanin at pH 1.64. It was not positive for neurosecretory material using Gomori's chrome-alum haematoxylin-phloxine (Smith's modification). It was negative for fat using Sudan IV in frozen and paraffin sections.

However, an occasional vacuole contained small P.A.S. positive granules (Fig. 41), and a little faintly argentophilic dust was not infrequently seen in some vacuoles after Holmes' method.

Apart from these occurrences none of the staining methods mentioned above revealed any specific substance in the vacuole.

The five vacuoles recorded in healthy control animals were observed in paraffin embedded material stained with haematoxylin and eosin, and no material was seen in them.

Multinucleation of neurones. Neurones with double nuclei were very rare (Fig. 13). One was seen in each of 3 control and in 2 scrapie sheep.

Intracytoplasmic granules in neurones. Two types of intracytoplasmic bodies were seen in the spinal cord neurones.

One type appeared as small granules of uniform size, typically in a cluster at one side of the nucleus (Fig. 31). They were faintly yellow in unstained sections. They stained black with Sudan Black and appeared brick red with Sudan IV in both frozen sections of formalin fixed material, and in sections of paraffin embedded material. The granules stained strongly with aldehyde fuchsin and with the periodic acid-Schiff reagent, which were found to be the ideal methods to demonstrate them. The P.A.S. reagent worked well after most fixatives including routine formol-sublimate, but aldehyde fuchsin stained the background pink after this fixative. Bouin was a better fixative for the latter. They give a very faint reaction with Feulgen's method.

The granules were also acid fast, and this property was more easily demonstrated by the Wolf/Pappenheimer (1945) modification than by classical acid fast methods. One hour in carbol-fuchsin at 60°C. was adequate to demonstrate acid fastness, as after longer periods (Cowdry, (1952) recommends overnight staining at 60°C.) it was found difficult to decolorise the Nissl substance. The granules stain brownish red or deep red rather than the brighter red seen with acid fast bacteria or the acid fast material sometimes seen in the adrenal glands and livers of sheep. They could be stained by methyl green using the method of Popper *et al.* (1944). They were positive with the argentaffin reaction (Gomori, 1952) and sometimes argentophilic with the Holmes and Bielschowsky-Glees methods.

These granules did not stain with eosin, phloxine, iron haematoxylin, methyl green pyronin, Giemsa, chrome-alum haematoxylin phloxine, or the Nissl stains (toluidine blue, cresyl fast violet, eosin-methylene blue, and Einarson's gallocyanin).

The granules were either absent, or seen only as a fine purple stippling or violet tinge to the cytoplasm by Gomori's

method, in young sheep under eighteen months of age. In sheep of natural scrapie age (usually 3 years) they were present as a clearly delineated cluster, mostly in the motor cells. In older sheep, which were available up to 7 years of age, they were present as one or two large aggregates in nearly all the larger cells of the ventral horns, but never reached such dimensions as to constitute a "pigment sac".

These granules were considered to be a lipofuscin. There was no difference in their occurrence in control and scrapie animals.

The second type of intracytoplasmic bodies were single or multiple granules of varying sizes which were diffusely scattered or arranged in loose groups in the cell body and sometimes the processes (Fig. 32). They were intensely phloxinophilic, resisting differentiation by tartrazine long after the red stain had been removed from the blood corpuscles. They stained red with Giemsa, picro-Mallory, and eosin-methylene blue. They also stained with Heidehain's iron haematoxylin.

They were negative with the periodic acid-Schiff reaction, the Feulgen method, Kurnick's methyl-green pyronin, aldehyde fuchsin, acid-fast methods, and with Sudan IV in both paraffin and frozen sections. They did not stain with the Nissl stains, including Einarson's gallocyanin at pH 1.64. They were negative for glycogen after alcohol-formalin fixation using both Best's carmine and the Bauer-Feulgen methods. Perl's method for iron gave negative results. They stained faintly pink with chrome-alum-haematoxylin phloxine (Gomori's (1941) method and Smith's (1951) modification). They did not stain with cresyl fast violet after treatment with citric acid (Papez, 1944).

The bodies were spherical and measured from less than 0.5 microns up to 5 microns in diameter. Small bodies less than 0.5 microns in diameter were found in all groups of sheep, but larger bodies seemed more numerous in some, but not all, of the natural scrapie group. The average diameter of fifty large inclusions which were measured was 3.7 microns. Counts

of the number of cells containing inclusions in transverse sections stained by the phloxine-tartrazine method in 15 control and 15 natural scrapie sheep, revealed that large inclusions (more than 2.5 microns in diameter) were present in 2 of the former animals and 10 of the latter. Furthermore, the total number of cells containing large inclusions was only 2 in the controls, but 132 in the scrapies. Medium size inclusions (between 0.5 microns and 2.5 microns in diameter) were seen in 7 out of 15 control and in all 15 scrapie sheep. The total number of cells containing these medium inclusions was 27 in the controls and 376 in the scrapies. Although the total number of cells containing small inclusions (less than 0.5 microns in diameter) was greater in the scrapies (scrapies 4,848; controls 2,402) the range in individuals of both groups was considerable (Table X).

The number of large and medium sized inclusions in each positive cell was also generally greater in the natural scrapies than in the controls. In both scrapies and controls the larger cells of the ventral horns, particularly in the intumescencia, were most frequently affected, and a cell which contained large inclusions usually had small and often medium sized bodies present also. Although the larger size of phloxinophilic bodies were more numerous in these 15 natural scrapie cases than in the controls, they were rare in vacuolated cells, and their frequency bore no obvious relationship to severity of vacuolation or clinical symptoms (Table XI).

Subsequently, an equal number of sections from a further 15 control animals were examined without attempting to count the inclusions. There were no large inclusions and only relatively small numbers of medium inclusions present.

Sections from 10 experimental cases of scrapie were counted by similar methods, and large inclusions were seen in seven cells from two of the cases.

Glia elements. There was no increased satellitosis in the scrapie cords. In the control animals, perineuronal satellites were more numerous in the intumescencia than elsewhere,

and in the lumbo-sacral intumescentia as many as 12 cells surrounded some neurones in a single transverse section. The majority of the satellites were oligodendroglia, but a few microglia were usually present, and occasionally an astrocyte was seen.

A general increase in glial elements was not seen in H. and E. preparations. In the cases of Louping ill and Russian spring summer encephalitis which were available as controls, histiocytic proliferation was obvious throughout the grey matter, and neuronophagia of degenerating neurones by numerous mesenchymal elements was marked (Fig. 33). Sometimes only a nodule of histiocytes was left where a neurone had originally been (Fig. 34). Nothing resembling this picture was seen in scrapie, and only in the severest cases was a rare degenerated neurone observed being phagocytosed by many twisted microglia (fig. 35). Small glial nodules (Rasen) were seen, particularly in the lumbo-sacral intumescentia, of the older controls, but these were composed principally of oligodendroglia with few microglia and astrocytes.

Never the less, definite neuronophagia does occur in scrapie and is not uncommon, but only neurones in the more advanced stages of severe cell change or necrosis were involved, and then by only one or two phagocytes. Often one had to examine a diseased cell in serial section to find a single twisted, or sometimes elongated, microglia close alongside or sunk into the cytoplasm of the cell or its processes (Fig. 26).

On the other hand, severely degenerating neurones were seen, both vacuolated and unvacuolated, in which no evidence of neuronophagia was found. The cell seemed to be disappearing by a process of cytolysis, and, presumably, absorption of the liquefied products of degeneration by the tissues.

Vacuolation per se did not seem to stimulate phagocytosis (Fig. 36).

Neither clasmatodendrosis nor gliosis, generalised or in specific nuclei, were seen by the Cajal gold sublimate

methods. Astrocytes were numerous in all areas of the grey matter in the controls, and formed quite a definite feltwork in older animals. It was not possible to differentiate unlabelled Cajal preparations of scrapie and control spinal cords (Figs. 37 and 38).

Astrocytes were seen in the grey matter in haematoxylin and eosin preparations showing usually one, sometimes two, faintly eosinophilic intranuclear inclusions (Figs. 39 and 40). These bodies, which displayed strong phloxinophilia and were P.A.S. negative, sometimes distended the nucleus to two or three times its normal diameter until only a faint rim of chromatin surrounded them. A clear halo seemed to surround the smaller bodies. They were present in both scrapie and control animals, but were extremely rare, except in one scrapie, (S15) where they were slightly more numerous.

Clusters of fine P.A.S. + ve granules were seen in the cytoplasm of the glia in both scrapie and control animals (Fig. 40). These glial cells usually had medium sized spherical nuclei with a rather light chromatin content, and were thus presumed to be oligodendroglia. These cells were often seen in the adventitia of the bloodvessels, and the P.A.S. + ve material in such cases was often in large globules. In some cases large globules of this material in the adventitia did not seem to be associated with a nucleus.

In four cases of scrapie, (S12, S15, S17, S23) a few glial cells contained neutral fat granules. These lay close to blood vessels, and the fatty material was in well defined bright rose-red granules in Sudan IV preparation of frozen sections, while the P.A.S. + ve material was in large orange to brick-red globules.

The Vascular system. Perivascular collections of cells resembling lymphocytes were present in both the scrapie and control groups of sheep (Figs. 41, 42, 43).

They were found in more sheep with natural scrapie than in experimental or control sheep; thus when 200 sections per case were examined they were observed in 55% of natural

cases, 25% of experimentals, and 33% of controls. Examination of 50 sections per case revealed less positive cases in all groups, but natural scrapies were still the most frequently affected (Table IX).

However, except in three natural cases of scrapie (S6, S35, S36) the infiltrations were not more severe or extensive than in the controls. In all groups it was unusual to see more than three layers of infiltrating cells, which had the appearance of lymphocytes, lying in the Virchow-Robin space (Fig. 44). This infiltration was either circumscribed or a small focal accumulation along the vessel wall. Again, excluding the above three natural cases, only one or two affected vessels were usually seen in any group even though 200 sections from different levels of the cord were examined.

There was no difference in the degree or extent of perivascular infiltrations in advanced as compared to non-advanced naturally occurring scrapie.

The perivascular accumulations occurred in both the grey and the white matter. In the natural cases 54% of affected vessels were in the white matter, 38% in the grey matter, and 8% partially in both. In the experimentals and controls affected vessels were rather more numerous in the grey matter. When in the grey matter, they occurred in the two horns about equally.

Although more cases of natural scrapie were affected than were controls and experimentals, even in the most severe of the three natural cases mentioned above, only 12 affected vessels were seen in 200 sections. This case was clinically advanced and many vacuoles were present. Mild perivascular cuffing by cells which were predominantly lymphocytes, of only 12 vessels in 200 sections was not comparable as an inflammatory reaction with the large number of vessels, surrounded by massive cuffs of histiocytes, which were seen in sections of the spinal cord in the cases of Louping ill.

Apart from perivascular accumulations of lymphocytes no other abnormalities of blood vessels were seen which were considered significant.

Thus diapedesis of red blood corpuscles into the Virchow-Robin space of the smaller arteries, or the shrinkage space round capillaries, was often seen in all groups. Sometimes quite extensive cuffs of corpuscles were present.

The vessels were not congested in the scrapie cases. Polymorphs were quite numerous in the lumen in a few animals of both groups. Eosinophilic amorphous material was sometimes observed lying close to the capillaries in all groups, but was rather prominent in a few cases of scrapie. It appeared to be serum which had diffused outside the vessels.

In the older animals of all groups the paradventitial tissue, particularly around the commissural arteries, was quite extensive. The endothelium, elastic tissue, and muscular coats were not observed to be abnormal in any of the scrapie spinal cords examined.

Large globules of material which stained orange-red with Sudan IV and red with Gomori's aldehyde fuchsin, and were P.A.S. positive, were seen in the adventitial tissue close to blood vessels in older sheep from all groups. This material was probably lipofuscin. Sometimes smaller globules were seen in the cytoplasm of cells lying close to the blood vessels. In four cases of scrapie a small number of cells containing definite droplets of neutral fat in their cytoplasm was seen close to blood vessels in the grey matter. Oligodendroglial perivascular satellites were not increased in the scrapie cases.

The ependyma and the central canal. The elongated ependymal cells were arranged in a fairly regular palisade round the central canal. Phosphotungstic acid haematoxylin staining of the routine formol-corrosive sublimate fixed sections revealed blepharoplasts near the ciliated border. Glial fibres were present on the pointed side of the cell furthest away from the canal. The oval or elongated nuclei, which contained several dark staining dots of chromatin, were not particularly regularly arranged - viewed in transverse section they appeared as two or three irregular layers of nuclei lying in that half of the cell furthest from the central canal.

An occasional nucleus was close to the ciliated border. There was some variation in the number of nuclei seen in transverse sections in different individuals.

Sudan IV staining of frozen sections from older sheep sometimes showed globules of faint orange-red staining material in one or two ependymal cells. Usually only one globule was present in the cytoplasm of each cell and this was about the same size as the nucleus. This material resembled lipofuscin.

In one control sheep a small ependymal lined cavity lay dorsal to the central canal.

No difference was observed between the ependyma of scrapie or control sheep.

In both scrapie and control sheep, the central canal often contained an eosinophilic network in which was pink granular material and occasionally one or two red circular masses. This material was almost certainly coagulated cerebro-spinal fluid. Occasionally one or two lymphocytes or macrophages were present in the canal in both groups.

The meninges. Small subpial infiltrations of leucocytes, involving only a limited region of the meningeal circumference, were seen in one advanced and three non-advanced cases of natural scrapie. No meningeal lesions were seen in the experimental disease.

The white matter. Examination of the spinal white matter by the Sudan IV, Spielmeyer and Bielschowsky-Glees methods revealed degenerative changes in certain of the scrapie cases and in some controls. These animals could be divided into three groups on the basis of the extent of the changes.

In the first group degeneration of the neural tubes was so extensive that it was easily distinguished in transverse sections stained by myelin or fat stains (Fig. 45). This was seen in only two out of 43 natural cases examined in transverse section, and was present in none of the 12 experimental or 17 healthy control sheep. In these 2 cases the degeneration of myelin and axis cylinders occurred in the medial region of the ventral funiculus and to a lesser extent in the lateral

funiculus, and in one of the cases it also occurred in the dorsal funiculus of the upper cervical region. The changes in the former two funiculi extended from the cervical to the lower lumbar region.

In longitudinal section the degeneration was seen as chains of oval cavities (the digestion chambers) containing spheroidal masses (the myelin ovoids)(Fig. 46), which were the remains of the neural tube, together with one or more nuclei. Myelophages laden with neutral fat lay both in and outside the digestion chambers. Two types of cell were present on the wall of these digestion chambers; one had a rather large oval nucleus resembling an astrocyte nucleus, while the other had a small elongated and rather dark staining nucleus resembling a microglia nucleus. These nuclei were curved round the wall of the digestion chamber as if their cytoplasm actually formed the wall. Sometimes droplets of neutral fat were present in their cytoplasm. The nuclei of the myelophages in the digestion chambers were usually small, round and dark staining and their cytoplasm was filled with droplets of fat. Some of the nuclei were karyorrhectic and pyknotic. Cells containing droplets of fat were also present outside the digestion chambers. In paraffin embedded sections clear vacuoles were present in the cytoplasm of all these fat containing cells, so that they were typical Gitter cells (Fig.47).

Breakdown of myelin to neutral fat was not so far advanced in all fibres; in some, the greater part of the myelin ovoids were rather more orange to red coloured than normal myelin but only a few droplets of neutral fat and one or two small round nuclei of myeloclasts were present in the mass (Fig. 48). In others, ovoids were present but the colour of the mass was either the same as normal myelin or slightly more orange to red. The small nuclei of myeloclasts were only occasionally present.

Bielschowsky preparations of longitudinal sections showed the axis cylinders to be considerably swollen and covered with argentophilic granules, or beaded and vacuolated, or broken into fragments (Figs. 49, 50, 51, 52).

In some places there was an obvious loss of axons due to their complete destruction, but healthy fibres could be seen amongst the degenerated ones in the affected areas.

It was not always easy to distinguish axonal fragments in the myelin ovoids, as the whole sphere took on a deposition of argentophilic granules. Some of these granules may have been axon remains, while others may have been due to an increased argentophilia of the myeloclasts' cytoplasm.

In the second group the type of lesion and their distribution in the ventral funiculi was similar to the preceding two cases, but very seldom were more than a few fibres in each longitudinal section affected. Neutral fats could be demonstrated in all these cases. This degree of involvement was seen in six out of seventeen natural scrapie cases in which longitudinal sections were examined and in one control sheep.

In the final group occasional chains of myelin ovoids of an orange-red colour in contrast to the brick red of normal myelin, were seen in longitudinal sections. In some of the ovoids, haematoxylin staining showed myeloclasts without fat droplets, but in no case could neutral fat droplets be demonstrated. Although these lesions were as a whole rather rare, the general impression was that chains of myelin ovoids were more frequent, particularly in the ventral funiculus in the scrapie cases than in the controls. Degeneration of this extent was seen in six natural cases of scrapie and in six control sheep out of the seventeen scrapies and seventeen controls examined in longitudinal section.

Significant changes could not be detected in the white matter in the twelve cases of experimental scrapie, the remaining three cases of natural scrapie or in the ten control sheep.

Occasional rather large, empty, oval or round holes were seen between the nerve fibres in the white matter in both paraffin and frozen sections of the majority of cords from both scrapie and control sheep. In longitudinal sections only one hole was usually present, although sometimes a short chain of two or three was seen. These holes, which never contained

myelin ovoids, neutral fat, or gitter cells, should not be mistaken for digestion chambers.

Section II.

THE INCIDENCE AND DISTRIBUTION OF VACUOLATED NEURONES.

The distribution of the neurones and the angio-architecture in the normal spinal cord.

When counting vacuoles the difficulties associated with identifying specific nuclei were overcome by dividing the grey matter into three zones. In order to determine the position of the nuclei and the main characteristics of their neurones in each zone, and any vascular peculiarities of the zones, thick sections from normal cords as well as 7 micron paraffin sections were examined.

Examination of the 100 micron thick frozen sections stained with cresyl fast violet (Figs. 53, 54) showed that the three zones contained the following nuclei:-

Zone A.

- Nucleus proprius cornu posterioris.
- Nucleus reticularis spinalis.
- Nucleus cornucommissuralis posterioris.
- Cellulae disseminatae posteriores.
- Clarke-Stilling's column. (Nucleus dorsalis).
- Nucleus cornucommissuralis anteriores.
- Nucleus intermedio medialis.
- Cellulae disseminatae intermedio.
- Cellulae disseminatae anteriores.
- Cellulae subst. grisea centralis.

Zone B.

Medial aspect.

- Nucleus motorius medialis ventralis.
- Nucleus motorius medialis dorsalis.

Lateral aspect.

Nucleus motorius lateralis ventralis.

Nucleus motorius lateralis intermedius.

Nucleus motorius lateralis dorsalis.

Zone C.

Nucleus intermediolateralis.

The size of the nuclei varied in different segments of the cord, and some of them did not occur in all segments.

Of the 10 segments examined in this study, Zone C was present only in T₆ and L₃, and consisted, as seen in a single transverse paraffin section, of rarely more than 6 medium sized bipolar cells in the lateral horn. In transverse section the cells were round; in longitudinal section oval. In about two thirds of them there was a zone devoid of Nissl close round the nucleus.

