CONTRIBUTIONS TO COMPARATIVE NEUROPATHOLOGY

by

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CONTENTS

INTRODUCTION AND ACKNOWLEDGEMENTS

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In 1959 the author of this thesis was awarded the degree of Doctor of Veterinary Medicine and Surgery of the University of Edinburgh for a thesis entitled "Observations on Swayback Disease of Lambs in South-East Scotland". That work comprised field and laboratory studies of naturally-occurring swayback with emphasis upon the neuropathology of the disease. One of the conclusions reached was that contemporary ideas on the pathogenesis of the lesions in the central nervous system of affected lambs were unlikely to be correct and a more probable alternative hypothesis was advanced.

Since that time concern with the pathogenesis of swayback has been sustained, and a more general interest has been developed in comparative neuropathology as a whole, especially in diseases of the very young animal, wherein developmental processes may interact with the more familiar pathological patterns of inflammation and degeneration. Growth of these interests has been facilitated by employment since 1960 in the Department of Experimental Pathology of the Moredun Research Institute. The papers incorporated in this thesis represent a substantial proportion of the author's work of the past decade.

The material falls naturally into five sections:- copper deficiency and swayback in sheep; copper deficiency and swayback in goats and swine; border disease of lambs; miscellaneous neuropathological conditions and

developmental considerations. A continuous but sometimes tenuous thread of concern for the interactions of disease and development runs through the published work, culminating in an attempt to relate specific examples of such interactions to general concepts of teratology. This formed the basis of a lecture to the Royal Society of Medicine, an abstract of which is included amongst the publications.

Over the past ten years, the author has benefited immeasurably from the scientific help, discussion and criticism of colleagues in his own and other disciplines. The nature of the contributions of those whose names appear as co-authors in several of the papers is clear and is most gratefully acknowledged. Their willingness to co-operate conceptually, materially and technically has removed obstacles to the progress of several studies. The debts to those whose names do not appear are innumerable and collectively no less important. They are acknowledged in the papers to which their work contributed and it would be invidious to single out a few for further mention here. However, a special debt of gratitude is owed to Dr. J.T. Stamp whose restrained direction of the work and firm criticism of the conclusions reached at all stages, have done much to shape these studies.

SUMMARY AND CONCLUSIONS

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This thesis embodies the published results of a decade's experience in comparative neuropathology. The approach has been predominantly morphological, and most of the reports concern ruminants, though a variety of species both domestic and feral are included. Some studies of normal structure and development have also been carried out mainly to illuminate the background of pathological processes.

At first an attempt has been made to describe, define, and analyse a given disease in terms of pathogenesis and aetiology, proceeding thereafter to research on particular aspects of the problem. Full development of this pattern has been achieved only in a few instances, notably swayback and Border disease. In swayback a general understanding of the process has been reached, and the remaining problems appear to centre around species and tissue differences in mitochondria and the effects of copper and other substances on the enzyme systems contained therein. With Border disease many broad avenues of research still require to be explored before a clear picture of the condition can be obtained.

No general conclusions are applicable to the work as a whole.

The diseases which have been considered appear to have a high degree of host specificity and it is fortunate that the natural hosts can be used for experimental work. The problems and dangers of extrapolating experimental results and hypotheses from one species to another have been amply

demonstrated in the work on copper deficiency.

COMMENTARY

SECTION A. - SWAYBACK AND COPPER DEFICIENCY IN SHEEP

The work in this section can be best considered under two headings:- pathology and aetiology.

1. PATHOLOGY

(a) The Pathogenesis of the Lesions in the Central Nervous System:

From 1940 the accepted view was that swayback "is essentially a cerebral demyelination with consequent destruction of axis cylinders and later cavitation, and secondary degeneration of the motor pathways". Such was the interpretation of J.R.M. Innes (1940). The re-examination of the problems of swayback by the author of the present thesis revealed pathological changes which were irreconcilable in the terms of this hypothesis, and an alternative theory was proposed, which considered that during the later phases of maturation of the central nervous system the normal development of nerve cells, myelin sheaths and glia may be inhibited. In an earlier thesis (D.V.M. & S. University of Edinburgh, 1959) it was suggested that "the lesions of swayback are due to neurodysgenesis involving nerve cells and glia in addition to myelin sheaths".