The motor nuclei of Zone B were very well developed in the intumescencia, particularly in C₇, C₈ and L₆, where about 50 to 60 large polygonal cells could be seen in a single transverse paraffin section. About three quarters of them lay in the lateral groups. In T₆ and L₃ only about 6 to 10 motor nuclei could be seen and they were not divisible into lateral and medial groups. Only small numbers were present in C₄.

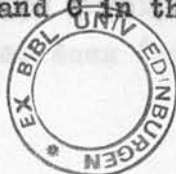
In Zone A the small and medium sized polygonal and spindle shaped cells of the zona intermedia were common. They were present in all segments of the cord, and were particularly numerous in the intumescencia. Sometimes large polygonal cells were seen in this zone. The cells of the zone could not, even in the thick frozen sections, be easily divided into cellulae disseminatae posteriores, intermedio, and anteriores, though they may be classed in these groups by their position in relation to the grosser anatomy of the grey matter. They extended from below the substantia gelatinosa Rolandi down to the motor neurones, and in the intumescencia were present between the lateral and medial motor groups. The nuclei cornucommissuralis consisted of small and medium sized cells, four or five being present in each nuclei in all

sections. In the intumescentia larger cells may occur in the anterior commissural nucleus.

The cells of the nucleus proprius cornu posterioris were medium to large sized and polygonal and lay in the head and neck of the dorsal horn. Most occurred in the intumescentia, but even here, only two and three per section were seen. The cells of the nucleus reticularis spinalis were oval and most common in the thoracic and upper lumbar region, where they lay just dorsal to the lateral horn on the lateral cervix of the dorsal horn. A small group of large polygonal cells was sometimes seen in T_6 and L_3 where the grey commissures join the grey matter, the nucleus intermedio medialis, and in all regions one or two oval cells lay in the grey matter near the central canal, the cellulae grisea centralis. Clarke-Stilling's column probably occurred throughout the thoracic and lumbar regions, but as only one or two cells were seen in most sections it was difficult to differentiate it, except in L_3 where five or six medium to large round cells were usually seen in each transverse section. In the sheep, there was no marked perinuclear area devoid of, or with only very fine, Nissl substance in the cells of Clarke's column.

In addition to the neurones of the three zones, the cellulae postero-marginales were seen round the dorsal and lateral edge of the dorsal horn of all segments. These were either rather prominent large spindle shaped cells, of which not more than two or three were seen in each section, or medium sized oval cells, with a clear nucleus and not much Nissl substance. The cells of the substantia gelatinosa Rolandi were very small and round or oval without much Nissl substance. Although insignificant looking cells they were very numerous in all sections.

With the Lepehne-Pickworth method of staining blood vessels, the capillary bed appeared to be equally rich in all areas of the grey matter. However, arteries and precapillaries were noticeably more numerous in the centre of each grey column, in the region of zone A and C in the present study, than in the



motor areas of the ventral horns or in the dorsal horns (Figs. 55, 56). The vessels forming this arterial net seemed to derive from the commissural arteries and the dorso-lateral and median-lateral arteries. The capillary supply of the ventral motor areas presumably derived from the commissural and median-lateral arteries after these had passed through zones A and C, and to a lesser extent from the relatively inconspicuous ventro-lateral arteries.

The incidence of vacuolated neurones.

The following information on the incidence and significance of vacuolated neurones was obtained by counting the number of vacuoles seen in serial sections of the spinal cord.

1. Vacuoles were found in the spinal cords of more cases of scrapie than of the controls. Thus they were seen in 97 out of 98 scrapies (experimental and natural) which were examined but in only 5 out of 34 clinically healthy animals.
2. Vacuolation was a constant occurrence in natural cases of scrapie. It was seen in 97% of experimental scrapie.
3. The number of vacuoles observed was much greater in the scrapie animals than in the controls. When 200 sections were examined from each of 20 natural cases of scrapie (Tables XII and XIII), a total of 18,145 vacuoles were counted giving an average of 4.5 vacuoles per section. When 200 sections were examined from each of 20 experimental cases of scrapie (Table XIV), a total of 413 vacuoles, giving an average of 0.103 vacuoles per section were seen. However, when 200 sections from 15 healthy control sheep were examined only 5 vacuoles were seen giving an average of 0.0017 vacuoles per section. When 50 sections were examined from 50 natural cases of scrapie (Table XVIII), 7,517 (average per section = 3) were seen. When 50 sections were examined from 19 healthy controls, only one vacuole was seen (average per section = 0.001).

4. Vacuoles were more numerous in advanced than in non-advanced cases of natural scrapie. Thus when 200 sections were examined from each of 10 advanced cases (Table XII), a total of 14,336 vacuoles were counted, giving an average of 7.2 vacuoles per section (range 0.8 to 34.6). When 200 sections were examined from each of 10 cases of non-advanced scrapie (Table XIII), a total of 3,809 vacuoles were counted, giving an average of 1.9 vacuoles per section (range 0.1 to 13).
5. Only small numbers of vacuoles were seen in the spinal cords in experimental scrapie (Table XIV). When 200 sections were examined from 20 cases a total of 413 vacuoles were counted giving an average of 0.103 per section (range 0 to 0.35).

The distribution of vacuolated neurones.

At different levels of the spinal cord.

1. The majority of vacuoles in all groups of scrapie occurred in the intumescentia. There was no evidence that the lumbo-sacral intumescentia was more or less severely affected than the cervical intumescentia.
2. Counts of neurones at different segments of normal cords showed that where vacuoles were numerous, as in the intumescentia, large numbers of neurones were normally present. Where vacuoles were few, as in the mid-thoracic region and upper cervical region, there was normally a small neurone population.
3. There was no evidence that in early or advanced cases of scrapie one level of the cord was more severely affected than any other.

In different zones of the grey matter.

1. From 89% to 92% of vacuoles occurred in Zone A in all groups of scrapie:

Natural advanced scrapie (200 sections from 10 cases)	91.3%
Natural non-advanced scrapie (200 " " 10 ")	92.4%
Natural scrapie (50 sections from 50 cases)	89.4%
Experimental scrapie (200 sections from 20 cases)	89.2%

2. Not more than 7.8% of vacuolated neurones occurred in Zone B, which contains the motor neurones.

Natural advanced scrapie	(200 sections from 10 cases)	7.8%
Natural non-advanced scrapie	(200 " " 10 ")	4.2%
Natural scrapie	(50 " " 50 ")	6.1%
Experimental scrapie	(200 " " 20 ")	4.8%

3. Slightly more vacuolated neurones occurred in the medial than the lateral motor groups in both advanced (65%) and non-advanced (59%) natural scrapie. This was although there are three to four times as many neurones in the lateral motor groups. Also the lateral groups are distinctly demarcated, whereas it is not easy to be sure whether all the medial group are in fact motor neurones or some are large members of nucleus cornucommissuralis anteriores or part of the cellulæ disseminatæ anteriores.
4. Zone C, although only a relatively small group of neurones restricted to the thoracic-lumbar region, contained a large percentage of vacuoles. When 200 sections per case were counted the proportion of all vacuoles in the entire cord in 10 advanced cases of scrapie was 0.9%, in 10 non-advanced cases 3.4%, and in 20 experimental cases 6.0%. When 50 sections were counted from 50 cases of natural scrapie, 4.4% of all vacuoles occurred here.

In the 10 cases of advanced scrapie the vacuoles in Zone C comprised 9.8% of all the vacuoles in T₆ and L₃, in the non-advanced cases 29%, and in experimental scrapie 49%.

5. Zone C was affected by vacuolation in 43 out of 50 cases of natural scrapie (86%) and degenerative changes without vacuolation were seen in a further 6 cases. Therefore, in all, 49 out of 50 cases (98%) showed either vacuolation or degeneration in Zone C. In the experimental cases vacuoles were present in Zone C in only 10 out of 20 cases, but other degenerative changes were seen in 80% of the 20 cases.

All distinguishable nuclei in Zone A appeared to be

affected in some degree but certain groups of cells were affected repeatedly (Figs. 57 to 61). These were, in order of frequency:-

1. The nucleus cornucommissuralis anteriores.
2. The zona intermedio, particularly the cellulae disseminatae intermedio, and the cellulae disseminatae posteriores and last the cellulae disseminatae anteriores.
3. The cornucommissuralis posteriores.
4. The cellulae substantiae grisea centralis.
5. The nucleus proprius cornucommissuralis.
6. The nucleus intermedio-medialis.

The other nuclei of Zone A were also affected occasionally.

In Zone B, as noted above, there were slightly more vacuoles in the n. motor medialis than in the n. motor lateralis. If the lateral group was affected it was much more likely to be the n. motor lateralis intermedius, particularly in the lumbosacral intumescencia, or the n. motor dorsalis, than the other groups.

Zone C was the nucleus intermedio-lateral. As noticed previously this important nucleus is of small extent, so that a relatively large proportion of its neurones were degenerated or vacuolated (Figs. 62, 63, 64).

An occasional vacuole was seen in the cellulae postero-marginales and the cells of the substantia gelatinosa Rolandi (Gierke's cells) of most cases of natural scrapie. These vacuolated neurones were not included in the above counts. As in the case of the other degenerative changes in neurones, vacuolation in all zones was often bilaterally symmetrical.

The numbers of vacuoles counted in zones A, B and C in each case of natural and experimental scrapie are shown in Tables XV to XIX inclusive. The examination, without vacuole counts, of a further 18 cases of natural and 10 of experimental scrapie, entirely confirmed the above findings on the histological changes and the incidence and distribution of vacuoles in the spinal cords. Over the whole series

vacuolation was constantly present in all 68 cases of natural scrapie. The majority of the degenerated and vacuolated neurones were in Zone A, they were very rare in Zone B, but were very prominent in 97% of cases in the small but important intermediolateral nucleus of Zone C. Vacuolated neurones were seen in 96.7% of the 30 experimental cases, and, although they had essentially the same distributions as in the natural cases, they were much less frequent. Vacuoles were seen in the intermediolateral column of 46.6% of the 30 experimental cases, and degenerative changes of varying degree in 83.3% of the cases.

Microscopic observations.

The sensory ganglia of the sheep consist of aggregated neurones enclosed in a connective tissue capsule. Each neurone is surrounded by a capsule consisting of a layer of satellite cells, or neuroglia. Surrounding and lying between the neurones is a fine interstitial connective tissue containing with the capsule of the ganglion, and containing the intraganglionic blood vessels and lymph spaces. The axons of the nerve cells are collected into bundles which pass to the periphery of the nerve as afferent and efferent fibres respectively.

The neurones.

The round, unipolar neurones of the ganglia were of varying size, and are usually classified in anatomical descriptions as large, medium and small. In the spinal ganglia the average maximum and minimum diameter of 50 large neurones from 3 control sheep was 65.4×31.7 microns. The average diameter of 50 of the smallest neurones seen was 19.5×17.25

THE PERIPHERAL NERVOUS SYSTEM.

Section I.

THE SPINAL SENSORY GANGLIA AND THE GASSERIAN GANGLIA.

Macroscopic observations.

The spinal ganglia are grey nodular enlargements on the dorsal root fibres of the spinal nerves. The largest ganglia occur at the intumescencia of the cord, and the smallest at the upper cervical and mid-thoracic region. The Gasserian ganglia were more fibrous and rather pinker in colour than the spinal ganglia. There was no naked eye difference between the spinal ganglia, the nerve roots, and the Gasserian ganglia of the scrapie and the control groups of sheep.

Microscopic observations.

The sensory ganglia of the sheep consist of aggregates of neurones enclosed in a connective tissue capsule. Each neurone is surrounded by a capsule consisting of a layer of satellite cells, or amphicytes. Surrounding and lying between the neurone capsules is a fine interstitial connective tissue continuous with the capsule of the ganglia, and containing the intraganglionic blood vessels and lymph spaces. The axons of the nerve cells are collected into bundles which pass to the periphery or the cord as afferent and efferent fibres respectively.

The neurones.

The round, unipolar neurones of the ganglia were of varying sizes, and are usually classified in anatomical descriptions as large, medium and small. In the spinal ganglia the average maximum and minimum diameter of 50 large neurones from 5 control sheep was 66.6 x 51.9 microns. The average diameter of 50 of the smallest neurones seen was 19.5 x 17.25

microns, and of 50 medium sized neurones was 42.3 x 34.5 microns. However, there were many intermediate sizes between small and large. The sizes of the neurones in the scrapie cases were not obviously different from those seen in the control sheep. In all groups of sheep the large cells stained rather palely in haematoxylin and eosin preparations compared to the medium and smaller cells. The Nissl substance was usually in fine grains evenly distributed throughout the cytoplasm, although in an occasional cell it was arranged in coarse whorles, streaks, or commas round the nucleus. The nucleus was rather large, round and centrally situated, and its size was greater in relation to the size of the cytoplasm in the smaller as compared to the larger cells. The nucleus contained a single, large, centrally situated nucleolus and some fine chromatin granules. Occasionally an accessory body was also present. Very occasionally two nucleoli were seen in one nucleus. The nucleolus sometimes contained one or two small vacuoles. The neurofibrils as seen in sections prepared by the Holmes' method formed a distinct reticulum, which was rather condensed round the nucleus (Fig. 69).

Vacuolation. Vacuoles were occasionally seen in the cytoplasm of the neurones in natural scrapie, experimental scrapie and control sheep. These vacuolated cells lay both near the surface and deep in the ganglion. Adjacent neurones were morphologically unaltered. The vacuolated cells were not chromatolytic or degenerated in any way; apart from the presence of the vacuole, cytoplasm, Nissl substance and nucleus were quite normal (Figs. 65, 66). The neurofibrillary network was normal although rather condensed close around the vacuole. The vacuoles not infrequently contained eosinophilic material (Fig. 65), usually in the form of a pink reticulated fragment, but sometimes as fine granules or a large faintly eosinophilic amorphous mass. Distinct eosinophilic spheroids, like those seen in the vacuoles of neurones in the spinal cord in scrapie sheep, were not observed. Apart from this material, no specific contents could be identified in the vacuoles and the same

histochemical and staining methods as used for vacuoles in the spinal cord were all negative. They included methods for fat (Fig. 67) and glycogen, the P.A.S. reaction, the Feulgen reaction, the methyl green-pyronin Y method, and the method for chrome alum-haematoxylin positive neurosecretory material.

The vacuoles were not more common in either natural or experimental cases of scrapie than in the control sheep.

When the numbers of vacuoles in spinal ganglia were counted in 48 sections from each of 10 controls, 10 experimentals, and 30 natural cases of scrapie (Tables XX, XXI, XXII), vacuoles were observed in 90% of controls, 70% of experimentals, and 83.3% of natural cases. The average number of vacuoles seen in each case (i.e. in 48 sections), was 6.2 (range 0 to 15) in the controls; 5.6 (range 0 to 19) in the experimentals and 6 (range 0 to 18) in the naturally occurring cases.

The majority of vacuoles were seen in the ganglia of the intumescencia (80.7% in controls; 85.7% in experimentals; and 73.3% in naturals) and most were unilocular (56% in controls; 89% in experimentals; and 80% in naturals), although multiple vacuoles, particularly two vacuoles in one cell, and multilocular vacuoles were also seen in all groups of sheep. Vacuolation of the cell processes was never seen. It was difficult to say with certainty which group of cells was most affected by vacuolation because the vacuoles distorted them but taking into account the size of the nucleus and the cell capsule, most vacuoles occurred in the large size of neurone, and they were rare in the small size. Vacuoles were of varying sizes, but were often large; one very large unilocular vacuole seen in a control animal measured 138 x 99 microns.

Vacuoles were not counted in the Gasserian ganglia. They were common in the control sheep, in one of which they were very numerous (Fig. 68), and they were certainly not more frequent in the scrapie sheep. The vacuoles and their contents were similar to those in spinal ganglia.

Chromatolysis and necrosis. Apart from vacuolation, variations from the usual appearance of the neurones were extremely rare in all groups of animals.

Chromatolysis was very uncommon, and the occasional cells seen occurred both in control and scrapie sheep. There was marked eccentricity of the nucleus and both central and diffuse chromatolysis were seen. Occasional cells showing eosinophilic necrosis were seen in both groups. Occasional spheroidal hyaline, eosinophilic masses, measuring up to about 20 microns in diameter, were sometimes seen lying in the cell capsule, often in a concavity in the neurone's cytoplasm. They were not more frequent in the scrapie sheep and were uncommon in all groups of sheep.

Atypical neurones. The Cajal silver impregnation method showed that the unipolar type of neurone was the commonest in all groups of sheep. The glomerulus had usually not more than one loop in the large cells. Atypical neurones (Figs. 70, 71, 72) were very uncommon, although fenestrated cells with ansiform and dendriform loops, truncated processes, knobbed processes (end bulbs), and neurones surrounded by pericellular skeins were observed. A rare neurotised residual nodule was seen. No difference was observed in the occurrence of these phenomena in scrapie and control sheep. It should be emphasised that these atypical occurrences were very uncommon; all the abnormal types of neurones together certainly constituted less than 1% of the total neurones seen.

Intracytoplasmic bodies. Two types of intracytoplasmic inclusions were seen in the neurones (Fig. 73) and these corresponded to those seen in the neurones of the spinal cord.

Lipofuscin pigment was very common, particularly in older sheep, where it occurred as one or two large aggregates of granules at the periphery of the cell or, probably less often, close to the nucleus. Although pigment was present in larger amounts than in the spinal neurones of the same case, it never distorted the surface of the neurone sufficiently to form a "pigment sac". The individual granules often seemed of

larger size than in the spinal neurone and they gave a brighter acid fast reaction. Otherwise the staining properties and histochemical reaction of the granules at the two sites were identical. No difference was observed in the occurrence of these intracytoplasmic granules in the control, natural or experimental scrapie groups.

So called "black" pigment was not seen in the spinal or Gasserian ganglionic neurones of these sheep. However, extra capsular pigment was observed lying near blood vessels and between the nerve fibres of one scrapie and one control sheep and this was darkish brown in colour, although it had the same staining reactions, including being acid fast, as the intracytoplasmic lipofuscin.

Phloxinophilic bodies with similar staining properties and histochemical reactions to those in the spinal neurones were also observed. They were more frequent in the ganglia than in the cord, and both large and other sizes of inclusions were seen in all three groups. No attempt was made to determine the incidence by counting these inclusions as in the cord, for as far as could be seen, there was no indication of any difference in their occurrence in any group of sheep.

The nucleolus sometimes lay outside the nucleus, and often a track was seen where it had passed out of the nucleus and through the cytoplasm (Fig. 73). As the rest of the cell appeared quite normal, and the occurrence was seen in both scrapie and control sheep, this was not thought to be a pathological reaction, but to indicate that the nucleolus was more resistant to the microtome knife, and had been mechanically pushed out of its normal position.

The cell capsule.

The amphicytes had oval nuclei which appeared circular on transverse section, and they contained moderate amounts of chromatin. The number in each capsule seemed to be related to the size of the neurone. Thus when the number of amphicytes

visible in a single transverse section of 30 neurones from each of 10 control sheep were counted, the large neurones were surrounded by, on the average, 14 satellites (range 6 to 30), the medium sized neurones by an average of 7 (range 3 to 14), and the small neurones by an average of 3 satellites (range 1 to 6). In an exceptional case in a control animal, one large but morphologically normal neurone was surrounded by 48 amphicytes.

Sometimes a capsule containing no visible neurone but an accumulation of amphicytes was seen. This so called residual nodule could be followed in serial section, whereas when a plain of section cut through the top of a cell capsule so that the superficial layer of satellites resembled the residual nodule, the next section in the series usually showed the parent neurone. An isolated neurone was sometimes phagocytosed by small rather irregular shaped cells (Fig. 74), and sometimes an obvious proliferation of amphicytes was seen in an isolated capsule. These phenomena - residual nodules, neuronophagia, and proliferation of amphicytes, were, however, extremely rare, and there was not the slightest indication that they were more common in either of the scrapie groups than in the control group of sheep.

The Ganglion capsule, the interstitial connective tissue, and the blood vessels.

The capsule of the ganglia and the interstitial connective tissue surrounding the neurones consisted of collagenous and reticulin fibres which were continuous with the endoneurium of the nerves. In older sheep the connective tissue surrounded the larger intraganglionic blood vessels in a relatively thick layer. The connective tissue component of the spinal ganglia was not different in scrapie and control sheep.

No constant abnormality was observed in the blood vessels of the spinal or Gasserian ganglia. In one scrapie animal two vessels lying external, but close to the spinal ganglionic capsule, showed a periarteritis with recanalisation of the organised thrombus present in one.

The nerve fibres of the ganglia and the radices.

Sudan IV and Holmes' preparations did not reveal degeneration of myelin or axis cylinders of nerve fibres in the ganglia or their attached roots. However, when the roots were cut prior to fixation the axons near the incision frequently formed swellings, rings, and corkscrew appearances which simulate the forms of axon degeneration often described in the literature (Figs. 75, 76). In the present cases they were artefacts. The nerve fibres of the spinal roots and sympathetic ganglionic trunks seemed much more susceptible to artefacts due to post mortem interferences than were the fibres in the spinal cord.

Section II.

THE SYMPATHETIC GANGLIA.

Macroscopic observations.

There were no macroscopic changes in the sympathetic ganglia or trunks of the natural or experimental scrapie group of sheep.

Microscopic observations.

The ganglia of the sympathetic nervous system consist of aggregates of multipolar neurones, each neurone being enclosed in a cellular capsule. As in the spinal ganglia, the whole ganglion is surrounded by a connective tissue capsule, fine branches of which ramify between the cellular capsules of the neurones. In this interstitial connective tissue are the intraganglionic blood vessels and lymph spaces. There are many nerve fibres in the ganglia, both in large bundles and as a network between the cell capsules. These are afferent pre-ganglionic fibres derived from the neurones of the intermediolateral column of the spinal cord which enter the ganglia via

the white rami communicantes, and efferent fibres derived from the neurones of the ganglia and running via the grey rami communicantes to the periphery.

The neurones.

The neurones of the anterior cervical, stellate, thoracic trunk, coeliaco-mesenteric, and posterior mesenteric ganglia of the sheep were usually round in outline in haematoxylin and eosin preparations of paraffin sections. Occasional cells were oval and rather elongated. Considerable variations in size of the cell diameter from about 10 x 10 microns up to 32 x 40 microns were observed.

Chromatolysis. The Nissl substance was present in the cell either as granules uniformly dispersed throughout the cytoplasm, or as a condensed peripheral zone of larger Nissl bodies with fine granules elsewhere in the cytoplasm. There were also intermediate forms in which the granules were uniformly dispersed as in the former group, but larger Nissl bodies occurred at the cell margin. Cells showing the former diffuse distribution of Nissl bodies were much more common than those showing the peripheral, or "ring", distribution. However, the latter represented a distinct normal occurrence and was seen with all types of fixative and in both control and scrapie sheep. "Ring" types of Nissl arrangement were more frequent and more pronounced in the older sheep, where the central Nissl was like very fine dust, so that it was important to differentiate it from central chromatolysis. The following changes were also seen in chromatolysis and helped to differentiate the pathological from the normal state:-

1. The central area of the cytoplasm was generally quite free of Nissl granules, and had a hyalinated appearance staining eosinophilically with haematoxylin and eosin, and like ground glass with Einarson's galloxyanin.
2. There was sometimes an accumulation of Nissl on the nuclear membrane ("nuclear caps").

3. The nucleus was very eccentric, and was sometimes even partly extruded from the cell.
4. The cell body was swollen.

Rare chromatolytic cells (Fig. 77) were seen in both the control and scrapie groups of sheep. They were observed in 53% of the control sheep; in 38% of the natural scrapie sheep; and in 44% of the experimental scrapie sheep, but usually not more than one or two affected cells were present in the 48 sections in which vacuoles were counted. However, in one case of natural scrapie (S26) relatively large numbers of chromatolytic cells were seen in the ganglia of the thoracic trunk. There were never less than two in every section and in several sections as many as six chromatolytic cells were seen. Although this is comparatively more abnormal cells than in the controls, it was noted that each transverse section of the ganglia contained approximately 1,200 neurones, so that the proportion of diseased neurones was really very small.

Apart from this case of scrapie, chromatolysis was not more frequent in scrapie than control sheep.

Necrosis. As well as chromatolysis, a few necrotic neurones were seen (Figs. 78, 79). They were observed in 31% of the natural scrapie cases and 40% of the controls, but only one or two were seen in 48 sections from each animal. In these cells the cytoplasm stained acidophilically with haematoxylin and eosin, the perikaryon had shrunk away from the cell capsule and lost its rounded outline, and the nucleus was eccentric, shrunken, and dark staining. The nucleolus was usually darkly stained and still visible. Sometimes small vacuoles were seen in the cytoplasm of the cell. Necrotic neurones were not more common in natural and experimental scrapie sheep than in the controls.

Neurofibrils were well demonstrated by the Holmes' method after both formol-sublimate and alcoholic fixation (Fig. 80). As in the spinal ganglia, they formed a more

prominent reticulum than in the neurones of the cord. They were rather compact round the nucleus. They were not abnormal in any group of sheep, except where they were fragmented in the rare necrotic neurones which were seen in both scrapie and control sheep.

Eosinophilic hyaline masses. Single eosinophilic hyaline masses were sometimes seen lying in the capsule besides a neurone. These bodies were usually circular or oval in outline and were often partially enclosed by a concavity in the neurone's perikaryon. They were usually quite large in size, being up to about a third the size of the neurone concerned. They were seen in 26% of the healthy control group of sheep, 20% of the natural scrapie and in none of the experimental group of sheep. Only one or two of these hyaline masses were seen in each case. This material may have been the remains of a degenerated process or end bulb, or a form of secretion. Very large end bulbs sometimes lay partially in a concavity in the neurone's cytoplasm, both in sympathetic and sensory ganglia (Fig. 72), and in Haematoxylin and eosin preparations such structures may appear eosinophilic and hyaline. However, in some cases they seemed to be formed by degeneration and necrosis of a part only of the neurone's cytoplasm, while the remainder of the perikaryon containing the nucleus survived. This process could be followed in serial section (Fig. 81), and the impression was obtained that ultimately the necrotic piece of cytoplasm would be detached and remain as an eosinophilic hyaline mass alongside or in the resultant concavity in the neurone.

There was no difference in the occurrence of these hyaline masses in scrapie or control group of sheep.

Vacuolation. Vacuoles were seen in the cytoplasm of neurones in the sympathetic ganglia of scrapie and control sheep (Fig. 82), but less commonly than in the spinal ganglia. They were observed in 70% of the natural cases of scrapie; 50% of experimental cases, and 66% of control sheep. The average number of vacuoles per case (i.e. in 48 sections) was 2.1 (range 0 to 10) in the

naturals, 0.6 (range 0 to 2) in the experimentals, and 1.8 (range 0 to 7) in the controls. Most vacuoles occurred in the stellate ganglia (natural scrapie 52%, experimental scrapie 66.6%, and controls 44%) but vacuoles were seen in all ganglia. The majority of the vacuoles were unilocular, except two (3.2% of all vacuoles) seen in a natural case of scrapie, and two (7.5% of all vacuoles) seen in a control sheep which were multilocular. The two neurones containing these multilocular vacuoles in the natural case were chromatolytic, whereas all the other vacuolated neurones seen in the sympathetic ganglia, were, apart from the vacuole, quite normal in appearance. As in the spinal ganglia, the vacuoles often contained eosinophilic material, but neither fat nor other specific substance could be demonstrated.

These vacuoles were counted in the stellate, sympathetic trunk ganglia, and coeliaco-mesenteric ganglia (Tables XXIII, XXIV and XXV). They were not counted in the anterior cervical or posterior mesenteric ganglia for, although similar vacuoles were seen in these structures in both scrapie and control sheep, they were not obviously more numerous in either group.

Nucleus and nucleolus. The nucleus in the control sheep was large, round, and contained relatively little stainable material. The nuclear membrane was well stained. Only one nucleus was seen in each cell, and in about half the neurones this was eccentric. The nuclei of the small neurones were, compared to the difference in the size of the cell bodies, only slightly smaller than in the large neurones. Thus the average maximum and minimum diameters of 100 cells from alcohol fixed stellates of 10 sheep was 25 x 19.1 microns and the average maximum and minimum diameter of their nuclei was 11.9 x 10.4 microns. The largest cell seen measured 40 x 33 microns and its nucleus 13 x 10 microns, while the smallest cell measured 15 x 12 microns, and its nucleus 10 x 10 microns.

In all these respects the nuclei of scrapie and control sheep did not differ.

In the majority of cells the nucleolus was single and situated in the centre of the nucleus. However, in nearly all cases (93% of natural scrapie; 77% of experimentals, and 93%

of controls) two nucleoli of equal size were seen in a few cells. They were then situated roughly in each half of the nucleus. If only one nucleolus was present it was larger than when two were present, and it was spherical and stained basiphilically with haematoxylin and eosin. Sometimes a small vacuole was seen in the nucleolus. "Mulberry" forms were not observed. This appearance of the single or double nucleoli applied to the control sheep, the experimental, and all but three of the natural cases of scrapie, in which the nuclear contents were different from the others.

In one case (S6), an unusually large number of neurones in the coeliaco-mesenteric ganglia had double nucleoli, one of which was larger than the other and was surrounded by a clear halo (Fig. 83). Both were basiphilic after haematoxylin and eosin staining.

Intranuclear inclusions. In two cases the nucleus contained several bodies as well as the nucleolus (Fig. 84). In one case (S25), these were seen in only a very few cells of the stellate ganglion and not more than three bodies were present in a nucleus. In the other case (S20) up to five cells in each transverse section of both the thoracic trunk ganglia and the coeliaco-mesenteric ganglia contained as many as eight inclusions in each nucleus. These ganglia were sectioned completely from end to end, and affected nuclei occurred throughout the ganglia. The inclusions were nearly always intranuclear, but in a few cases they were also present in the cytoplasm. They were visible without staining, when they were faintly refractile. They were intensely basiphilic with haematoxylin and eosin, and were spherical and about a third to a half the size of the nucleolus, which was often seen to be present and of normal size and appearance. The affected cells appeared otherwise quite normal, and no other changes were seen in the ganglia of either case.

The intranuclear inclusions had the following staining and histochemical reactions:-

1. Unstained decerated sections - faintly refractile.
2. Routine haematoxylin and eosin - strongly basiphilic.

3. Heidenhain's iron haematoxylin - strongly basiphilic.
4. Lendrum's haematoxylin-phloxine-tartrazine - not phloxinophilic, but stained strongly with haematoxylin.
5. Picro-Mallory - stained pinkish-red while the nucleolus stained blue.
6. Giemsa - stained paler blue than the nucleolus.
7. Eriocyanin A-safranin O - stained faintly pink.
8. Einarson's gallocyenin-chromalum -
 - a. Negative at the recommended pH of 1.64. At this pH the nucleolus and Nissl substance were prominently stained.
 - b. By altering the pH with N. NaOH., they were very faintly stained at pH 1.8, while at pH 2 they stained with the same intensity as the nucleolus and the Nissl substance.
9. Silver methods - argentophilic with Holmes' method. With this method they stain much darker than the nucleolus, which was usually stained faintly red-brown.
10. Perl's reaction for ferric salts - negative.
11. Sudan IV - a. frozen sections - negative.
b. paraffin sections - negative.
12. Feulgen's reaction - a. with hydrolysis - negative.
b. without hydrolysis - negative.
13. Periodic-acid-Schiff reaction - negative.
14. Methyl green-pyronin method - the inclusions gave a faint positive reaction, staining the same pink to red colour as the Nissl and rather deeper pink than the nucleolus. All the available tissue had been fixed in formalin and post fixed in corrosive sublimate, and these two fixatives were always found to give inferior results with the methyl green-pyronin reaction compared to alcoholic fixation.
15. Gomori's chrome alum haematoxylin phloxine - stained pale blue. The nucleolus stained bright red.

Intracytoplasmic bodies. Intracytoplasmic bodies, which resembled in their staining and histochemical reactions those seen in the cord and spinal ganglia, were found without difference in sympathetic ganglia from scrapie and control sheep. Large and small phloxinophilic inclusions were seen in all groups of sheep and were not obviously different in the controls and scrapie sheep. Lipofuscin granules were numerous, especially in older sheep and were arranged in the cell in a manner very similar to those seen in spinal ganglia. Extracellular lipogenous pigment was also seen (Fig. 85). In addition in one case (S20), a few neurones contained intracytoplasmic bodies which appeared similar to those seen in the nuclei of the same ganglia, and which have been previously described in detail.

The cell capsule.

The amphicytes were small cells whose nuclei appeared round or oval in transverse section. They were generally less numerous than in the spinal ganglia, and were not proliferated or abnormal in appearance in the scrapie cases.

In both scrapie and control groups, some neurones were replaced by a collection of amphicytes forming residual nodules (Fig. 86). The occurrence was infrequent in all groups. Also very occasionally, in both scrapie and control sheep, neuronophagia of an isolated neurone was observed.

The ganglion capsule, the interstitial connective tissue, and the blood vessels.

No difference in these structures was seen in the scrapie as opposed to the control group of sheep. The amount of intraganglionic connective tissue particularly around the blood vessels was noticeably increased in the seven year old control sheep.

In two cases of natural scrapie and three control sheep

blood vessels outside the ganglia and closely related to the capsule were surrounded by circumscribed infiltrations of cells resembling lymphocytes. In one of the control sheep, a few polymorphs and eosinophils were also scattered more diffusely round the circumscribed vessel.

Intraganglionic diffuse and perivascular infiltrations of leukocytes were seen in a few sections from both scrapie and control sheep. When the infiltrations were perivascular and more compact, the majority of the cells resembled lymphocytes. In the case of the more diffuse infiltrations (Fig. 87), some cells were small and rather like lymphocytes, whereas others had oval, elongated, and rather twisted nuclei. Neither type of infiltration was common, nor did their presence seem to be related in any way to scrapie. They were seen in 31% of the natural scrapies, 11% of experimental scrapies, and 46% of control sheep.

Paraganglia-like structures.

Two aggregations of atypical cells measuring in transverse section 195 x 75 microns and 69 x 54 microns respectively were seen in the stellate ganglia of one control (C33), and one scrapie sheep (S25). In the former the cluster of cells lay close to the axial vessels, and was surrounded by a connective tissue capsule which blended with the adventitia of the vessels. From this capsule, fine but distinct strands of interstitial connective tissue ramified between the cells, and round a large capillary which ran longitudinally through the centre of the structure. The cells themselves had rather coarsely granular cytoplasm which was faintly basiphilic, and a small round nucleus containing a prominent central nucleolus. Moderate numbers of elongated nuclei were also present, and these may have been either endothelial cells or associated with the connective tissue framework. No cells were seen which resembled either smooth muscle cells or transitional stages of smooth muscle cells.

The cell cluster might have been paraganglia or other

structures. Unfortunately, both clusters were of small size, and the greater part of each was already serially sectioned and stained with routine haematoxylin and eosin. Only one unstained slide was available for histochemical study.

After post chroming this single slide for one hour in 3% potassium bichromate, it was stained by Lillie's modification of Schmorl's ferric ferricyanide reduction method. The cytoplasm of the cells in the structure failed to reduce the ferricyanide to give the characteristic greenish-blue colour. Adrenal medulla was used as a control.

The cover slips were removed from sections already stained by haematoxylin and eosin and they were decolorised and restained by Masson's trichrome using aldehyde fuchsin as an elastic tissue stain, by the periodic acid Schiff method, and by post chroming in 3% potassium bichromate for three hours to give the chromaffin reaction of Lison. The latter method was negative, and P.A.S. positive material was not seen in the cytoplasm of the cells. Masson's trichrome revealed fine strands of interstitial connective tissue, but elastic staining was indistinct. Because of the previous decolorisation procedures, all these three methods were considered inconclusive.

The nerve fibres of the ganglia and sympathetic trunks.

Large and small myelinated fibres were common in the sympathetic trunks and ganglia, but Sudan IV and Holmes' preparations did not reveal degeneration of myelin or axis cylinders in any case. Artefacts similar to those seen in the spinal ganglia were observed near the cut ends of the nerve fibres.

The neurones of the adrenal medulla.

Only the neurones of the adrenal medulla, which are usually taken to be sympathetic neurones, were included in the present study. Probably because only a small number of sections

were cut from each gland, these infrequent neurones were seen in the adrenals of only 19 out of 112 control sheep, 5 out of 57 natural scrapie cases, and none out of the 13 experimental sheep available. In one of the control sheep a hyaline eosinophilic mass similar to those reported in the sympathetic ganglia, was seen close to a neurone. In one natural scrapie sheep, a necrotic neurone was seen. There was no indication of any significant difference between the neurones of the control and scrapie sheep.

Despite the small number of cases in which neurones were seen however, the interesting observation was made that vacuolation of the neurones was present in apparently healthy animals. Vacuoles, usually unilocular, were seen in one natural case of scrapie (20% of those in which any neurones were seen) and 10 control sheep (52.6% of cases showing neurones). Furthermore, taking into account the fact that never more than a small number of neurones was present, it seemed possible for a large proportion of neurones to be vacuolated. Thus in one control animal nine neurones were seen of which three were vacuolated, and in another control eleven were vacuolated out of 46 neurones seen. In haematoxylin and eosin stained sections, the vacuoles sometimes contained material of a similar appearance to that seen in the sympathetic ganglia. In both scrapie and control sheep, the vacuolated neurones were otherwise morphologically unaltered.

DISCUSSION.

THE SPINAL CORD.

The neurones.

The work of previous investigators of the nervous system in scrapie disease has been reviewed in the introduction to this thesis. These earlier studies were mainly concerned with the brain, and although several observations have a bearing on the present study, the work of Zlotnik (1958¹, 1958²), is of particular interest because he first described the pathology of the neurone in detail. This worker showed that as well as vacuolation, neuronal changes such as chromatolysis, acute swelling, pyknosis, severe cell change, necrotic cells and ischaemic cell change occurred in the brain stem. These neuronal changes are essentially similar to those described in the spinal cord in the present study. The occurrence of a similar disease process in both the brain stem and cord provides, in contrast to the findings of those workers who considered changes in the central nervous system negligible or of little significance (Bosanquet, Daniel and Parry, 1957, Parry, 1957, and Delez, Gustaffson and Luttrell, 1958), further evidence of the involvement of the central nervous system in scrapie.