This hypothesis forms the point of departure of the present work. In Paper 1 the neuropathology of 190 cases of swayback was re-examined in greater detail and the changes in 27 clinically severe cases in 9 age

groups were compared. The results supported the hypothesis and revealed patterns of degeneration the significance of which was not fully appreciated at the time. These patterns concerned both nerve cells and nerve fibres especially in the brain stem and spinal cord. The intensity of the disease process in both components was similar and increased from birth to about 8 weeks of age and thereafter declined with the gradual formation of glial scar tissue. It was concluded that the morbid process in spinal cord may continue after birth. The limitations of routine histological methods however, did not permit any assessment as to whether the nerve cells were damaged concomitantly with nerve fibres or whether their degeneration was of a primary or secondary nature.

Until this time the attention of pathologists had been focussed predominantly upon the striking lesions in the cerebral and spinal white matter, but in view of the above findings it seemed appropriate to examine in greater detail the affects of the swayback process upon the nerve cell (Papers 2 and 7). The association of a low copper status and swayback had long been known and it had recently been established that the cytochrome oxidase activity of brain was depleted in copper deficiency and swayback. By histochemical methods (Paper 2) it was found that depletion of cytochrome oxidase activity in grey matter was severe in cases up to 12 weeks of age and that depletion was most marked in those cell groups in which morphological evidence of neuronal degeneration most frequently occurs. This result gave rise to the idea that copper deficiency might have

widespread neuronal effects though only some nerve cells showed degeneration in routine histological preparations.

It was therefore decided to try and quantitate the extent and degree of damage in defined anatomical sites. For this purpose particular segments of spinal cord were chosen from the entire collection of over 200 cases of swayback and compared with similar tissues from 20 normal lambs (Paper 7). Methods of counting cells and scoring the degree of damage were developed and it was found that pathological change in nerve cells was, in fact, widespread but the chance of nerve cells becoming irreversibly damaged was greater in the cervical and lumbosacral enlargements than elsewhere. In swayback lambs over 16 weeks of age there was a significant reduction of nerve cells in the cervical enlargement. The majority of these cells were in the ventral horn and therefore mainly lower motor neurons. In the enlargements many such cells are concerned with limb movements and the greater damage to them might be due to specific functional stresses of locomotion. As clinical ataxia progressed the stresses on surviving cells would be increased. If these neuronal changes were secondary to myelin sheath changes, extensive damage to peripheral nerve myelin would be expected, especially when the gradual nature of the neuronal degeneration is considered. However, all studies of swayback have shown that peripheral nerve changes are mild and not found consistently. Thus the possibility that slow degeneration of the nerve cells might be the basis of the pathogenesis of swayback began to be more seriously entertained; degeneration of the myelin sheath would

occur when the cell was no longer able to support its own axon. In other words, the myelin changes in spinal cord might represent a Wallerian-type degeneration.

The pathological findings in so far as the brain stem and spinal cord are concerned supported this view, but at first sight the cerebral lesions, assuming the mechanism of their formation to be similar, did not fit the hypothesis. Cortical neuronal necrosis and the products of degeneration of cerebral myelin are, in the amounts found, trivial and inconsistent with the size of the lesions in the cerebral white matter (Paper 2). These objections would be largely overcome however, if it could be shown that cerebral axons degenerated prior to myelination; were this the case the stimulus to myelin formation would be lost. The relative preservation of cortical nerve cell bodies in the vicinity of the lesions can be explained on the basis that if the fibres are unmyelinated at the time of axonal degeneration the cell itself would be unlikely to have developed its full functional capability and hence be less liable to functional stresses. Disruption of the axon - the peripheral cytoplasm of the cell - due to oxidative enzyme depletion would remove the burden of maintaining this remote cytoplasmic territory and preservation of the cell body would be favoured - albeit as a non-functional unit.

The electron microscope has been the tool by means of which strong evidence in support of these ideas has been assembled (Papers 10, 11 and 12). The cystic and gelatinous cerebral lesions have been found to contain

numerous degenerate unmyelinated neural processes and insignificant amounts of myelin degeneration products. In the spinal cord degenerate neural processes of similar morphology have been found, but in this situation many have been associated with intact sheaths or sheaths showing only the earliest signs of degeneration. When severely degenerate myelin sheaths were encountered the axons contained by them were usually in the process of being phagocytosed; the ultrastructural appearances were consistent with those of Wallerian degeneration. Conversely intact axons surrounded by such degenerate sheaths were never found. These observations can only be interpreted as evidence that in swayback myelin degeneration is secondary to degeneration of the axon.