Four of the neuronal changes observed in the cord, chromatolysis, sclerosis, multinucleation, and vacuolisation, require further comment.

Chromatolysis. In the present study, central and diffuse chromatolysis were seen in the neurones of the spinal cord; central chromatolysis was far more common than diffuse. Zlotnik (1958¹) saw both central and peripheral chromatolysis in the brain stem, but he does not state which type of change was the most frequent.

In the spinal cord early central chromatolysis was followed by eccentricity of the nucleus, but peripheral Nissl substance was usually still present when more advanced forms of degeneration were beginning to affect the cell. In contrast

to this, progressive chromatolysis is said to show a primary central chromatolysis followed by uniform fractionation of Nissl particles throughout the cell and finally eccentricity of the nucleus (Campbell and Novick, 1946). This diffuse chromatolysis resembles that of human poliomyelitis, and was seen in the present study in the spinal cords of sheep affected with Louping ill and with Russian spring summer encephalitis. According to Bodian (1948), when central chromatolysis occurs in poliomyelitis it is a manifestation of the recovery of the neurone, and it has generally been considered that the reparative phase in the regression of chromatolysis begins by reconstitution of Nissl bodies near the cell and nuclear membrane (Bielschowsky, 1932).

In scrapie, however, while it may be that some of the cells showing central chromatolysis were recovering, the presence of necrotic and liquefied neurones and the relative infrequency of diffuse chromatolysis suggests that further degeneration of the chromatolytic neurones was probably in progress. It may be that diffuse chromatolysis, which is common in those neurotropic virus diseases mentioned earlier, represents the acute reaction of the neurones, while the central chromatolysis which predominated in scrapie, represented a chronic reaction in which Nissl substance was both slowly used up and gradually reconstituted, but with ultimately a fatal outcome. Such a theory would be compatible with the long drawn out course of the disease.

Sclerosis. It may be questioned whether the sclerotic neurones seen in the present study represented genuine pathological change, a normal occurrence, or an artefact, and, in fact, their pathological significance has been doubted in many diseases.

The morphological difference between certain forms of chromophile, pyknotic and sclerotic cells is very slight, possibly only one of degree, so that Spielmeier (1922) for instance, suggests the first may lead to the last. Nissl (1896) knew that "pyknomorphic" neurones occurred in normal brains but considered them an artefact. On the other hand Cowdry (1910),

who showed the chromophilia to be due to an increase in mitochondria, thought they were normal, and Cajal (1897), quoted by Greenfield (1958), and Einarson (1933) considered them a histological expression of inactivity. Dolley (1910) thought they were in an initial stage of fatigue. In the opinion of Miller (1949) they were in a state of increased activity, but Endstrom (1957) could not produce the condition by experimentally increased activity in the neurones of guinea pigs.

On the other hand, there is no doubt that they may be an artefact, due to cutting the tissue (Scharrer, 1938), to post mortem pressure on tissue (Weil, 1946), to fixatives (Wolf and Pappenheimer, 1942, Greenfield, 1958), and due to excessive or prolonged heating in the paraffin oven (Speilmeyer, 1922). In the present study, initial investigations of processing methods showed that this type of artefact could be produced by excessive time in the paraffin oven. Failure to take all these possible causes into account, has sometimes led to faulty conclusions (Harvey and Perryman, 1944).

In the present study however, there were several factors indicating that sclerotic neurones were a genuine pathological change in scrapie cords:-

1. Their comparative rarity in control tissues processed similarly to scrapie tissues.
2. Their occurrence in scrapie tissues processed by different methods.
3. Serial sections stained by different methods still showed them.
4. The presence of other pathological neuronal changes in the same scrapie cords.
5. Reports of the occurrence of pyknotic neurones in other parts of the C.N.S. in scrapie (Zlotnik, 1958¹).

Multinucleation. Binucleate cells were rare in both scrapie and control sheep. Zlotnik (1958¹) also noticed infrequent multinucleated neurones in the medulla cord junction. According to Bielschowsky (1932), they may be seen in normal human neurones

but in pathological conditions they are often to be met with. However, they were not significantly numerous in scrapie.

Vacuolation. The occurrence of small numbers of vacuolated neurones in the medullas of healthy sheep has been recorded by Holman and Pattison (1943), and Palmer (1957²), Zlotnik and Rennie (1957, 1958), drew attention to the occurrence of small numbers of vacuolated but otherwise normal neurones in the medullas of an appreciable proportion of healthy sheep of various breeds. In the healthy sheep of the present study, vacuoles were very rare in the spinal cords, but were much more numerous in the spinal, Gasserian, and sympathetic ganglia and in neurones in the adrenal glands. They have also been reported in other apparently healthy animals; the spinal ganglia of rabbits (Field, 1952), and the human cerebellum (Spielmeyer, 1922). The latter considered them physiological because the vacuoles were well demarcated and the cells otherwise normal.

No specific substance was found in vacuoles at the above sites. This is in contrast to the neurosecretory material present in certain vacuolated neurones of healthy invertebrates and vertebrates, including the dog (Jewell, 1953), and the domestic fowl (Wight, unpublished work; see fig. 90).

Vacuoles containing fat have been recorded in spinal ganglia in senile animals, (Truex and Zemer, 1942).

It thus seems that vacuoles may occur in the neurones of healthy animals, but that judged by the appearance of the Nissl substance and the rest of the cell, they are unlikely to be of pathological significance and may be physiological. The number of "normal" vacuoles in neurones varies according to the tissue - in the ganglia and in adrenal glands they were relatively numerous, small numbers were present in the medulla, while they were very rare in the spinal cord.

Autolytic changes and defective processing are known to produce vacuoles. Weil, (1946), says it is sometimes impossible to say whether vacuolation is due to post mortem changes in the absence of glial and mesenchymal reactions, but if the nucleus

is well preserved it suggests post mortem changes, while concomitant degeneration of the cell and its nucleus suggest disease in life. A frequent criticism of the significance of vacuolation in scrapie is the possibility that they may be artefacts (Gordon, 1957), so that the following observations in the present study were most important in disproving this and indicating that vacuolation is part of the disease process.

1. Many vacuolated neurones also showed cytoplasmic and nuclear degeneration.
2. Neuronophagia, although not conspicuous, was seen in necrotising vacuolated cells.
3. Neurones which were not vacuolated were undergoing other forms of degeneration.
4. Vacuoles were present in cords processed by several different methods.
5. Vacuoles were extremely rare in control cords processed by the same methods.

Finally similar findings to these have been reported in the medulla by Bertrand *et al.* (1937), Palmer (1957²) and Zlotnik (1958¹).

Pathological vacuolation of neurones does not only occur in scrapie. In the sheep it has been reported in Louping ill (Brownlee and Wilson, 1932), and occasionally in conditions other than scrapie (Holman and Pattison, 1943). In man and other animals it is recorded in the literature in many unrelated conditions, for example, in toxæmias due to infections (Grinker and Stone, 1928), after organic and inorganic toxins (Grandell and Weil, 1933, and Hassin, 1921), in inanition (Andrews, 1940), after death due to freezing (Benders, 1928), in inherited conditions such as the familial lipidoses or pseudo-lipidosis of calves (Whittem and Walker, 1957), in transsynaptic degeneration (Greenfield, 1958), in deficiency disease (McCrea and Tribe, 1956), and in infectious conditions (Spielmeyer, 1922). The occasional occurrence of ballooning vacuolation during degeneration of neurones has been known for some time (Alzheimer, 1904) although

in degenerating cells vacuoles are usually, as in severe cell change, small peripheral cavitations in the liquefying cytoplasm, so called "foamy" vacuolation. This latter vacuolar degeneration may occur in many kinds of cell, (Cameron, 1952). According to Hadlow (1959), the neuropathological changes in Kuru, a disease occurring amongst the natives of New Guinea, are remarkably similar to those in scrapie, and include vacuolated neurones of the kind characteristic of scrapie. In most of the above conditions however, vacuolation like that seen in scrapie is rare. In scrapie, the typical vacuoles are a prominent feature of cellular degeneration.

In the present study, vacuoles were classified into two broad groups; those in otherwise normal cells and those in abnormal cells. Zlotnik (1958¹), although suggesting that a definite classification was difficult, made a list of six general types of vacuolation which occurred in the brain stem. Apparently similar types were now observed in the spinal cord.

Intravacuolar material. As recorded above, no specific material could be demonstrated in the vacuole. Eosinophilic bodies inside the vacuolated neurones of the medullas of scrapie sheep were originally reported by Holman and Pattison (1943), but Palmer (1957¹) attached considerable importance to their presence in scrapie. He suggested that they might be inclusions or products of cellular degeneration. However, Zlotnik and Rennie (1957) and Zlotnik (1957¹) have reported similar intravacuolar material in the medullas of control animals. In the present study, while intravacuolar material was frequently found in scrapie cases, none was seen in the controls, but so few vacuoles were found in the cords of the latter that one cannot attach specific significance to the absence of contents. Intravacuolar material was often seen in the ganglia of healthy sheep, and although it was not often in spheroidal form, this may have been due to the different type of cell or even to the physical effect of the different surrounding tissues. Nucleic acid could not be demonstrated in the material in the vacuoles in scrapie cords, which indicates that the bodies are not themselves virus and,

in view of the above findings, one can only state that there is no evidence that the bodies in the vacuoles are either virus or specific to scrapie. They are probably merely products of cellular degeneration, although future methods may show that more specific material is also present.

Palmer (1957¹) apparently also considered this spheroidal intravacuolar material to be the same as eosinophilic bodies which he found in the cytoplasm of medulla neurones in scrapie. He thought (1957²) that the bodies first formed in the cytoplasm, and as vacuolation proceeded, they increased in size remaining in the vacuole. The material was therefore, thought to be associated with the process of vacuolation. Four points emerged from the present study of the spinal cord which contra-indicated the probability of such an association between these intracytoplasmic bodies and vacuolation:-

1. Intracytoplasmic bodies, but not intravacuolar spheroids, were strongly phloxinophilic by Lendrum's (1947) method, which suggests they are of a different nature.
2. Intracytoplasmic bodies were not more numerous in spinal cords showing severe vacuolation.
3. Intracytoplasmic bodies were commonest in the large motor neurones in which vacuoles were found to be relatively infrequent.
4. In the cord, very small patches of Nissl substance and neurofibril dissolution were seen in some neurones, and these small areas might have been the site of early vacuole formation. No preliminary eosinophilic bodies were seen in these small patches.

Breakdown of nucleoprotein in chromatolysis produces increased intracellular osmotic pressure with resultant turgidity and viscovity changes in the cell (Gersh and Bodian, 1943), and it is possible that in scrapie this results in onkosis, the altered ability of the cell to control fluid transport producing a vacuole. The few P.A.S. positive granules seen in an occasional vacuole were presumed to be cytoplasmic lipochrome which the encroaching vacuole had engulfed.

Intracytoplasmic granules.

Lipofuscin granules. The histochemical and staining reactions of the lipofuscin granules in the neurones of the spinal cord were similar in many respects to those reported for certain granules in human neurones. Thus they were insoluble in the common lipid solvents, they had an affinity for fat stains, stained with methyl green, and were acid fast (Wolf and Pappenheimer, 1954). They were periodic acid-Schiff positive and stained with aldehyde fuchsin, Sudan black (D'Angello, Isidorides, and Shanklin, 1955), and faintly with Sudan IV, but not with haematoxylin and eosin or toluidine blue when they appeared faint yellow (Dixon and Herbertson, 1950¹). They gave a positive argentaffin reaction, and, according to Pearse (1953), lipofuscins have this property of reducing silver solutions. They were also similar in staining reactions to the granules seen in the neurones of the brain and cord of the rabbit (Dixon and Herbertson, 1950²). For many years it has been thought that in man a relationship exists between these granules and ageing (Schultz, 1883), and, because of their increase in older sheep and the histochemical reactions outlined above, the granules would seem to correspond to the "senility" or "wear and tear" pigment of human neurones. Kähler, (1952) refers to them as an intermediary metabolic waste product which the cell cannot remove due to age or other conditions. Pearse (1953) suggests lipofuscin is the best term for the pigment -- 'fuscin referring to their yellow-brown colour, and the prefix lipo' suggesting their origin.

"Black pigment", or melanin, occurs in human spinal ganglionic neurones (de Castro, 1932), but it was not identified in the present study. The extraneuronal deposits of pigment in the ganglia appeared rather darker brown than those in the cord but they were acid fast which melanin is not (Gomori, 1952). According to Gomori, all the lipogenous pigments are derived from oxidation and polymerisation of unsaturated fatty acids, and represent various stages of one process. He states that,

since it is impossible to specify what variations in certain staining properties may mean, it is doubtful whether any classification within the group is biologically meaningful. Therefore, despite their darker colour these extraneuronal pigments are probably also a form of lipofuscin.

Pigment atrophy, a pathological increase in the formation of pigment already present in the cell, often occurs in old age, familial and toxic neuropathies in man - for example senile dementia (Alzheimer, 1904), Alzheimer's disease (Bielschowsky, 1932), and Korsakoff's syndrome (Carmichael and Stern, 1931). Increase in lipofuscin has been recorded in experimental deficiency diseases in rats (Pappenheimer and Victor, 1946). In human sympathetic ganglia it is said to be a constant accompaniment of certain pathological states (Kuntz, 1938).

However, there was no difference in the occurrence of the lipofuscin in the neurones of the cords or ganglia of scrapie and control sheep. Intracytoplasmic granules with several histochemical similarities to the spinal lipofuscin were seen in the neurones of the medulla by Zlotnik (1957¹, 1958²) and by Palmer, (1957²), but they did not find any difference in their occurrence in scrapie or control sheep.

Eosinophilic intracytoplasmic bodies. Interest was first directed to the presence of eosinophilic intracytoplasmic inclusions in the neurones of scrapie sheep by Stockman (1926). This worker compared them with Negri bodies, and considered them the most striking neuronal change in scrapie. Subsequently they were reported in the C.N.S. in scrapie by Schofield, (1938), Plummer (1946), Wagner *et al.* (1954), Palmer (1957^{1,2}) and Zlotnik (1957¹, 1958¹).

Plummer (1946) attached exceptional importance to the bodies considering them one of the significant microscopic lesions in scrapie, and a strong indication that the cause of the disease was a virus. Inclusion bodies do not, of course, invariably signify virus aetiology (Cowdry, 1934). In the present study, nucleic acid could not be demonstrated in the bodies, which indicates that they may not themselves be composed of virus

particles. Because of the presence of apparently similar, although less numerous, bodies in the controls it seems unwise to call them the significant microscopic lesion of scrapie.

Palmer's views (1957^{1,2}) on the association of eosinophilic intracytoplasmic bodies with the process of vacuolation have already been discussed (see page 80).

Stockman (1926) observed similar eosinophilic bodies in the spinal ganglia in swayback, Brownlee and Wilson (1932) in the spinal ganglia in Louping ill, and Zlotnik (1957¹) in the medulla of both healthy and scrapied sheep. It is possible that they occur in other animals and other diseases, as similar bodies have been reported in grass sickness in the horse by Obel (1955), who considered that the same kind of structure could be seen in bovine malignant catarrh. Innes and Sanders (1957) have suggested that they may correspond to "lyssa" bodies. The bodies studied in the spinal cord and the ganglia had the same histochemical and staining reactions as those reported by Zlotnik (1957¹), but the latter (1958^{1,2}) found no difference between their occurrence in scrapie and healthy sheep medullae.

In the cord, the larger type of inclusion was found to be unusually numerous in some cases of scrapie. The observation that the size and incidence of the inclusions in the spinal and sympathetic ganglia of scrapie sheep was not different from the control sheep, and their infrequent presence in the control cords, means that they are not specific for scrapie; it does not mean however, that they have no significance in the spinal cord. As large bodies were most often found in unvacuolated motor neurones, it may be that these cells are in a state of unusual excitation because of the degeneration or vacuolation of their related internuncials, and that the inclusions represent an abnormal production of a metabolic secretion. Zlotnik (1958¹) ^{has} suggested that this inclusion may be a secretion. Neurosecretory material found in the nucleus supra-opticus of the rabbit (Palay and Wissig, 1953), and the hypothalamic neurones of several vertebrates (Bargman, 1949, Smith, 1951, and Slopper, 1955) stains black, not pink, with Gomori's chrome-alum haematoxylin phloxine (C.A.H.P.). However, although C.A.H.P. negative, the bodies

may still be classed as a type of secretion, for Smith (1951) has restated the concept of neurosecretion as "any granule or other type of neuronal inclusion which is not identifiable as Nissl substance, mitochondria, or neurofibrillae, and is not the result of a pathological process, is provisionally eligible for inclusion in the category of neurosecretory material".

The present study gives no definite indication of the nature of these inclusions. It is however, possible to say that they are not specific for scrapie, but that the larger type may be unusually numerous in some, but not all, cases of the disease.

The Glia.

Palmer (1957²) was unable to establish significant changes in the glial elements of the brain, but Zlotnik (1958¹) saw neuronophagia of many degenerating neurones in the brain stem, and, in the more advanced cases, oligodendroglial and microglial perineuronal rosettes and focal and diffuse infiltrations. He found pronounced satellitosis in most scrapie animals. This seems a much more severe glial reaction than occurred in the spinal cord, where neuronophagia was occasionally present, but was not prominent. In the cord, dead and dying cells did not attract more than one or two phagocytes, so that the reaction may be described as a "low grade" neuronophagia. This unimpressive reaction may be associated with the chronic or "slow" (Sigurdsson, 1954) nature of scrapie, and contrasts markedly with the intense neuronophagia seen in an acute encephalitis like Louping ill.

On the other hand, when neurones were fatally diseased, but no evidence of phagocytosis was seen, it is possible that the products of cytolysis were absorbed into the tissues without obvious glial proliferation. Innes (1957) has said that if scrapie is due to a virus it has none of the lesions characteristic of any other virus encephalitis, as, amongst other negative features, there is no inflammation or, at most only inflammation of a very minor grade. However, it is known

that under certain circumstances such as following the administration of ACTH, viruses which normally cause acute destruction of neurones with prominent neuronophagia, may still destroy the neurones, but with negligible glial reactions (Aronson and Schwartzman, 1953). The absence of cellular reaction is therefore, not evidence that neuronal degeneration in the C.N.S. is not due to a virus.

No evidence of astrocytic proliferation was found in the spinal cord, which is contrary to the findings of Bertrand *et al.* (1937), who reported an astrocytic gliosis in the anterior horns. Astrocytic proliferation has been reported in higher centres, especially the medulla, by Bertrand *et al.* (1937), Wagner *et al.* (1954) and Zlotnik (1958¹), but Palmer (1957²) could not find any astrocytic changes in the brain. The slow destruction of neurones, which takes place in scrapie, is the type of reaction likely to stimulate a gliosis (Hicks, 1947), but not even in the most severe case was there any evidence for this in the present study.

The phloxinophilic intranuclear inclusion bodies seen in the astrocytes do not appear to have been recorded previously, unless they perhaps correspond to those seen by Spatz (1918) in the glia of new-born rabbits. The rare occurrence of this inclusion in both scrapie and control animals suggests it is of little significance in the disease.

The P.A.S. positive granules seen in the cytoplasm of some glial cells and in the paradventitial cells are almost certainly lipofuscin waste material being transported from the neurones to the blood vessels. Rio-Hortega (1932) has stated that such scavenger cells are microglia. In the present study the transporting cells were like oligodendroglia in P.A.S. - haematoxylin preparation and they may be so, for Field (1957) has shown that the functions of the glial elements may be different in different species. The phenomenon has no significance in scrapie.

The vascular system.

Bertrand *et al.* (1937) recorded that perivascular infil-

rations in the C.N.S. were rare in scrapie cases killed in the terminal stages of the disease. Wilson et al./described (1950) lesions of subacute meningo-encephalitis with perivascular infiltrations in the brains of sheep with experimental scrapie. Zlotnik (1958¹) found the incidence of infiltrations in the brain stem declined with the advancement of the disease; they were most severe in healthy sheep and least severe in advanced scrapie. He also (1958²) found no difference in vascular changes in the brain stems of experimental scrapie and healthy sheep and did not consider them to be significant. In the present study, perivascular infiltrations were not different in type from those in healthy controls, but in the natural scrapie cases as a group they were rather more frequent than in the healthy control group. However, in only three natural cases were they more severe than in the controls; in fact in all four groups - advanced and non-advanced natural scrapie, experimental scrapie, and healthy controls - the difference in incidence and severity was very slight.

In trying to assess their significance, the following points must be taken into account:-

1. Perivascular infiltrations, especially of lymphocytes, do not signify an infectious process, and they are now recognised to occur in a variety of non-infectious conditions (Courville, 1950).
2. All the infiltrating cells were lymphocytes. Greenfield (1958) says that in acute demyelinating diseases lymphocytes might be present, but polymorphonuclear leucocytes and plasma cells are usually absent or very scarce. If plasma cells are prominent then it is strong evidence of an infectious aetiology. Rare and small lymphocytic infiltrations do not constitute an encephalitis or inflammatory disease (Globus and Strauss, 1928).
3. Perivascular infiltrations were seen in healthy sheep and the type of cell was the same as in scrapie. These accumulations in the healthy sheep may represent cell "rests" which can be activated under certain circumstances, or they may be the residue of an infiltrative reaction occurring in an earlier, possibly common but subclinical encephalitis of sheep.

4. The incidence was slightly greater in the natural scrapie group, but the individual severity was greater than in the controls in only three spontaneous cases. As two of these three cases were clinically advanced and showed severe vacuolation and degeneration of neurones, it might be that the rapid breakdown of nervous tissues had excited the lymphocytic exudation (Greenfield, 1958).

From these points it seems fairly safe to draw the following conclusions:-

1. The infiltrations provide no positive evidence for the infectious nature of scrapie. It is not possible to say whether or not they are due to an infection of the spinal cord.
2. The severity, incidence, and nature of the infiltration are not typical of an inflammatory reaction. Based on them, scrapie is more an encephalopathia than an encephalitis.
3. In most cases of scrapie, the infiltrations are rare and do not differ from those occurring in healthy sheep. However, in a few cases infiltration were more severe; these may have been due to the products of degeneration of large numbers of neurones.

Proliferation and hyalinisation of the media of small brain arteries, which was observed in the brain stem of scrapie sheep by Zlotnik (1958¹), was not seen in the spinal cord. The diapedesis of red blood corpuscles and the effusion of plasma-like material seen in the cords of scrapie and control sheep, may have been related to the method of killing the animals. "Haemorrhagic encephalitis" of man, even in the classical form, shows quite a different histological picture from these cuffs of red blood corpuscles (Alpers, 1928).

The endyma and the meninges.

The central canal was quite normal in scrapie. The small endymal lined cavity seen in a control sheep was of

interest, since similar structures have been observed in the spinal cord of human infants by Roser (1959). Lichtenstein (1949), says that they are frequently seen in the occipital white matter of man, and are of no significance pathologically.

As small meningeal infiltrations were observed in only four scrapie sheep, they cannot be considered of much importance in the disease. Bertrand *et al.* (1937) have noted the occurrence of meningeal infiltrations in the brain stem in natural scrapie, and Wilson *et al.* (1950) observed a meningeal reaction in the cerebrum of experimental sheep, in consequence of which the latter authors called the disease a subacute meningo-encephalitis. The histological picture seen in the present study of both natural and experimental scrapie cords, does not, in this author's opinion, justify classifying the disease as a meningitis.

The white matter.

The scattered degeneration of myelin sheaths and axis cylinders in the white matter followed the usual histological picture of secondary degeneration (Spielmeyer, 1922). After the primary myeloclastic processes are completed, myelophages break down the myelin sheath to neutral fat. These fat containing cells have been called Fettkoernchen cells alpha when they lie in the digestion chambers, Fettkoernchen cells beta when on the walls of the digestion chambers, and Fettkoernchen cells gamma when outside the broken down neural tube (Jakob, 1904). Because of their vacuolated appearance in paraffin sections, it is more usual to refer to all these cells as gitter cells.

The empty oval or round holes sometimes seen in paraffin and frozen sections of the white matter, should not be mistaken for demyelinated fibres. Their nature was not apparent, but they seemed to be of equal occurrence in both control and scrapie sheep. Similar cavities have been reported in the white matter of the cord of several animals, including the goat, by Trowell (1943), who quotes Ostertag (1911) as considering them due to post mortem swelling of the myelin sheath.

Secondary degeneration of fibres in the white matter was marked in two cases of scrapie, but on the whole the positive cases of scrapie showed no more than occasional degenerating fibres discretely scattered in the ventro-medial part of the ventral funiculus and to a lesser extent in the lateral funiculus. Although the breakdown of myelin to fat is a slow process in animals and man (Jakob, 1904, Hurst, 1925, Cramer and Alper, 1932), extensive myelin breakdown would be easily detected in a prolonged disease like scrapie. This absence of tract degeneration is in agreement with the findings of Cassirer (1898), Stockman, (1926), and Brownlee (1940) all of whom suggested however, that occasional degenerating fibres may occur. These workers used the Marchi method which is known to be capricious in the sheep, and consequently the findings were not considered significant. Although age and chronic wasting conditions tend to cause degeneration of small numbers of fibres (Duncan, 1931), the peculiar localisation of the fibres in the scrapie cases suggests they may not be due to these incidental factors. King (1911) observed degenerating tracts in similar regions subsequent to experimental medulla lesions in the sheep, and as the neurones of the brain stem are definitely involved in scrapie (Zlotnik, 1958¹), it is possible that the degeneration seen in the spinal white matter may be secondary to these lesions.

The incidence and distribution of vacuolated neurones.

The cell groups in Zones A, B, and C of the control cords.

Division of the grey matter into three zones for vacuole counting purposes had the following advantages:-

1. The zones were easily demarcated in any segment.
2. It was unnecessary to identify small nuclei, which in thin paraffin sections is extremely difficult.
3. Even in thick frozen sections of the spinal cord a great number of nerves cells do not seem to belong to

- any recognisable nucleus. Again, they can be easily grouped in the zones.
4. Degeneration and vacuolation alter the appearance of the neurones so that they cannot be placed in nuclei by cytological similarities, but they can be grouped into broad zones.
 5. The nerves cells of the three zones have significant functional differences.

The examination of thick frozen sections was merely an aid to establishing which nuclei occur in the respective zones, and requires little discussion. The findings showed that the cell groupings of the sheep's cord differed in detail rather than in principle from those reported in other animals (Bok, 1928, Kappers, Huber and Crosby, 1936, Mettler, 1948, Ranson, 1957, Rexed, 1954). Recently Goller (1958), has studied the structure of the sheep's spinal cord. To designate the cell groups he used the older terminology of Waldeyer (1888) and Jacobsohn (1908), whereas in the present study the terminology used is that of Bok (1928). This is important, for as has been pointed out by Rexed (1952), there is considerable variety, and even confusion, in the names used to describe the cell groups of the spinal cord.

Even if the nuclei of the spinal grey matter could have been accurately identified, their individual functions are not certain. However, the majority of the cells of the three zones appear to have respectively different functions.

Most nuclei in Zone A were composed of internuncial cells (Fulton, 1943), intercalated singly or in short chains between the incoming fibres, or fibres descending from higher centres, and the final motor efferent neurones of the same or the opposite side. There may be a few preganglionic sympathetic neurones in the central part (Zona intermedio) of the zone (Kuntz, 1934), and possibly a few motor neurones were included in the ventral regions, but the majority of the cells were internuncials.

Most of the neurones of Zone B were motor efferent neurones. However, Sprague (1951) has shown in the monkey that,

despite their distinctive position, size, and cytological similarity to one another, some of the so called motor neurones are really internuncials.

Zone C was functionally the most accurately demarcated as the cells were naturally isolated by the projection of the lateral horn into the white matter. It consisted exclusively of preganglionic sympathetic neurones. Rexed (1952) claims that only this zone (n. intermediolateralis) is indisputably sympathetic, and consequently those other neurones noted above, whose sympathetic function is not absolutely certain, were not included.

The three zones thus have the following functions:-

1. Zone A - predominantly internuncial.
2. Zone B - predominantly motor efferent.
3. Zone C - preganglionic sympathetic.

The incidence of vacuoles. Following the original observations of Besnoit and Morel (1898), the majority of workers have accepted vacuolation of the neurones of the C.N.S. as a most significant histological change in scrapie. Some observers found only vacuolation (Brownlee, 1940), whereas others also saw concurrent neuronal disease or other phenomena which they thought important (Bertrand *et al.* 1937, Holman and Pattison, 1943, Plummer, 1946, Lucam *et al.* 1950, Wagner *et al.* 1954). In suggesting a myopathic causation for scrapie, however, Bosanquet *et al.* (1956) and Parry (1957), reported that they found only occasional vacuoles in the medulla and cord which they considered of little significance because of their presence in normal sheep and in humans affected by various diseases. Delez *et al.* (1957) examined the medullas of 20 scrapie sheep and reported that vacuolation was equally prominent in normal control sheep.

The occurrence of occasional vacuolated neurones in normal sheep has never been seriously disputed, and only Brownlee (1940) has suggested that vacuolation was specific for scrapie. Holman and Pattison (1943) found vacuoles in the medullas in one out of 59 healthy sheep which they examined, while they

were present in 73% of normal medullas examined by Zlotnik and Rennie (1957), up to 7 per section being seen. In a further survey Zlotnik and Rennie (1958) found they occurred in the medullas of healthy sheep of several different breeds. In the present study they were often observed in normal sympathetic and spinal sensory ganglia, and they were noted to be particularly common in the normal Gasserian ganglia and the neurones in the normal adrenal medulla. They also occurred in the normal spinal cord, but in contrast to some of the other tissues mentioned, they were so rare that they can almost be ignored.

In healthy animals therefore, it seems as if vacuolated neurones are more numerous in some parts of the nervous system than in others. However, apart from the above reports of Bosanquet et al. (1957), Parry (1957), and Delez et al. (1957), which contain no quantitative data, comparisons of the incidence of vacuolated neurones in the medullas of healthy and scrapie sheep have shown them to be significantly more numerous in scrapie. They were found mostly with ease in 75 medullas from scrapie sheep examined by Holman and Pattison (1943), as against the single vacuole they observed in 59 healthy sheep. Zlotnik (1957², 1958¹) compared the incidence of vacuoles in the medullas of control sheep with that in natural scrapie, and found it to be very significantly greater in the latter. In the present study vacuoles were constantly more numerous in the spinal cords of scrapie than of healthy sheep. The average number of vacuoles per section in the scrapie cords was 4.5 and in the controls 0.0017 when 200 sections per case were examined, and when 50 sections per case were examined the findings were 3 and 0.001 vacuoles per section respectively. Thus, in contrast to the statements of Bosanquet et al. (1957) and Parry (1957), vacuolation must be considered very significant in the cord in scrapie, while vacuolation in the cord of normal sheep is very insignificant.

As added evidence, vacuoles were found in greater numbers in the cords of experimental scrapie sheep than in the controls. They were however, not frequent (average per section 0.103).

The number of vacuoles in the diseased cords was lowest in experimental scrapie group and highest in the advanced natural

scrapie group. There were individual exceptions to this, the cord of one clinically non-advanced case containing more vacuoles than the majority of the advanced group. Similar findings have been reported in the medulla (Zlotnik, 1958¹). These individual exceptions may be due to different centres being affected with varying severity in different cases, or, as the history prior to arrival at the Moredun was often incomplete, to faulty interpretations of the symptoms. Innes and Sanders (1957), pointed out how inadequate is the technique of neurological examination in animals. On the whole, however, when groups of animals were considered, there was a relationship between the severity of clinical symptoms and the degree of vacuolation in naturally occurring scrapie.

Vacuolated neurones were less numerous in experimental scrapie cords than in non-advanced natural scrapie. A similar observation has been reported in the brain stem by Zlotnik (1958²), and Wilson et al. (1950) found no vacuoles in the large neurones of the cord in experimental scrapie. These results are interesting because most of the experimental sheep must be considered to have shown severe clinical symptoms - in many cases pruritus had been intense and severe incoordination and ataxia were often followed by paresis. On the other hand the clinical picture was not entirely identical with natural scrapie, because the progress of the disease was more rapid than in the latter. This suggests that perhaps the neurones appear normal by the histological methods used but are non-functional.

These observations on the incidence of vacuolation in the advanced and non-advanced cases suggest that the functions of the vacuolated neurones are impaired. This is certainly so when the cell is severely degenerated, very ballooned by vacuolation, or, as chromatolytic neurones are temporarily non-functional (Bodian, (1948), chromatolytic.

The significance of the distribution of vacuoles, with particular reference to the symptoms of scrapie. According to Bertrand et al. (1937) and Brownlee (1940) the large neurones of the anterior horns

were principally affected in scrapie, and Lucam et al.(1950) specifically recorded that the motor neurones were affected. Wilson et al.(1950) could not find any vacuoles in the large neurones of the spinal cord in experimental scrapie, but they do not state whether vacuoles occurred in small and medium sized neurones. The present results, however, show that only a very small proportion of vacuoles were found in the motor areas where the larger cells of the cord occur (Zone B, 4.2% to 7.8%). This finding confirms the suggestion of Cassirer (1898) that clinically scrapie is a derangement of coordination, not akinesia but parakinesia, and that, on a symptomatic basis, the motor proto-neurone may be excluded. It also well explains the fact that neither Bosanquet et al.(1957), nor Hulland (1958), found any evidence of motoneuronal patterns in the skeletal muscles.

The majority of lesions, both vacuoles and various forms of degeneration, were present in Zone A. As stated previously this zone contains mostly internuncial cells. The majority of dorsal root fibres terminate on these internuncials, and further passage through a series of internuncials is usually necessary in all but the simplest reflexes in order to reinforce the stimulus. Probably not more than 2% or 3% (Fulton, 1943) of dorsal root fibres actually pass directly to the ventral horn motor neurones, and this same primary passage through internuncials applies to motor impulses emanating from higher centres. Furthermore, the internuncials not only prolong the activity of the motor neurones, but also cause widespread diffusion of activity throughout a number of individual motor nuclei, and act as a valve mechanism permitting or preventing influence upon the motor neurones. Lesions of the internuncials could have important clinical effects for, as Fulton (1955), puts it "failure of the interneurone to respond, or prevention of its response, to incoming activity leaves the motoneurone uninfluenced by that activity."

Consequently, this vacuolation and degeneration of the internuncial neurones may be one of the factors causing incoordination in scrapie by interference to the passage of ipsilateral and contralateral reflexes. Involvement of the nuclei

cornucommissuralis and the neurones of the substantia grisea centralis particularly suggests that contralateral reflexes may be affected. Incoordination, fasciculation of muscles, and muscle spasms in certain forms of poliomyelitis are thought to be due to degeneration of internuncials (Kabat and Knap, 1944). Release of the motor neurone from inhibition and resultant interference to the "trigger mechanism" level may be responsible for the rapid, jerky flexion of the "cuddy trot" in scrapie.

Although Brownlee (1940) says that the large cells of the anterior horns showed vacuolation, an appendix to his work shows that some vacuoles were seen in the intermediolateral column. Remarkably, Brownlee does not mention this in the text, but says "the distribution of nerve cell systems in the sheep is not yet sufficiently well known to permit the inference that selective injury has been inflicted on the cells of any particular nerve cell group or groups". In contrast to Brownlee's statements, the counts of vacuoles made in this study clearly show that there is selective injury of certain cell groups; namely, the spinal internuncials (Zone A) and the intermediolateral nucleus (Zone C). Furthermore, although there is no experimental evidence in the sheep that the neurones of the distinctly demarcated intermediolateral nucleus are sympathetic preganglionics, similar groups of cells are known to be central sympathetics in several animals, and cells occurring in this unusual location are found not only in many mammals, but also in birds and reptiles (Kappers et al. 1936). Parry (1957) has also stated that vacuolation can in no way explain the symptoms, but this also appears incorrect.

Bertrand et al. (1937) suggested that the pruritus in scrapie may be due to lesion of the autonomic nervous system, and whether or not this is so, the present study shows the preganglionic neurones (Zone C) were frequently and sometimes severely affected. Billingsley and Ranson (1918) have shown that the ratio of preganglionic to ganglionic neurones in the superior cervical ganglion is one to thirty two. Each degenerated neurone in Zone C has therefore a much magnified effect for, as Kuntz (1934) says of the sympathetic ganglion

neurones, "Their normal physiological activity requires the integrity of the preganglionic neurones".

Vasomotor, pilo-erector and sweat centres certainly occur in the preganglionic neurones (Zone C of the present study) of the mammalian spinal cord and interference to the normal functions of these may account for the pruritus. However, it is perhaps worth noting that a centre associated with itchiness and itchy skin is situated below the acoustic nucleus in cats (Rothman, 1941), and that it is in the caudal part of the medulla that Zlotnik (1958¹) has recorded particularly severe vacuolation in scrapie sheep.

Some of the less characteristic symptoms of scrapie suggest sympathetic involvement, particularly exophthalmos, regurgitation of ruminal contents, and thirst. In the present study it was noted that death often followed changes in climatic conditions, and fatal cases of scrapie have previously been reported to be more frequent in winter (Stockman, 1913, Gaiger, 1924). Sawyer and Schlossberg (1933) have shown experimentally that in the absence of functional sympathetic nerves, cats are unable to withstand changes in temperature, and are highly sensitive to cold. The results of sympathetic derangement may be more widespread than this however, for homeostasis, or the maintenance of the matrix in a constant state, mainly depends on the functional integrity of the sympathetic nervous system (Fulton, 1943).