Histological and histochemical comparisons of the lower motor neuron in swayback and in experimental peripheral nerve section have shown a remarkable degree of similarity. In both conditions the neuron shows chromatolysis, a lowering of oxidative enzyme activity, retispersion of the Golgi apparatus and a reduction in lysosomes (Papers 1, 2, 8 and 14). Axonal reaction i.e. the neuronal changes which follow nerve section, was all the more severe when the copper status of the experimental lambs was low. It would be imprudent to interpret these findings as proof that in swayback the neuronal lesions are due to axonal reaction. However, there are compelling reasons for considering that spontaneous rupture of axons may occur in swayback and contribute to the overall pattern of disease. The disease appears at times of rapid growth of the foetus or lamb, the lesions

occur in cells whose metabolism may be depressed by lowered respiratory enzyme activity, and whose axons pass through regions which undergo rapid local growth in which a high rate of fibre elongation is required (Paper 33). Not only are respiratory enzymes lowered but the mitochondria in which they are located are the organelles which show structural abnormalities at a very early stage of the degenerative process (Papers 9, 10 and 11). Axonal flow is from the perikaryon distally (Weiss, Taylor and Pillai, 1962). Thus the more remote the portions of the cytoplasm are from the cell body the earlier they are likely to become hypoxic and their structural integrity compromised.

It has also been observed (Paper 33) that the oligodendroglial myelin-forming cells of the normal foetus contain demonstrable cytochrome oxidase activity only during myelination. Presumably depletion of enzyme activity in these cells would have a depressing effect upon the rate of myelinogenesis, and thus make a small additional contribution to the overall process of swayback.

In summary, it is clear that swayback is not a demyelinating disease in the true sense of the term. The lesions in the white matter result, in the main, from destruction of the axon. If, as in the cerebrum the axon degenerates before myelination then no myelin forms, but should the process occur when the axon has already myelinated, as in many spinal fibres, then a Wallerian-type degeneration occurs. However, depression of the activity of the myelin forming cells may play a supplementary role. Both axonal

degeneration and depressed oligodendroglial activity may be the result of respiratory embarrassment at cellular level, the neuron being incapable of providing for the demands of rapid growth especially when combined with those of function. As the locus of cytochrome oxidase, and an organelle which shows structural changes early in the degenerative process, the mitochondrion may be of fundamental importance in swayback. In short the pathogenesis of this disease would appear to be "a neurodysgenesis involving nerve cells and glia in addition to myelin sheaths".

(b) The Cardiovascular System:

Cardiovascular lesions involving mainly the elastic laminae of the blood vessels are common sequelae of copper deficiency in the pig and domestic fowl. No significant extraneural pathology has been described in swayback, though an unusual appearance of blood vessels, mainly arterioles, in the white matter of the cerebral hemispheres, di-encephalon, and grey matter of the spinal cord was noted in some cases of swayback (Paper 1). In view of this it seemed that a systematic examination of the cardiovascular system by morphological, biochemical and biophysical methods should be undertaken. The opportunity arose in connection with a visit to the United States when co-operation with workers experienced in the techniques of connective tissue biochemistry and tensile strength measurements was obtained. However, no significant deviations from the normal patterns

were found in either copper deficient sheep or lambs affected with swayback (Paper 13). The reasons for this are puzzling and obscure, but one obvious speculation centres around the differences in amount and anatomical distribution of elastica in the major vessels of the ruminant compared with other species.

2. AETIOLOGY

Since 1937 when Bennetts and Chapman in Australia first demonstrated the prophylactic effects of dosing with copper salts, outbreaks of swayback have been consistently associated with deficiency of this metal in the affected lamb and its mother and sometimes also in the herbage and soil. Synthetic diets deficient in copper have been used extensively in experiments in monogastric species, but the lack of a suitable copper deficient source of roughage has, until recently, prevented the use of such diets in ruminants. This lack of a suitable experimental diet for ruminants has delayed the direct examination of aetiological mechanisms in swayback. Until the difficulties were overcome investigations were carried out in flocks with naturally-occurring disease, or in laboratory experiments using selected natural foodstuffs low in copper and heavy molybdenum and sulphate supplements (Papers 3, 4, 5). These systems rarely gave reproducible results. The main problem of interpretation lay in determining whether copper deficiency alone was responsible for the disease or whether other

unknown factors were acting in concert, either directly, or upon the ability of the pregnant ewe to absorb, retain, or utilise dietary or stored copper. Many of these problems remain unsolved at the present time but it has been shown (Paper 15) that swayback can be reproduced in housed sheep by feeding a semisynthetic diet low in copper.