The present status of ovine neurology and of knowledge of the physiology of the sheep's central nervous system, only allows one to suggest, and not to state with certainty, the effects of lesions in specific nuclei. Furthermore, lesions in parts of the central nervous system other than the spinal cord certainly play a most important part in the symptomatology. The possible involvement of a centre in the medulla concerned with skin itchiness has been mentioned. Centres of respiration and rumination in the medulla of the sheep (Amoroso, Bell and Rosenberg, 1951, Bell and Lawn, 1955) appear to lie in a similar area to that which Zlotnik (1958¹) often found involved in scrapie. Neurone degeneration has often been noticed in

the reticular formation of the medulla (Holman and Pattison, 1943, Zlotnik, 1958¹, and Palmer, 1959) and, in experimental cats and monkeys, this formation has been shown to be concerned, not only in maintaining wakefulness, but in the discharge to motor neurones (Magoun, 1950). The clinical disease of scrapie would, therefore, appear to be a neurological complex brought about by degenerative lesions affecting a chain of centres in the neuraxis, each centre being involved to a greater or lesser degree in the individual case.

The relationship of the distribution of vacuoles to the angio-architecture of the grey matter. Involvement of the internuncial and preganglionic sympathetic neurones and sparing of the motor neurones, suggests that the disease shows a degree of predilection for certain areas of the spinal cord. Electivity is common in diseases of the nervous system, and suggested reasons for this phenomenon include the specific vulnerability of certain groups of neurones ("Pathoklithis"), inherited factors, restricted movement of viruses along certain nerve pathways, and phylogenetic differences (Hiller, 1928). Speilmeyer (1926) suggested that peculiarities of the angio-architecture may lead to great variability in blood supply to certain areas, this either bringing more toxin to these areas or less blood to support them in distress, although distance from the arterial end of the blood vessels, rather than the actual abundance of vessels, may be important (Krogh, 1945, 1950). Temporary ischaemia in the cord of dogs has been shown to cause degenerative changes in the internuncials (Kabat and Knap, 1944). In the present study, examination of sections stained by the Lepehne-Pickworth method suggested that the areas of greatest vacuolation may be nearer the arterial blood supply. The bilateral symmetry often noted in the location of degenerated and vacuolated neurones also suggests that there may be a relationship to the angio-architecture. Even so, it is difficult to draw conclusions as to the exact part that the blood supply plays, or its relation to the aetiology of the disease. One can only say that in the spinal cord the local predilection of the lesions may be related to the vascular architecture.

The spinal cord in the routine histological diagnosis of Scrapie.

The need for an accurate method of post mortem diagnosis (Stamp, 1956), has now been satisfied by the studies of Zlotnik (1957¹, 1958^{1,2}) and Zlotnik and Rennie (1957, 1958) on the medulla. Although the occurrence of vacuoles in normal medullas has to be taken into account, in general Zlotnik (1958^{1,2}) found a larger average number of vacuoles per section of the medulla, particularly in experimental scrapie, than were found in the present study in an average section of the spinal cord. Furthermore, the brain of the sheep is more easily removed at autopsy than is the cord. In consequence, the spinal cord is probably inferior to the medulla for the histological diagnosis of scrapie. However, as vacuolation is so very rare in the cords of healthy sheep, sections cut from two blocks from the cervical intumescentia and two blocks from the mid-thoracic region so as to include the intermediolateral column, would form a valuable adjunct to diagnosis, when the routine pathologist has time to expose these regions of the cord.

THE SPINAL GANGLIA.

Although pruritus is such a prominent symptom of scrapie, there are very few reports on the histology of the spinal sensory ganglia in the disease.

Besnoit and Morel (1898) claimed that the ganglia were congested and infiltrated as a secondary reaction to the peripheral neuritis, which they believed to be so important. M'Gowan (1914) found no abnormalities in the ganglia. They were examined by Stockman (1926), who observed eosinophilic inclusions, and by Brownlee (1940), who saw vacuoles in the neurones of the spinal ganglia in both scrapie and control sheep, but does not record their respective incidence or significance in scrapie. Field (1952) had seen vacuoles in the spinal ganglia of normal rabbits which he thought resembled those in the medulla

of scrapie sheep, but he failed to find any vacuoles in the spinal ganglia of normal sheep.

The neurones.

Vacuolated neurones. In the present study vacuoles were found in a large proportion of both scrapie and control groups of sheep and the similar incidence in all groups strongly indicates, in complete contrast to the findings in the spinal cord, that vacuolation of neurones in the spinal ganglia is not of significance in scrapie. Furthermore, the vacuolated neurones at the two sites were not so similar as Field suggested, since in the spinal cord in scrapie they were very often chromatolytic or otherwise degenerated, whereas in the spinal ganglia of scrapie sheep they were otherwise normal, and did not differ morphologically from those in the ganglia of control sheep.

Vacuoles were found in 90% of control sheep, and, as in those seen by Field in 70% of normal rabbits, they did not contain fat or glycogen. No specific substance could be demonstrated in these vacuoles in the scrapie or control groups of sheep. As adjacent neurones and the ganglion as a whole appeared quite normal, no definite explanation can be given for their occurrence. Earlier in this discussion it has been suggested that they may^{be} a physiological occurrence. It is interesting to note that after innoculating herpes zoster virus directly into the sciatic nerve of rabbits, Field saw "considerable numbers" of vacuoles in ganglia of both the same and opposite sides, and he suggested that in these cases they might be due to irritation of the cell.

Vacuolated neurones have also been found in the spinal ganglia of man, cat and rat, but they were associated with senescence and contained granules of lipoid (Truex and Zwemer, 1942). This lipoid, which stained black with osmic acid, yellow to orange-red with both Sudan IV and scarlet red in frozen sections, and was soluble in the alcohol and xylol used in routine processing, was said definitely not to be

lipofuscin, but true fatty degeneration. The vacuoles were both unilocular and multilocular.

Atypical neurones. Other changes in neurones were very infrequent in scrapie. Atypical forms of ganglionic neurones are often said to be more common in animals than man, and de Castro (1932) says that what is atypical in man may be normal in other animals. Cells with knobbed processes and pericellular nests are said to occur in the ox and be abundant in the horse (Dogiel, 1908), and fenestrated, multirouted and multipolar neurones are described in the dog (Ranson, 1912). Maximow and Bloom, (1948) illustrate the ganglionic neurone of a sheep which has its process originating from numerous anastomosing loops. In the present study, however, these atypical forms were uncommon in the sheep's spinal ganglia. Truex (1941) has suggested that these atypical forms are not a normal multipolar type of cell, but degenerate forms. He found them more commonly in man than in cats, rats and chickens. Atypical forms may be associated with pathological conditions (de Castro, 1932), but in the present study their incidence was not different in natural or experimental scrapie or control groups of sheep.

Significance of the histological findings.

The complete absence of significant morphological differences in the three groups of animals examined, convincingly indicates that lesions of the spinal ganglia were not a direct cause of the pruritic symptoms in scrapie, nor was there any evidence that the ganglia were involved in the disease in any way.

THE SYMPATHETIC GANGLIA.

Changes seen in the sympathetic ganglia may be divided into those common to both the scrapie and the control group, and those seen in only the scrapie sheep. Before discussing

their general significance, it is worth examining certain of the changes in more detail.

The Neurones.

Neurone degeneration. Occasional chromatolytic and degenerating neurones were seen in the sympathetic ganglia but they were not thought to be related to scrapie, and probably represented stages in the gradual loss of sympathetic neurones which occurs throughout life in other species (Kuntz, 1938). Very pale hypochromatic neurones are known to occur normally in man (Ingersoll, 1934, Truex, 1951), and Hollinshead and Clark (1935) have stated that it may even prove difficult in man to distinguish true chromatolysis from the frequent "ring" arrangement of the Nissl substance. However, genuine chromatolysis was easily identified in the sheep. In any case, there was no indication that very pale neurones or "ring" forms of Nissl substance arrangements were any different in occurrence in the scrapie sheep or control sheep.

Hyaline eosinophilic masses were seen inside the cell capsule in both natural scrapie and control sheep, and it is therefore interesting to note that Köhler (1952) regarded them as occurring only in the horse. He refers to the masses as deposits, whereas in the present study they sometimes seemed to be formed by a piece of necrotic cytoplasm becoming detached from the otherwise healthy perikaryon. The possibility has also to be considered that in some cases they may be large end bulbs.

Vacuolated neurones. Vacuolated neurones were only slightly more common in natural scrapie sheep than in the controls, and it is doubtful if the difference is significant. They were not so frequently observed as in the spinal ganglia. Equally as important as their respective incidence, is the fact that a large proportion of vacuolated neurones in the spinal cord were chromatolytic or degenerating, whereas all but two of those seen in sympathetic ganglia from natural and

experimental scrapie were quite normal apart from the vacuole. The majority of vacuoles in the sympathetic ganglia were also unilocular. They thus resemble the vacuoles seen in the spinal ganglia of healthy sheep, rather than the pathological vacuoles seen in the spinal cord in scrapie.

Brownlee (1940) examined a few sympathetic ganglia from scrapie sheep and saw one vacuole. Vacuoles have, however, been observed in the sympathetic ganglia of several apparently healthy domestic animals (Köhler, 1952, Nüssel, 1957), including the sheep (Godina, 1950). Köhler thought that homogenous material inside the vacuoles indicated that they contained an albuminoid fluid and neurofibrillary remains, but, in contrast to those seen in the spinal ganglia by Truex and Zwemer, (1942) there was no evidence that they contained fat. He suggests that they occur primarily as small vacuoles between the neurofibrils and that secondarily they enlarge, tearing apart the fibrils and packing them together round the edges. This type of vacuole seems very similar to those seen in the ganglia in the present study. They undoubtedly start as small cavities and there is probably some local destruction and peripheral condensation of neurofibrils, but as the latter form a very much finer reticulum during the life of the cell (Dawson, Hossack, and Wyburn, 1954) it is unwise to speculate as to how or where the vacuoles start originally. Although numerous investigations show that vacuoles occur in healthy animals, Köhler believes that their number is significantly greater in individuals whose vegetative nervous system has suffered damage, and he thought they were more frequent in foreign body peritonitis and serous tuberculosis in the bovine, colic in the horse, and other non-specific conditions. This suggests that a slightly increased incidence of vacuolation in the sympathetic ganglia in only an occasional case of scrapie might be a non-specific change.

The nucleus of the neurones. Double nucleoli, similar in their position and size in relation to single nucleoli to those in the present study, were observed in the horse, cow, and dog by

Köhler, who thought they were more common in young animals. He suggested that they were expelled from the nucleus in later life. There was no evidence that they were more common in young animals in the present study, and when nucleoli were seen in the cytoplasm they were presumed to have been pushed into this position by the microtome knife. As the cytoplasm is semi-fluid in life, when distinct tracts were seen it was considered to be evidence that the movement was post mortem.

On the other hand, Hagen (1942) considered that double nucleoli in man were a degenerative change. Occasional double nucleoli of equal size were seen in the majority of sheep from all groups in the present study and were thus considered normal. However, in that case in which many double nucleoli, one of the pair being haloed, were seen, the neurones may have been in an abnormal condition, although there was no evidence, such as chromatolysis or necrosis, of progressive morphological degeneration. Mulberry forms and vacuolation of the nucleolus, which are often said to represent degenerative processes in man (de Castro, 1932), were absent or infrequent.

The multiple bodies seen in the nuclei of two cases of scrapie do not correspond to the intra-nuclear viral inclusions described by Cowdry (1934), as the stages of development leading to ultimate death of the cell which characterise Cowdry's type A inclusions, were absent. They may be similar to Cowdry's type B, although they were neither haloed nor acidophilic, but there is no definite evidence that this type are of viral aetiology. They may be similar to the nucleolar extrusions which Vogt and Vogt (1947) thought represented a nucleolar product which forms the ribose nucleoprotein of the Nissl substance, and which they frequently observed in senility and diseased conditions of the human nerve cell. Truex (1951) observed somewhat similar bodies in occasional neurones from normal human sympathetic ganglia. Although no staining or histochemical reactions are recorded, he describes them as discrete clumps of nuclear chromatin and says that they may be transported through the nuclear membrane to replenish the

cytoplasmic chromatin. However, although the present structures had several positive and negative staining reactions in common with the nucleolus and Nissl substance, including a faint positive reaction with methyl green-pyronin, they were not apparently histochemically identical with those structures. For instance, they did not stain with galloxyanin solutions at the same pH as that which strongly stained the Nissl particles and nucleolus, but by increasing the alkalinity only slightly they could be stained. According to Einarson (1933, 1935), at pH 1.64 galloxyanin chromalum staining indicates the degree of "genuine basophily", and this depends directly on the quantity of nucleic acids present. At pH 1.8 co-staining begins, and as the alkalinity is further increased various non-specific structures, such as the axon and the neuroglia, may be stained. This suggests some difference between the nucleic acid content of the inclusions and the nucleolus.

The incidence of these inclusions was too small to indicate any relationship, even indirectly to scrapie.

Intracytoplasmic granules and extraneuronal pigment. Intracytoplasmic phloxinophilic bodies and lipofuscin granules occurred in the sympathetic neurones of scrapie sheep, but differed in no way from those seen in the controls. Their nature and the significance of the extra-cellular pigment has already been discussed in detail (see pages 81 to 84).

Cellular infiltrations.

Two types of cellular infiltrations were seen in the sympathetic ganglia. The first were perivascular infiltrations of lymphocyte-like cells occurring in all groups of sheep and probably corresponding to those seen in the autonomic ganglia of various domestic animals by Köhler (1952), and considered by him a normal physiological reserve of cells. Köhler also saw infiltrations of polymorphs in a case of bovine traumatic peritonitis, which he thought were a definite inflammatory reaction, while in man, Mogilniziky (1923) has associated

infiltrations with several diseases. Although polymorphs were not seen, diffuse infiltrations of cells with twisted nuclei were present in both scrapie and healthy sheep. These infiltrations may be associated with past non-specific disease processes such as Kuntz (1938) has seen in the human subject, or even be similar to those associated with age in man (Craig and Kernohan, 1933). Whatever their cause, neither type of infiltration was frequent, extensive, or associated with scrapie.

Paraganglia-like structures.

Aggregations of chromaffin cells, or paraganglia, have been known to occur in sympathetic ganglia since first identified by Kohn (1899), and subsequently they have been reported in several domestic animals (Köhler, 1952). Köhler does not report their occurrence in sheep. Their neurogenic origin has often been doubted (Maximow and Bloom, 1948), and Nonidez (1942) has pointed out that structures in the stellate ganglia of dogs resembling paraganglia were, in fact, arterio-venous anastomoses.

The Schmorl ferric ferricyanide reduction test and the periodic acid Schiff reaction are stated by Lillie (1954) to be the best methods of demonstrating chromaffin. Both tests were negative in the present study, but this may have been due to unsuitable fixation of the small amount of material available, or to prolonged interference in removing the cover slip and decolorising the sections. The latter procedure may have interfered with Lison's test or, as Nonidez (1935) observed in the glomus aorticum of guinea pigs and rabbits, the cells may not give a typical chromaffin reaction. De Castro (1926) did not think the granules were chromaffin, but lipid. If the present structures were arterio-venous anastomoses, they were much less common than Nonidez (1942) found in the dog. Neither mature nor transitional forms of plain muscle fibres such as Nonidez saw in the dog, were seen. Red blood corpuscles were found only in the lumen of the solitary capillary identified in each case.

The morphology of the structures thus suggests that they are paraganglia, which, because the chromaffin reactions were negative, is probably a better term than chromaffin cells.

Nerve fibres of the ganglia and sympathetic trunks.

The preganglionic fibres of the sympathetic nervous system of vertebrates are relatively well myelinated, while the post ganglionic efferent fibres are only finely, or not at all, myelinated (Kuntz, 1934). Some of the myelinated fibres seen in the sympathetic trunks in the present study were presumably preganglionic, and the advanced degenerative changes and death of neurones of the intermediolateral column observed in the spinal cords in scrapie would, theoretically, be followed by degeneration of their axons and myelin sheaths in the sympathetic trunks. No evidence of degenerating fibres or of myelin changes were found. There are, of course, many times more post ganglionic than preganglionic fibres in the sympathetic trunks, and it is possible that any fibres which were at a stage of degeneration which could be histologically identified were overlooked.

Significance of the histological findings.

Changes common to both scrapie and control sheep include a considerable number of non-specific variations, most of which may be due to age, to non-specific diseases past or present, or to variations within normal individuals. Similar variations undoubtedly account for many of the divergent opinions expressed in the literature on pathological changes in human sympathetic ganglia (Truex, 1951). Kuntz (1938) says of the human subject "autonomic ganglia exhibit certain variations common to all the ganglia in that age group, but the ganglion of certain individuals in every age group exhibit a wider range of variations than others". He includes in changes due to age excessive deposition of pigment, exhaustion of the Nissl substance, degenerative changes, necrosis, and

hyalinization in occasional ganglion cells, neuronophagia of moderate degree, and moderate progressive increase in the quantity of interstitial connective tissue from birth onwards. However, when these same changes are marked and occurred in considerable numbers he considered them pathological.

Many of the above changes listed by Kuntz were observed in the present study, but in general they were not more pronounced in the scrapie cases than in the controls. In no single case could the changes be described as marked or as occurring in large numbers of cells. Even in the one scrapie case in which chromatolytic cells were relatively frequent, not more than six cells out of an estimated 1,200 visible in the same transverse section were involved.

The few changes exclusively found in the scrapie group were large haloed nucleoli and intranuclear bodies. They may represent a reaction of the nuclear contents to abnormal conditions, but as they occurred in only 3 out of 30 natural cases of scrapie, and in none of the 10 experimental cases it is not possible to relate them to scrapie with certainty. They may be, as are so many sympathetic ganglion cell changes, "not causes but accompaniments of the disease in question" (Kuntz, 1938), or they may even be caused by some completely unrelated condition.

One may thus say that this study revealed no evidence of direct or indirect morphological involvement of the sympathetic ganglia in scrapie disease of sheep.

How may these negative findings be related to the frequent involvement of the neurones of the intermediolateral column of the cord in scrapie? The neurones of the intermediolateral nucleus are to the ganglionic neurones much the same as the internuncials are to the motor neurones of the cord, and consequently degeneration of one part of the chain does not mean that morphological changes will follow in the other. Definite transsynaptic degeneration is known to occur in only a few centres (Greenfield, 1958), and the sympathetic nervous system is not one of these. It is possible therefore, for the preganglionic neurones to be severely degenerated

while the ganglionic neurones appear quite normal, but it is not possible for the latter to function adequately without the integrity of the former (Kuntz, 1934).

This work records the results of a study of the spinal cord, spinal accessory ganglia, sympathetic ganglia, celiac ganglia and nerves in the adrenal glands of sheep affected with scrapie disease.

No macroscopic differences were found between affected tissues from scrapie sheep and from healthy control sheep.

The structure of the spinal cord in both experimentally induced and natural scrapie disease showed degenerative changes, which included chromatolysis, vacuolation, axonal change, calcareous and calcareous. Vacuolation was a significant change in natural scrapie, and when it was severe than other degenerative changes were usually also severe. Vacuoles were also present in experimental scrapie but early chromatolysis was the most common change. These changes were discussed, particular reference being given to the significance of central chromatolysis in a disease with a prolonged course, and to the possibility that calcareous and calcareous vacuoles were identical. The different types of vacuolated neurones seen in scrapie were described in more detail than those reported in normal and other pathological conditions. It was concluded that vacuolation and chromatolysis were not significant changes in scrapie but that these changes were present in scrapie.