One of the functions of the Moredun Research Institute is to apply the results of research to the control of animal disease in the field. From studies of the pathogenesis and aetiology of swayback it has been possible to outline means by which losses from the disease may be limited by prophylaxis and treatment (Paper 6). Not only is dosing of the pregnant ewe of value in preventing losses from the disease but prophylaxis or treatment of lambs in flocks when the delayed form of the disease has appeared may be of value.

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SECTION B. - SWAYBACK AND COPPER DEFICIENCY IN GOATS AND PIGS

By virtue of its use for well over a century, the term swayback describes a specific disease of sheep. Reports of similar disorders in other ruminant species have appeared from time to time but none have been supported by neuropathological and biochemical examinations.

For these reasons the appearance of a disease in a kid (Paper 16) which showed the clinical signs and pathological changes characteristic of swayback was of considerable interest. The circumstances of the occurrence were in an experimental herd maintained for the study of vitamin A metabolism and it was hoped that close examination of this controlled situation would yield information of aetiological importance.

In the two subsequent seasons a few kids showed ataxia and histological and histochemical confirmation of the diagnosis of swayback was obtained (Paper 17). An inverse relationship between copper and vitamin A in the blood of the kids was found, but no particular significance has yet been attached to this observation.

As already mentioned in Section 1, the difficulty of establishing controlled copper deficiency in ruminants caused some experimental investigations into the aetiology of swayback to be carried out in monogastric animals. Methods of producing copper deficiency in pigs had been established for some years but no results of histological or histochemical examinations of the central nervous system had been published. Nor had nervous symptoms

in experimentally copper-deprived swine been described though copper deficiency has been implicated in naturally-occurring porcine ataxia (McGavin, Ranby and Tammemagi, 1962). It was felt that if a critical deficiency could be obtained in foetal or newborn piglets the chances of obtaining neurological changes would be maximised. It proved impossible to deplete adult sows of copper by simple dietary means and breeding stock with which to produce congenitally deficient piglets had themselves to be reared from a few days of age in a controlled state of copper deficiency (Paper 18). Six litters were produced by these means and all the piglets sampled had brain and liver copper levels well within the range associated with swayback in lambs. No clinical ataxia developed however, and only one pig showed any neuropathological changes when it was killed at 104 days of age (Paper 19). These consisted of lesions in the spinal tracts, the distribution, histological and ultramicroscopic appearances of which bore some similarity to those of swayback.

The low incidence of clinical and pathological changes in the nervous system in experimental copper deficiency in goats, pigs and later also sheep (Paper 15) suggests that factors other than copper deficiency per se e.g. climatic variations, dietary levels of molybdenum and sulphate and vitamin A and genetic factors may have a conditioning role in the development of swayback.

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SECTION C. - BORDER DISEASE OF LAMBS

The study of Border disease of lambs which was initiated in 1962 derives from and is complementary to the work on swayback in the sense that Border disease is another congenital disease of sheep the most characteristic clinical signs and pathological changes of which relate to the central nervous system.

At the beginning of the study the diagnostic criteria for the recognition of Border disease were poorly defined. Small "scruffy-looking" lambs with coarse hairy fleeces, sometimes abnormally pigmented and showing low viability may be products of bad management combined with a variety of intercurrent diseases. Only when a proportion of such lambs also showed the characteristic tonico-clonic spasms could a tentative diagnosis of Border disease be made. Defective myelin staining of central white matter was the only significant pathological change and this only correlated with the presence of neurological signs.

The first step in this study was to try and establish more definitive criteria for diagnosis. These were achieved as a result of intensive histological examination of 53 sheep (Paper 20). It was confirmed that hypomyelinogenesis was an appropriate term for the neurological lesion. It was also found that hypomyelinogenesis was present in a proportion of animals without obvious nervous symptoms and was associated with abnormalities of the interfascicular glia. Hypomyelinogenesis was most severe in newborn

and young lambs and was associated for the first few weeks of life with abnormal accumulations of interfascicular lipid droplets containing cholesterol and cholesterol esters. In slightly older lambs the clinical signs were usually milder and were associated with less severe hypomyelinogenesis and little or no interfascicular lipid. In short it appeared that the nervous symptoms and pathological changes might resolve with time.