No specific substance, including fat, lipid, carbohydrate, or protein could be demonstrated in the vacuoles. This was discussed with particular reference to the specific intracellular material reported in natural and diseased neurones in other animals. Intracellular spherules were seen in some vacuoles in scrapie, but there was no evidence that they were specific or represented virus material. It was suggested that they may be, at least in part, a product of cellular degeneration.

Virus inclusion bodies were not found. Intracellular

SUMMARY AND CONCLUSIONS.

This work records the results of a study of the spinal cords, spinal sensory ganglia, sympathetic ganglia, Gasserian ganglia and neurones in the adrenal glands of sheep affected with scrapie disease.

No macroscopic difference was found between nervous tissues from scrapie sheep and from healthy control sheep.

The neurones of the spinal cords in both experimentally induced and natural scrapie disease showed degenerative changes, which included chromatolysis, acute swelling, severe cell change, sclerosis and vacuolation. Vacuolation was a significant change in natural scrapie, and when it was severe then other degenerative changes were usually also severe. Vacuoles were also present in experimental scrapie but early chromatolysis was the most common change. These changes were discussed, particular reference being given to the significance of central chromatolysis in a disease with a prolonged course, and to the possibility that sclerotic and vacuolated neurones were artefacts. The different types of vacuolated neurones seen in scrapie were described and compared with those reported in normal and other pathological conditions. It was concluded that vacuolation and degeneration of neurones were most significant changes in the spinal cords of sheep affected with scrapie.

No specific substance, including fat, lipid, carbohydrate, or nucleic acid could be demonstrated in the vacuoles. This was discussed with particular reference to the specific intravacuolar material reported in normal and diseased neurones in other animals. Intravacuolar eosinophilic spheroids were seen in some vacuoles in scrapie, but there was no evidence that they were specific or represented virus material. It was suggested that they may be, at least in part, a product of cellular degeneration.

Virus inclusion bodies were not found. Intracytoplasmic

granules which, because of their staining and histochemical reactions were considered to be a lipofuscin, were more numerous in the neurones of older sheep, but were otherwise similar in healthy and scrapie sheep. A second type of intracytoplasmic bodies was intensely phloxinophilic. The significance which has been attached to these phloxinophilic bodies was discussed and, although they were not specific for scrapie, it was found that large inclusions were very numerous in some, but not all cases of scrapie. No association was found between them and the process of vacuole formation, and it was suggested that they may be a normal cellular secretion which was sometimes increased in the disease.

There was little glial reaction in the cord. Glasmotodendrosis, gliosis, increased satellitosis, and extensive neuronophagia were not observed, but there was sometimes neuronophagia of degenerated neurones by only one or two microglia. Sometimes cytolysis and disappearance of cellular remains without phagocytosis seemed to take place. These findings were discussed and it was concluded that the absence of extensive neuronophagia cannot be used as evidence for the non-infectious nature of the disease. It was suggested that the mild neuronophagia may be associated with the prolonged course of scrapie.

Infrequent intranuclear inclusions in glial cells were reported in both healthy sheep and those with scrapie.

Perivascular infiltrations with cells resembling lymphocytes were recorded in all groups of sheep, but in only three natural cases of scrapie were they slightly more severe than in the control group. These findings were discussed and it was concluded that, as perivascular infiltrations of lymphocytes provide no evidence of infection and because they were generally so mild in scrapie, they were of little positive significance.

Small subpial infiltrations of very limited extent were seen in only four natural cases of scrapie. In the remaining cases of experimental or natural scrapie there was no evidence of meningitis.

Secondary degeneration of myelin and axis cylinders was observed in the ventral and lateral funiculi. Usually a few fibres only were affected, but in two cases the degree of degeneration was marked. This was discussed and it was suggested that the location of these fibres may indicate that they originated from degenerated neurones which other workers have reported in the brain stem in scrapie.

The advantages of dividing the grey matter into three zones for vacuole counting purposes was discussed, and the major nuclei in the cord, particularly in the three zones, were determined by a study of thick frozen sections.

Vacuolated neurones were seen in the cords from all 68 natural cases of scrapie, in 29 out of 30 cords from experimental cases, and in 5 out of 34 healthy control sheep. They were very significantly more numerous in sheep with scrapie than in healthy control sheep. When the number of vacuoles seen in 200 serial sections from each of 20 sheep affected with natural scrapie, 20 sheep with experimental scrapie and 15 healthy control sheep were counted, the average number of vacuoles per section was 4.5, 0.103, and 0.0017 respectively. Counts of vacuoles in 50 serial sections from 50 sheep affected with natural scrapie and 19 healthy control animals, thoroughly confirmed the significance of vacuolated neurones in the spinal cords of sheep affected with scrapie.

Vacuolated neurones were more numerous in the cords of the group of sheep with clinically advanced natural scrapie (average per section 7.2 vacuoles), than the group with clinically non-advanced natural scrapie (average per section 1.9 vacuoles). These findings were discussed, attention being paid to the significant, but relatively small numbers of vacuoles observed in experimentally induced scrapie, in which the disease has a more rapid course than in natural cases.

Vacuoles were most numerous in the intumescentia of the cord, but the greatest number of neurones normally occur in these regions.

The location of the vacuolated neurones was of considerable

interest. Degenerated and vacuolated neurones had a similar distribution, and often showed bilateral symmetry. It was shown that the motor neurones were infrequently affected, not more than 7.8% of all vacuoles occurring in them in any group of sheep affected with scrapie. On the other hand, 89% to 92% of vacuoles occurred in the zones of the grey matter which contained internuncial cells, and vacuolation of the neurones of specific internuncial nuclei, such as those concerned with the transmission of contralateral reflexes, was often recorded. Finally the nucleus intermediolateralis was frequently involved. Vacuoles were present in the latter nucleus in 83% of the natural cases, and vacuolation or degeneration of neurones was seen in 98% of natural cases. In experimental scrapie, neurones of this nucleus were vacuolated in 50% of all cases, and degeneration or vacuolation was seen in 80% of the cases.

The above findings were discussed, and although the present limited stage of knowledge of ovine neurophysiology was stressed, it was suggested that they provide a possible basis for many of the symptoms of scrapie. The relative absence of lesions in the motor neurones is in agreement with the findings of clinicians and with the histological studies of the musculature undertaken by other workers, while lesions of the internuncial cells are a possible cause of the incoordination and abnormal gait observed in the disease. Many of the less characteristic symptoms may be attributed to sympathetic derangements resulting from lesions in the intermediolateral nucleus. Severe lesions here may interfere with the maintenance of homeostasis.

Studies of the angio-architecture of the grey matter suggested that regions of intense vacuolation lay near the largest blood vessels. The elective nature of the disease in the cord is discussed in relation to this observation.

The advantages and disadvantages of histological examinations of the cord in the routine diagnosis of natural scrapie were discussed.

In the spinal ganglia atypical and degenerated neurones

were very rare in all groups of sheep, and were not more common in those affected with scrapie. Occasionally neuronophagia and residual nodules were seen in both scrapie and control sheep. Intracytoplasmic lipofuscin granules and phloxinophilic bodies were present in some neurones, the former being more numerous in older sheep, but not being different in scrapie. The ganglion capsule, the interstitial connective tissue, the blood vessels, and the nerve fibres in the ganglia and radices showed no histological lesions which could be related to scrapie disease.

Vacuoles were found in occasional neurones from 70% to 90% of sheep in all the groups, and by counting the number of vacuoles seen in serial sections from natural scrapie, experimentally induced scrapie, and healthy control groups of sheep, it was shown that there was no significant difference between groups. Similar vacuoles were also observed in the Gasserian ganglia of healthy sheep. As in the spinal cord, no specific substance could be demonstrated in these vacuoles, but, whereas the vacuolated neurones of the cord in sheep with scrapie were often degenerated, in the spinal and Gasserian ganglia the vacuolated neurones were, apart from the vacuole, otherwise of normal morphology.

The above findings were discussed and it was concluded that, in contrast to the spinal cord, vacuolated neurones were not of significance in the spinal or Gasserian ganglia in scrapie disease. There was no evidence that the pruritus of scrapie was caused by morphological lesions in these ganglia.

The appearance of the neurones in the normal sympathetic ganglia of the sheep was described and discussed with particular reference to the arrangement of the Nissl substance. Occasional chromatolytic and degenerated neurones were recorded in both control and scrapie sheep, but in all except one animal they were not more numerous in scrapie. Hyaline eosinophilic masses, which were not specifically associated with scrapie, were described and it was suggested that they may originate from either large end bulbs or degenerated segments of the parent neurone.

Multiple intranuclear inclusions were recorded in two cases of natural scrapie and their histochemical and staining reactions were described. Double nucleoli, neuronophagia, and small cellular infiltrations into the ganglia were recorded in both scrapie and control sheep, but were not thought to be significant in scrapie disease. Lipofuscin granules and phloxinophilic bodies which had histochemical reactions similar to those in the spinal cord were recorded in the neurones of the sympathetic ganglia, but were not increased in sheep affected with scrapie. No degeneration of nerve fibres was found in the sympathetic trunks.

Paraganglia-like structures were recorded in the ganglia, although they appeared to be uncommon.

Vacuolated neurones were seen in the sympathetic ganglia of 50% to 70% of sheep from all groups, and by counting them in serial sections it was shown that they were not more numerous in scrapie. Similar vacuoles were recorded in the neurones of the adrenal medulla, where they were at least as numerous in control as scrapie sheep. As in the spinal ganglia no specific intravacuolar substance could be demonstrated and the neurones were, apart from the vacuole, morphologically unaltered.

It was suggested that many minor changes in sympathetic ganglia may be of a non-specific nature and it was concluded that there was no significant morphological difference, including vacuolation of neurones, between the ganglia of clinically healthy sheep and those with scrapie disease.

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THE HISTOPATHOLOGY OF THE SPINAL CORD IN
SCRAPIE DISEASE OF SHEEP

P. A. L. WIGHT

Moredun Research Institute, Gilmerton, Edinburgh

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P. A. L. WIGHT

Moredun Research Institute, Gilmerton, Edinburgh

INTRODUCTION

A detailed histological examination of the spinal cord in naturally occurring scrapie disease of sheep is not recorded in the literature.

Several investigators have noted abnormalities in the cord while engaged in more general observations on the whole, or more detailed work on other parts, of the central nervous system (Besnoit and Morel, 1898; Stockman, 1926; Bertrand, Carré and Lucam, 1937; Brownlee, 1940; Plummer, 1946; Lucam, Béchade and Saurat, 1950; Wagner, Goldstein, Doran and Hay, 1954; Palmer, 1957²; Zlotnik, 1958¹).

All the above workers are agreed that some involvement of the neurones may occur although opinions have varied as to whether this consists merely of vacuolisation or a more profound alteration. No attempt has been made to determine whether these lesions are constantly present in the disease. Glial activation, perivascular infiltrations and meningeal lesions have been thought significant by some investigators, while others have failed to observe these appearances. Similarly, considerable importance has sometimes been attached to the presence of eosinophilic intracytoplasmic inclusions, while, at other times, their specificity for scrapie disease has been denied. The occurrence of abnormalities in the spinal cord, and certainly the importance of vacuolisation, have not met with universal acceptance, for both in the past and in recent times hypotheses have been put forward suggesting that lesions of the skeletal musculature are of greater significance (McGowan, 1914; Bosanquet, Daniel and Parry, 1956; Delez, Gustafson and Luttrell, 1957).

This brief review of the literature suggests that it is necessary to investigate the histopathology of the spinal cord in scrapie with particular reference to the significance, constancy and distribution of the lesions.

MATERIALS AND METHODS

The material consisted of the spinal cords of 10 sheep affected with naturally occurring clinically advanced scrapie, 10 sheep with naturally occurring non-advanced scrapie, 16 sheep affected with experimentally induced scrapie and 18 apparently healthy sheep. The criteria upon which the designations "advanced" and "non-advanced" were based were mainly symptomatic, as the length of illness and history were in some cases unknown and in others unreliable. The non-advanced cases were kept under observation only long enough to establish a clinical diagnosis of scrapie; at the time of destruction they were affected with little more than slight pruritus, and in some cases mild ataxia. The

advanced cases were all affected at the time of destruction with ataxia and inco-ordination and had at some time shown varying degrees of pruritus. The experimental group of sheep all showed clinical signs of scrapie. This group contained 11 hogs inoculated intracerebrally at 6 months to 8 months of age and killed when 12 to 18 months old, 2 lambs inoculated intracerebrally at birth and killed when 6 months old, and 3 cast ewes killed about 9 months after subcutaneous inoculation. The inoculum was a suspension of pooled brains obtained from the serial passage of scrapie brains which originated from the successful transmission experiments of Wilson, Anderson and Smith (1950). The age range of the scrapie sheep was covered by the control group of sheep, the majority of which were between 2 and 4 years old, although 5 were more than 7 years and 3 were 8 months old.

All animals were killed by venesection and the cords fixed as soon after death as possible in 10 per cent. formol saline for 1 month. Transverse pieces of cord from the fourth, sixth, seventh and eighth cervical, first and sixth thoracic, third, fifth and sixth lumbar, and first sacral segments, were post-fixed for 48 hours in corrosive formol, dehydrated in alcohols, cleared in benzol and embedded in paraffin. Routinely, sections, 7μ thick, were stained with haematoxylin and eosin, but a variety of staining methods were used for specific purposes such as cresyl fast violet and eosin methylene blue for Nissl substance, Mallory's phosphotungstic acid for glia fibrils, Holmes' methods for axis cylinders and intracellular neurofibrils, Lendrum's phloxine-tartrazine, Gomori's aldehyde fuchsin, Feulgen's method, and the periodic acid-Schiff reagent, picro-Mallory, Heidenhain's iron haematoxylin, Giemsa for intracytoplasmic inclusions, and safranin O-eriocyanine A for degenerating neurones.

Vacuolated neurones were counted with the 4 mm. objective in 20 consecutive 7μ serial sections from each of the 10 segments of the spinal cord listed above. Two hundred sections were thus examined from each of the 20 cases of natural scrapie, the 16 cases of experimental scrapie and 10 control animals. At the same time the location of the vacuolated neurones was determined by arbitrarily dividing the grey matter into 3 zones. Zone A comprised all the neurones ventral to the subs. gel. rolandi and dorsal to the large neurones which lie in relatively well defined nuclei in the ventro-lateral and ventro-medial aspect of the ventral horn. The majority of the latter are motor neurones and they were designated Zone B. The final Zone, C, consisted of the nucleus intermedio-lateralis where it formed a well defined horn (T_6 and L_3).

The number of neurones containing phloxinophilic bodies larger than 2.5μ diameter was counted in 4 serial sections from each of 5 segments of the cord (C_4 , C_8 , T_6 , L_3 and L_6) in 15 natural cases of scrapie, 10 experimental ones and 15 control animals.

Astrocytes were demonstrated by the Cajal method in sections from the cervical and lumbo-sacral intumescencia and the mid-thoracic region. Transverse and longitudinal sections from the mid-cervical and mid-thoracic regions and the cervical and lumbo-sacral intumescencia were stained by the Sudan IV technique and Spielmeyer's method to demonstrate degenerating myelin, and the Bielschowsky-Glees method for degenerating axons. The longitudinal sections were taken at three depths so as to include the ventral, lateral and dorsal funiculi. Seventeen

natural scrapie animals 12 experimental scrapie and 17 control animals were examined by these methods.

RESULTS

Grey Matter

Degeneration of neurones. Various forms of degeneration of neurones ranging from slight central chromatolysis to complete necrosis were seen in all cases of natural scrapie. Chromatolysis was seen at all stages, from early central chromatolysis to complete dissolution of the Nissl granules with eccentricity of the nucleus and moderate swelling of the cell body. Acute swelling was not frequent, but severe cell change when the nucleus was pyknotic, the cytoplasm foamy and often eosinophilic, and the neurofibrils disintegrated and clumped, was relatively common. Sclerotic neurones, often with fine peripheral vacuoles, and necrotic neurones were both observed.

In the severe cases, large numbers of all the above forms of degeneration could be seen in any single section, usually together with large numbers of vacuolated neurones, but in some of the less severe cases it was often necessary to examine as many as 10 consecutive sections in order to find one degenerating cell. The neurones of the subs. grisea centralis, the posterior and anterior cornu commissuralis nuclei, and the cells of the intermediate zones were most affected. Degenerative changes were seen in the nucleus intermedio-lateralis in 95 per cent of the scrapie cords, and were sometimes marked in this nucleus when relatively slight elsewhere. The large motor neurones of the ventral horns rarely showed degenerative changes.

Similar forms of degeneration were seen in the same areas of the grey matter in the experimental animals, but were very rare, although occasional chromatolytic cells were seen in every case. At least one or two chromatolytic neurones were observed in the nucleus intermedio-lateralis in 75 per cent of the experimental animals.

Diseased neurones were rarely seen in the spinal cords of the control group of sheep, although in one particular case 5 cells in the 200 sections showed central chromatolysis and 2 showed severe cell change.

Vacuolisation of neurones. Vacuolated neurones were seen in all the cases of natural scrapie and in 15 of 16 experimental animals. The vacuoles were either single or multiple and their size varied from small structures which did not noticeably distort the outline of the cell body to extremely large unilocular or multilocular structures. The majority of vacuoles were situated in the cell body, although in some neurones the cell processes only were vacuolated. In the latter cases the vacuoles were either small or were large enough to cause extreme ballooning of the process. The cytoplasm of vacuolated neurones was either unaltered or showed various

forms of degeneration. In some cells a necrotic eosinophilic rim around the vacuoles was all that remained of the cell body. As a rule the structure of the nucleus was not altered unless actual degeneration of cytoplasm had taken place. Neurofibrils were often normal in the cytoplasm of vacuolated cells although slightly denser round the edges of the vacuole. They were never present within the vacuoles. In some cells disintegrated neurofibrils were seen in the form of argentophilic staining dust in small isolated areas or around the edge of the smaller vacuoles.

In all cases of natural and most cases of experimental scrapie, some vacuoles contained masses of pale eosinophilic material, sometimes spherical in shape and often situated at the periphery of the cavity. This material was not phloxinophilic when differentiated according to Lendrum's original method, and was P.A.S. negative, although small numbers of P.A.S. positive granules were occasionally seen in some vacuoles.

Two thousand sections from 10 control sheep were carefully examined and a total of only 4 vacuoles, an average of 0.002 vacuoles per section, each in a different animal, were seen. In 3 neurones these vacuoles were small and single and the cytoplasm was otherwise healthy, while the fourth neurone was sclerotic and contained a rather large peripheral vacuole. Details of vacuolation of neurones in the 3 groups of scrapie animals are given in Tables 1 to 3. It can be seen that while in the advanced natural scrapie groups vacuoles were fairly numerous with an average of 7.2 per section (range 0.8 to 34.6), in the non-advanced scrapie sheep vacuoles were less often found with an average of only 1.9 per section (range 0.1 to 13), while in the experimental animals there were only a few vacuoles, averaging 0.12 vacuoles per section (range 0 to 0.35).