Electron microscopy of the spinal myelin in Border disease (Paper 22) showed fewer and finer myelin sheaths than in normal controls of similar age. The interfascicular glia had features more usually associated with astrocytes than with oligodendroglia or microglia. The processes of these cells were found wrapped around otherwise naked axons, but not condensing into compact myelin lamellae. Some myelin sheaths of otherwise normal appearance showed splits at the intra-period line and cavity formation resembling the oedema of alkyl tin poisoning. Occasional lipid-containing macrophages indicated that demyelinating processes might also be involved.

A major step forward in Border disease research was the demonstration of its transmissible nature (Papers 21 and 23). These experiments also showed that early foetal death, mummification and abortion can be associated with the disease. In addition to the characteristic neuropathological changes seen in naturally-occurring Border disease, about 20% of experimental cases showed cavitation or gelatinous transformation of the cerebral white matter. These lesions superficially resembled those observed in swayback but in most instances they could be distinguished from the latter by the presence

of numerous compound granular corpuscles and P.A.S.-positive, non-argentophilic plaques; the lesions were of a more patently destructive nature than those of swayback.

At the present time the histological and ultrastructural evidence appears to indicate that the interfascicular myelin-forming cell may be an important site of the disease process in Border disease. One tentative hypothesis is that the differentiation of spongioblasts into oligodendroglia and astrocytes may be defective, resulting in retarded and abnormal sheath formation. These views along with those on swayback have been summarised and an attempt made to relate them to general concepts of teratology (Paper 24). However, studies on the aetiology, pathogenesis and epidemiology of experimental Border disease are still in progress and it may be that this working hypothesis will require refinement or revision in the future.

SECTION D. - MISCELLANEOUS NEUROPATHOLOGICAL CONDITIONS

The collected papers in this section of the thesis do not form a homogeneous group. They reflect a sustained personal concern with diseases of the newborn and a growing awareness of neurological disease in animals by the veterinary profession as a whole. In order to meet the increased interest and to attempt to define in pathological terms the conditions being encountered in farm animals a neuropathological diagnostic service was established in 1960 at Moredun, serving principally the Veterinary Investigation Service. Three of the seven papers in this section (Papers 25, 27 and 29) stem directly from the activities of this diagnostic service and report respectively an outbreak of congenital hydrocephalus of undetermined aetiology in Ayrshire calves, a retinal atrophy of hill sheep, and a cerebellar cortical degeneration in Aberdeen Angus calves. The last-mentioned condition is of interest for two particular reasons. The epidemiology strongly suggested a genetic aetiology with a dominant mode of inheritance - a rare occurrence in bovine genetics. Secondly, the investigation which was spread over seven years demonstrates the value of a central laboratory accumulating data from a large catchment area.

The study of ataxia in the red deer (Cervus elaphus) was the direct result of interest in swayback, the original motivation being to determine whether the two conditions were analogous or not. The results (Paper 26) showed that the similarities were superficial. This view was subsequently reinforced by histochemical and manometric cytochrome oxidase assays

which revealed no significant differences between affected and non-affected park deer and free ranging wild red deer from an area where the disease was unknown. The low cobalt status of the deer park was corrected by top-dressing with cobalt sulphate and the investigation was terminated due to failure of the disease to re-establish itself after the culling of all cases for pathological and enzyme studies (unpublished observations).

Work on swayback has also stimulated interest in other aspects of copper metabolism. Chronic copper poisoning in sheep is a commonly occurring condition the general pathology of which is well described. As in hepato-lenticular degeneration (Wilson's disease) in man, cirrhosis due to storage of excess copper in the liver is a frequent post-mortem finding. Brain damage in sheep chronically intoxicated with copper had not been reported however. The opportunity to examine brains from such sheep arose recently (Paper 31) and spongy changes affecting the white matter at all subcerebral levels of the central nervous system was found. Since publication, this finding has been confirmed in other instances of chronic copper poisoning and in a few animals subacutely intoxicated by copper (unpublished results). The nature and pathogenesis of these lesions have not yet been determined but further work is being planned.

The experimental pathology of scrapie and the possibly related transmissible encephalopathy of mink are interests acquired upon the resignation of Dr. I. Zlotnik. Transmissible mink encephalopathy has a long incubation period, protracted course, fatal termination and pathological

changes which closely identify it with scrapie. The host susceptibility pattern and passage characteristics however show differences, the significance of which has yet to be determined (Papers 28 and 32).

Both scrapie and transmissible mink encephalopathy have been classified amongst the slow virus diseases. This group includes visna in sheep, Creutzfeld-Jakob disease and kuru in man and provisionally also a number of other conditions of man and animals the aetiology of which is unknown. The long incubation periods which characterise members of the group have demonstrated that extraordinary care and control of technique are necessary in experimental pathological investigations.

In grass-sickness of horses, the aetiology is unknown but neuronal changes bearing some resemblance to those in scrapie have been described in autonomic ganglia. For this reason and because the epidemiology might be explained in terms of a "slow" viral cause, an attempt was made to transmit the disease to small laboratory animals in experiments similar to those which had previously proved successful with scrapie. In the course of this work which was not fruitful, efforts were also made to improve the criteria by which diagnosis of grass sickness might be confirmed. Pathological examination revealed the presence of hitherto unreported lesions in the central nervous system (Paper 30). These consisted of chromatolytic neurons selectively distributed in those particular brain stem nuclei relating to the cephalic portion of the parasympathetic component of the autonomic nervous system. Chromatolysis was less regularly found also among the

ventral horn cells of the sacral spinal cord. Since publication of this paper the lesions have been found consistently in a further series of cases of grass sickness and it is considered that they provide an acceptable additional criterion for the confirmation of the diagnosis. The widespread but selective involvement of the autonomic nervous system in grass sickness may be important in the further consideration of the nature of this condition.

SECTION E. - DEVELOPMENTAL CONSIDERATIONS

The literature contains admirable descriptions of the development of the mammalian nervous system considered from the viewpoint of the embryologist and anatomist, but the pathologist concerned with developmental disease is less well served. The study of morphogenesis and histochemical aspects of myelin in the nervous system of the foetal sheep (Paper 34) was undertaken to obtain a greater understanding of the possible nature of the disordered developmental processes which occur in both swayback and border disease. Two factors appeared to be of particular relevance to swayback, namely the development of intense cytochrome oxidase activity during differentiation of definitive cell types and the overall rate of change within the central nervous system between 70 and 110 days gestation.

With respect to Border disease the timing of the commencement of myelination at various levels of the nervous system and the marshalling of myelin-like lipids to the perivascular spaces and adjacent neuropil of the particular region just prior to myelination have been valuable in forming concepts regarding the possible nature of the pathological process.

The subcommissural organ is a clearly differentiated highly specialised zone of ependyma about which little is known. In the sheep it is a prominent and fairly extensive structure; it is bound to catch the eye and stimulate curiosity as to its function. The investigations described in Paper 33 arose essentially from curiosity at a time when material and personnel of diverse technical accomplishment were available. The work did not

throw any fresh light upon the possible functions of the subcommissural organ and the paper is essentially descriptive.

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FURTHER OBSERVATIONS ON SWAYBACK

I. Transitional Pathology

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INTRODUCTION

The neonatal ataxias of lambs known as swayback and enzootic ataxia, were first studied comprehensively by Innes and Shearer (1940). Other contributions to the pathology have been made by Stewart (1932), Bull, Marston, Murnane and Lines (1938), Bennetts and Beck (1942), McDonald (1942), Tabusso (1942), Schulz, van der Merwe, van Rensburg, and Swart (1951), Palsson and Grimsson (1953), Spais (1956), Dandemaev and Abramova (1956), Barlow (1958), Gracey and Todd (1958), Jensen, Maag and Flint (1958), Howell and Davison (1959), Barlow, Purves, Butler and Macintyre (1960), Schulz and Behrens (1960), Spais, Palsson and van Bogaert (1961). Consideration of the epidemiology, clinical signs, pathology and biochemistry leaves little doubt that all these authors were working with the same condition.