The distribution of vacuolated neurones in the various levels and zones of the spinal cord was as follows. (a) The majority of the vacuoles, irrespective of the scrapie groups, occurred in the *intumescentia*, both cervical and lumbo-sacral. (b) From 88 to 92 per cent of the vacuoles were found in Zone A. All distinguishable nuclei were affected to some degree, but the neurones of the *cornua commissuralis anteriores* and *posteriores*, the *substantia grisea centralis* and the intermediate zones were more severely vacuolated. (c) Zone B, which contains the motor neurones, was as a whole, very little affected, containing only 7.8 and 4.2 per cent of all vacuoles in the advanced and non-advanced natural scrapie groups respectively. In the experimental sheep 6 per cent of vacuoles were present in Zone B, mainly due to the relatively large proportion of vacuoles occurring in the motor area in one sheep. (d) In Zone C, although only a relatively small group of neurones restricted to the thoraco-lumbar region were affected, they contained a large per-

TABLE 3
EXPERIMENTAL SCRAPIE GROUP
Vacuolation of Neurons in the Spinal Cord

Case no.	Average number of vacuoles per 20 sections in regions of the cord						Total no. of vacuoles in 200 sections from the four regions of the cord			
	Mid-cervical region C ₄		Cervical intrumescentia C ₆ C ₇ C ₈ T ₁		Thoraco-lumbar region T ₆ L ₃			Lumbo-sacral intumescentia L ₅ L ₆ S ₁		
	Zone A	Zone B	Zone A	Zone B	Zone A	Zone B		Zone C	Zone A	Zone B
W20	3	—	8.5	—	2.5	—	1.5	8	—	69
W21	2	—	2.5	—	2.5	—	1.0	4.3	—	32
W24	2	—	3.75	0.5	0.5	—	0.5	3.7	—	31
W17	—	—	2.5	—	—	—	—	7.3	1	35
W18	3	—	0.5	—	—	—	0.5	5.3	—	22
W13	—	—	2.75	0.5	1.5	—	—	4.0	—	28
W23	1	—	2.5	—	1.5	—	0.5	1.0	—	18
W25	—	—	2.0	—	1.5	—	1.5	2.66	0.33	20
W22	—	—	0.5	—	3.5	—	—	3.0	4.3	32
W31	1	—	0.25	—	—	—	—	0.33	—	3
W19	—	—	—	—	—	—	—	—	—	0
W28	—	—	5.75	—	1	—	3.5	5.6	—	54
W29	—	—	0.75	—	0.5	—	2.0	1	—	11
W30	—	—	0.25	—	0.5	—	—	1	—	5
W32	1	—	0.75	—	—	—	—	0.66	—	6
W34	—	—	2.25	—	—	—	—	—	—	9

centage of vacuoles. In advanced scrapie the vacuoles comprised 9.8 per cent of the total number found in the region, in non-advanced scrapie 29 per cent, and in experimental scrapie 44 per cent. As noted earlier the neurones of this zone, which is the intermedio-lateral nucleus, are frequently affected by other degenerative changes.

Cytoplasmic granules. Lipochrome granules and phloxinophilic bodies were seen in the cytoplasm of neurones in both scrapie and control sheep. The lipochrome granules were faintly yellow in unstained sections, were well stained by Gomori's aldehyde fuchsin, were P.A.S. positive and stained brick red with Sudan IV in both frozen and paraffin sections. They stained faintly with Schiff without hydrolysis, but not with eosin or by Lendrum's phloxine-tartrazine method. These granules were most prominent in the 5 very old control sheep, but otherwise there was no difference in their occurrence in control and scrapie animals.

The phloxinophilic bodies were red when stained with Giemsa, picro-Mallory and eosin-methylene blue, and were stained by Heidenhain's iron haematoxylin. They were negative by the following staining methods: Feulgen, P.A.S., Gomori's aldehyde fuchsin and Sudan IV in both frozen and paraffin sections. These bodies were usually spherical in shape and their size varied from less than 0.5μ up to 5μ in diameter. Small bodies of less than 0.5μ diameter were found in all groups of sheep, but it appeared as if larger phloxinophilic bodies were unusually numerous in some, but not all, of the scrapie group. Thus in 2 out of 15 control sheep examined only 2 neurones contained inclusions larger than 2.5μ in diameter, while in 10 out of 15 natural scrapie sheep a total of 132 cells contained these large inclusions. The bodies were rare in vacuolated cells and their frequency bore no obvious relationship to severity of vacuolation or clinical symptoms. Furthermore, in the experimental disease large inclusions were seen in only 7 cells from the cords of 2 out of 10 sheep examined.

Glial elements. Neither an over-all increase in glial elements nor massive generalised neuronophagia was present in any of the scrapie cases, and there was no obvious increase in perineural satellites. The Cajal gold chloride method revealed neither clasmotodendrosis nor gliosis. However, severely degenerated neurones sometimes showed evidence of neuronophagia by one or a small number of microglia. Neuronophagia did not appear to be associated with vacuolated cells unless they were severely diseased, so that it seemed as if vacuolation *per se* did not stimulate phagocytosis.

Large and small phloxinophilic inclusions were infrequently seen in the nuclei of astrocytes of both the control and scrapie sheep. The smaller inclusions were often surrounded by a halo, whereas the larger bodies distended the nucleus to 2 or 3 times its normal

diameter. Occasionally P.A.S. positive material was seen in the cytoplasm of glial cells in both control and scrapie sheep.

Vascular system and the meninges. Perivascular infiltrations by cells resembling lymphocytes were present in 3 control animals, in 5 experimental cases of scrapie and in 6 advanced and 5 non-advanced natural cases of scrapie. In 14 of the scrapie animals infiltrations were infrequent and did not differ in degree or extent from those noted in the control sheep. They consisted at the most of 3 layers of infiltrating cells or a small focal accumulation along the vessel wall, and they were present in the grey or in the white matter close to the grey matter. In one advanced and in one non-advanced scrapie animal more extensive changes were visible in the form of moderately frequent perivascular cuffings.

Occasional fat-laden glia cells were seen close to a blood vessel in the grey matter of 4 cases of scrapie, and paradventitial deposits of material resembling lipochrome were seen in both scrapie and control animals. Small subpial infiltrations of leucocytes, involving only a limited region of the meningeal circumferences, were seen in one advanced and 3 non-advanced cases of natural scrapie. No meningeal lesions were seen in the experimental disease.

White Matter

In 2 natural cases of scrapie definite degeneration of myelin and axis cylinders in the medial region of the ventral funiculus was demonstrated by the methods of Bielschowsky, Sudan IV and Spielmeyer. In one of these cases the dorsal funiculus was also involved, but in the upper cervical region only. In transverse sections the changes in the former two funiculi were seen to extend from the cervical to the lower lumbar region. In longitudinal sections the degenerated myelin sheath was seen in the form of myelin ovoids, while myelophages laden with neutral fat lay both in and outside the digestion chambers. The axis cylinders were considerably swollen and covered with argentophilic granules, or were beaded and vacuolated, or broken into fragments. There was an obvious loss of axons due to their complete destruction, but healthy fibres could be seen amongst the degenerated.

In 6 other natural scrapie animals and in 1 control sheep discrete myelin and axonal lesions were present. The type of lesion and their distribution were similar to the preceding 2 cases, but very seldom were more than a few fibres in each longitudinal section affected. Neutral fats could be demonstrated in all these cases.

In a further 6 natural scrapie cases and in 6 control animals slight myelin changes were observed, but in no case could neutral fat be demonstrated. In all these cases occasional chains of myelin ovoids were seen in longitudinal sections, and in Sudan IV preparations these ovoids were orange-red in contrast to the brick-red coloration of normal sheep myelin. In some of the ovoids, haemat-

oxylin staining showed myeloclasts without fat droplets. Although these lesions were as a whole rather rare, the general impression was that the chains of myelin ovoids were more frequent, particularly in the medial aspect of the ventral funiculus, in the scrapie cases than in the control animals.

Significant changes could not be detected in the white matter in the 12 cases of experimental scrapie, in the remaining 3 cases of natural scrapie or in the 10 control sheep.

DISCUSSION

The results of the present study show that the most constant lesions in the spinal cord from both natural and experimental scrapie cases are degeneration and vacuolation of neurones. In this respect the results are similar to those in the medulla as recorded by many workers (Bertrand *et al.*, 1937; Holman and Pattison, 1943; Wagner *et al.*, 1954; Palmer, 1957²; Zlotnik, 1958). The present study does not, however, support the work of Brownlee (1940), who found no lesions, other than large vacuoles, in apparently healthy neurones of the spinal cord.

Careful examination of cords from healthy sheep revealed that neuronal changes, including vacuolation, are so rare that they may almost be ignored. The number of vacuoles in the diseased cords was lowest in experimental scrapie and highest in advanced natural scrapie, which is very similar to that observed in the medulla (Zlotnik, 1958¹, 1958²). Bosanquet *et al.* (1956) and Parry (1957), found only occasional vacuoles in the cord which they considered of little significance because of their presence in normal sheep. This opinion is not supported by the present study, for vacuolated neurones were found extremely rarely in the healthy animals while increasing numbers of vacuoles were found in the 3 groups of scrapie sheep.

According to Bertrand *et al.* (1937) and Brownlee (1940) the large neurones of the anterior horns were principally affected in scrapie, and Lucam *et al.* (1950) specifically recorded that the motor neurones were affected. Wilson *et al.* (1950) could not find any vacuoles in the large neurones of the spinal cord in experimental scrapie, but they do not state whether vacuoles occurred in small and medium sized neurones. The present results, however, show that only a very small proportion of vacuoles were found in the motor areas where the largest cells of the cord occur (Zone B; 4.2 to 7.8 per cent). This finding confirms the suggestion of Cassirer (1898) that on a symptomatic basis the motor protoneuron may be excluded, and it also explains the fact that neither Bosanquet *et al.* (1957) nor Hulland (1958) found any evidence of motorneuronal patterns in the skeletal muscles.

The majority of lesions, both vacuoles and various forms of degeneration, were present in Zone A. This zone contains in the main small and medium sized cells interposed between the in-

coming dorsal root fibres, or fibres descending from higher centres, and the motor neurones. Lesions in these intercalated neurones may be one of the factors causing inco-ordination in scrapie by interference with the passage of ipsilateral and contralateral reflexes, involvement of the nuclei cornu commissuralis and the neurones of the substantia grisea centralis particularly suggesting that contralateral reflexes are often affected.

Bertrand *et al.* (1937) suggested that central lesions of the autonomic nervous system may account for the pruritus seen in scrapie: whether or not this is so, the present survey shows that the intermedio-lateral nucleus (Zone C), which contains the preganglionic neurones of the sympathetic nervous system was frequently and sometimes severely affected. Some of the less characteristic symptoms of scrapie suggest autonomic involvement, and it is possible that severe degeneration of the preganglionic neurones may severely undermine the animals' ability to maintain homeostasis.

Eosinophilic intracytoplasmic bodies were described in the neurones from scrapie sheep by Stockman (1926) who considered them significant. Similar or identical bodies have been reported by Brownlee and Wilson (1932) in apparently healthy sheep and in those affected by louping-ill. Palmer (1957¹) reported eosinophilic structures inside vacuoles and in the cytoplasm of neurones in scrapie sheep, while Zlotnik (1957¹, 1958¹) found eosinophilic bodies in both healthy and scrapie animals. In the present study, phloxinophilic bodies were found in the neurones of the cord from both healthy and scrapie sheep, although in a proportion of cases of the natural disease increased numbers of large inclusions were present. The presence of these bodies in healthy sheep makes it impossible to consider them specific for scrapie, and the absence of the large bodies from the majority of the experimental cases and many of the natural cases suggests that they have little significance in the disease. However, their increase in some cases of scrapie may be due to altered neuronal secretion or metabolism in the disease.

Although slight neuronophagia of some degenerated neurones was observed in the scrapie cases, glial changes in the spinal cord were not a prominent feature of the disease and astrocytic proliferation was completely absent. In the latter respect the results do not agree with those of Bertrand *et al.* (1937) who reported an astrocytic gliosis in the anterior horns of the cord. Astrocytic proliferation has been reported in higher centres, especially the medulla, by Bertrand *et al.* (1937), Wagner, *et al.* (1954) and Zlotnik (1958¹). Palmer (1957²) could find no astrocytic changes in the brain.

Bertrand *et al.* (1937) recorded that perivascular infiltrations were rare in scrapie cases when these were destroyed in the terminal stages of the disease. Wilson *et al.* (1950) described lesions of subacute meningoencephalitis with perivascular infiltrations in experimental

scrapie. Zlotnik (1958¹) recorded that of 3 groups of advanced scrapie, non-advanced scrapie and healthy sheep, respectively, examined by him, the advanced scrapie were the least affected and the healthy sheep the most severely affected. Zlotnik (1958²) found no difference in vascular changes in experimental scrapie and healthy sheep and did not consider them significant. In the present study the incidence and severity of vascular lesions were more or less similar in both advanced and non-advanced natural scrapie groups. In healthy animals and experimentally infected sheep vascular changes were less frequently seen than in the natural disease, but the difference in incidence and severity in all 4 groups of sheep was so slight that it is doubtful if much significance can be attached to these lesions in the spinal cord.

Secondary degeneration of fibres in the white matter was marked in 2 cases of scrapie, but on the whole the positive cases of scrapie showed no more than occasional degenerating fibres discretely scattered in the ventro-medial part of the ventral funiculus and to a lesser extent in the lateral funiculus. This absence of tract degeneration is in agreement with the findings of Cassirer (1898), Stockman (1926) and Brownlee (1940). These authors suggested that occasional degenerating fibres may occur although, because of the capriciousness of the Marchi method in the sheep, these findings were not considered significant. Although age and chronic wasting conditions tend to cause small numbers of fibres to degenerate (Duncan, 1931), the peculiar localisation of the fibres in the scrapie cases suggests they are not due to these incidental factors. King (1911) observed degenerating tracts in similar regions subsequent to experimental medulla lesions in the sheep, and as the neurones of the brain stem are definitely involved in scrapie (Zlotnik, 1958¹), it is possible that the degeneration seen in the spinal white matter may be secondary to these lesions.

CONCLUSIONS

Vacuolated neurones were either absent or extremely rare in the spinal cords of healthy sheep.

The most significant lesion in the spinal cord in scrapie was vacuolation and degeneration of neurones.

All animals affected with the natural disease show neuronal changes in their spinal cords. In the experimental disease lesions were seen in only 94 per cent of animals.

Vacuolisation of neurones in the natural cases bore a more or less significant relationship to the severity of the clinical symptoms.

The most commonly affected cells were the intercalated neurones and the central neurones of the sympathetic system, while the motor neurones were relatively infrequently affected.

Intracytoplasmic phloxinophilic bodies were observed in both

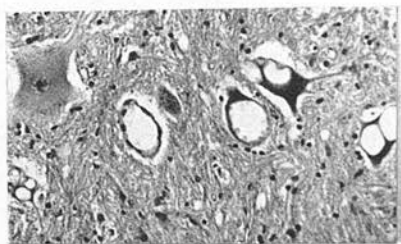


Fig 1

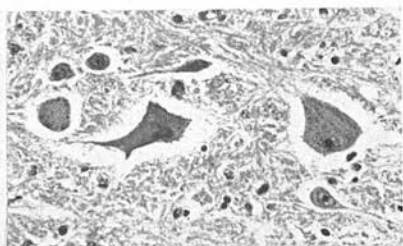


Fig 2

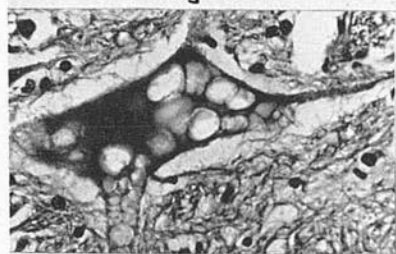


Fig 3

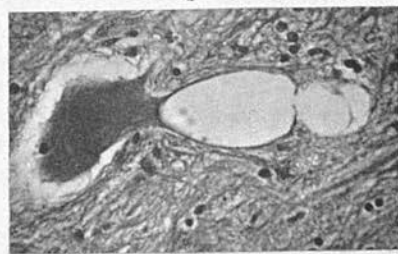


Fig 4

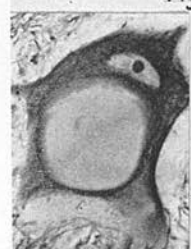


Fig 5a



Fig 5b

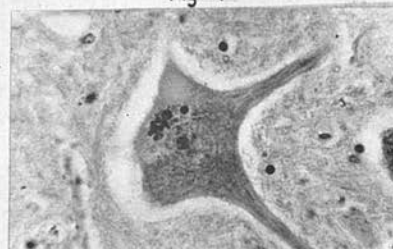


Fig 6



Fig 7

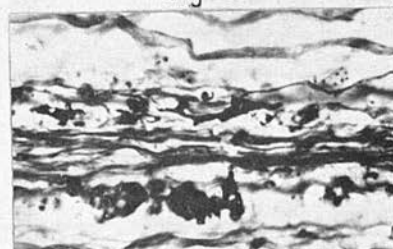


Fig 8



Fig 9

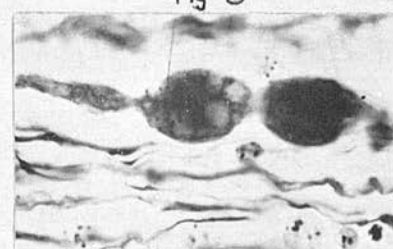


Fig 10

healthy and scrapie sheep. However, in some of the natural scrapie cases, large phloxinophilic bodies were unusually numerous.

Increased satellitosis and massive neuronophagia were rare, but occasional degenerated cells showed evidence of neuronophagia.

Secondary degeneration affecting both axis cylinders and myelin was found in the ventral and lateral funiculi. Usually few fibres were affected, and in only 2 cases was the degree of degeneration marked.

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LEGENDS TO ILLUSTRATIONS

- Fig. 1. Vacuolated neurones and neurone showing early acute swelling in the intermediate zone. H and E $\times 130$.
- Fig. 2. Degenerating and chromatolytic neurones in the intermediate zone. H and E $\times 130$.
- Fig. 3. Multilocular vacuolisation of a neurone and phagocytosing glia cells. H and E $\times 320$.
- Fig. 4. Vacuolisation of a process of a neurone. H and E $\times 320$.
- Fig. 5. (a) Vacuolated neurone with apparently normal neurofibrils elsewhere in the cell cytoplasm. (b) Early vacuole formation. Holme's method $\times 330$.
- Fig. 6. Phloxinophilic inclusions in the cytoplasm of a neurone. Phloxine/Tartrazine. $\times 320$.
- Fig. 7. Digestion chambers and myelin ovoids in the ventral funiculus. Spielmeyer's method $\times 80$.
- Fig. 8. Fragmented axons in the ventral funiculus. Bielschowsky method $\times 320$.
- Fig. 9. Swollen axon in the ventral funiculus showing granular argentophilic deposit. Bielschowsky method $\times 320$.
- Fig. 10. Beaded swelling and vacuolation of an axon in the ventral funiculus. Bielschowsky method $\times 320$.

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