Having discussed the possibility of myelin aplasia being involved in the pathogenesis, Innes and Shearer concluded that swayback was a demyelinating encephalopathy similar to Schilder's disease in infants. The basis of this conclusion was a certain resemblance of the cerebral lesion of swayback to a few cases of the congenital form of the latter condition (Mackay, 1940; Winkleman and Moore, 1942). This view was not challenged for about twenty years, when Behrens and Schulz (1959) and Schulz and Behrens (1960) suggested that the lesions of swayback resulted from venous stasis, oedema, and peri-vascular softening and necrosis. Spais *et al.* (1961) appeared to be in broad agreement with this view, though they used the term " spongy transformation ".

This assessment, however, only takes account of lesions of the cerebral white matter and Barlow (1958) and Barlow *et al.* (1960) found that whilst such lesions were almost pathognomonic for swayback, they were not an essential part of the disease, being absent from 59·5 per cent. of a series of 79 cases which they examined. These authors regarded scattered lesions of neuronal hyaline necrosis and fibre degeneration in the brain stem and spinal cord as the essential pathology of swayback. Such lesions were present in 100 per cent. of their cases and had been mentioned by the Australian workers (Bull *et al.*, 1937; Bennetts and Chapman, 1937; Bennetts and Beck, 1942; McDonald, 1942) and in England by Stewart (1932). The criteria used by Barlow and his colleagues for the diagnosis of swayback were (1) ataxia in the newborn or young lamb; (2) cavitation, or gelatinous lesions of the cerebral white matter and/or a characteristic histological picture of chromatolysis, neurone necrosis and myelin degeneration in the brain stem and spinal cord; (3) a low copper status, i.e. blood copper content of the affected lamb and its mother of less than 60 μg/100 ml. blood or liver copper content of the affected lamb of less than 80 ppm. copper D.W. (for lambs up to 9 weeks of age).

These authors made parallel studies of apparently normal lambs and

foetuses and were led to the hypothesis that an unknown biochemical defect might be acting as an inhibitor of normal development, or result in the degeneration of formed but immature nervous elements. They thought that cells, fibres and glia might be involved concurrently and they suggested the term neuro-dysgenesis to define the condition.

The present communication describes an attempt to study the changes in the lesions of swayback during the first nine months of post-natal life and this has been termed " transitional pathology ". This name has been chosen as both the progress and regress of the lesions have been considered.

Whilst it is not known whether the disease process is active at birth in those animals in which the onset of clinical signs is delayed, or that it is extinct by nine months of age, the data presented has the advantage of being derived from personally observed cases of approximately equal clinical severity. It reveals trends which may be both of importance in the greater understanding of swayback and of help in diagnosis. In addition, changes in the central nervous system are described which confirm and extend previous published findings.

MATERIALS AND METHODS

The material consisted of a collection of 190 cases of swayback assembled over 6 years from about 30 farms in Scotland and the north of England. Only material which satisfied the previously defined diagnostic criteria was included. For descriptive purposes the entire collection was used quite freely, but to illustrate the transitional pathology in detail only 27 lambs were considered. These 27 cases were drawn at random from those which had shown severe ataxia throughout the period of clinical observation, but which were able to walk about and feed themselves without assistance.

The cases were divided into 9 arbitrary age groups. Previous experience had shown that changes in the pathology might proceed at a greater rate in the younger animal, and thus four groups covered the first month of life and four groups the subsequent four months. In the last group, animals over five months of age, the number of cases fulfilling the necessary clinical criteria, was insufficient to allow random selection. This group consisted of one animal each at 6, 7 and 9 months of age.

Routine histological techniques were applied to standard blocks of tissue embedded in paraffin, supplemented where necessary by special neuropathological procedures on frozen sections from adjacent blocks. The stains used included Mayers haematoxylin and eosin, Giemsa or Spoerri's methods for Nissl granules, Holmes's method for neurofibrils and axons, phosphotungstic acid haematoxylin or Cajal's gold sublimate for fibrous glia (occasionally Anderson's Victoria Blue was used), van Gieson for collagenous connective tissue, Gordon and Sweet's method for reticulin, Sudan IV for neutral fat, Glees' variant of the Marchi technique for degenerating myelin, and the Smith-Quigley or Spielmeyer methods for normal myelin.

In an effort to minimise the subjective variations in interpretation, 3 separate assessments of the material in the 27 selected cases were made during a period of 12 months. Those features which showed a degree

of variation from one assessment to another have been omitted from the table but will be described in the text.

RESULTS

The results of the examination of the 27 selected cases are summarised in Table 1. As the major histological features of swayback have been described in an earlier paper, only a brief account of these will be included here, more attention being devoted to less prominent and more rare histological changes and to tracing the development and fate of the lesions.

TABLE 1

AGE	NO	Cong·tal or Delayed	CEREBRUM		HIPPO-CAMP'S	CEREBELLUM		Brain Stem Nuclei.	SPINAL CORD.			
			Grey Matter	White Matter		Central Nuclei.	Cortical Neurones		Grey Matter	White Matter. Fat	Demyel.	Gliosis
BIRTH	1	C	—	Mac. G	—	■	—	■	■	■	—	—
TO	2	C	—	—	—	—	—	■	■	■	—	—
48 hrs	3	C	—	Mic. G	—	—	—	■	■	■	—	—
48 hrs	4	C	—	Mic. G	◣	—	◣	■	■	■	—	—
TO	5	C	—	Mic. G	—	◣	◣	■	■	■	—	—
1 WEEK	6	C	◣	Mac. C	—	—	—	■	■	■	—	—
1 WEEK	7	C	—	—	—	—	—	■	■	■	■	—
TO	8	C	◣	Mac. C	—	—	—	■	■	■	■	—
2 WEEKS	9	C	—	—	—	—	—	■	■	■	■	?
2 W'KS	10	C	—	Mic. C	—	—	—	■	■	■	■	■
TO	11	C	—	—	—	◣	◣	■	■	■	■	■
4 W'KS	12	D	—	—	—	—	—	■	■	■	■	■
4 W'KS	13	C	◣	—	◣	◣	◣	■	■	■	■	■
TO	14	C	◣	Mac. C	—	—	—	■	■	■	■	■
8 W'KS	15	D	—	—	—	—	—	■	■	■	■	■
8 W'KS	16	C	—	—	—	—	—	◣	◣	■	■	■
TO	17	C	—	—	—	—	—	◣	◣	■	■	■
12 W'KS	18	D	—	—	◣	—	◣	■	■	■	■	■
12 W'KS	19	D	◣	—	◣	◣	◣	■	■	■	■	■
TO	20	D	◣	—	◣	◣	◣	■	■	■	■	■
16 W'KS	21	D	—	—	—	◣	◣	■	■	■	■	■
16 W'KS	22	D	—	—	—	—	—	■	■	■	■	■
TO	23	D	—	—	◣	—	◣	■	■	■	■	■
20 W'KS	24	D	◣	—	◣	—	◣	■	■	■	■	■
OVER	25	D	◣	—	◣	—	—	■	■	—	■	■
20 W'KS	26	C	◣	—	—	—	—	■	■	—	■	■
	27	C	◣	—	—	—	—	■	—	■	■	■

Mac. G – Macroscopical gelatinous lesions
Mic. G – Microscopical " "
Mac. C – Macroscopic cavitations
Mic. C – Microscopic "

◣ Slight cell and fibre changes
■ Moderate focal cell and fibre changes
Severe " " " "
■ Severe cell and fibre changes throughout

Pathological details of swayback lambs from birth to 9 months of age.

Cerebrum

Cerebral congestion and oedema were notable in the youngest cases. The oedema was often accompanied by histiocytic proliferations within the pia arachnoid which, in the oldest lambs, showed as localised fibrous tissue thickenings of the meninges (Fig. 1). The cortical neurones were also oedematous showing vacuolation, hydropic distention of the nuclear membrane, or appearing as nuclei surrounded by haloes of watery cytoplasm (Fig. 2). The laminar architecture of the cortex was sometimes inapparent, the cells being uniformly small and closely packed, rounded and with large vesicular nuclei and little Nissl substance. A few cells were apparently necrotic.

In these very young lambs gelatinous lesions or cavities of the cerebral white matter were common, but over the whole series they were present in less than 50 per cent. of cases. They were rarely seen in animals more than three weeks old and never in lambs over 8 weeks of age. When large, these lesions replaced most of the cerebral white matter, but smaller lesions tended to be confined to the axis of the convolutions of the occipital, temporal and parietal regions. The detailed appearance of these lesions has been described previously (Barlow *et al.*, 1960) and the present study has yielded no further information regarding their nature. In lambs more than 8 weeks of age lesions in the cerebral hemispheres as a whole were inconstant and slight.

Hypochromasia and shrinkage of neurons in the Vth layer of the occipital cortex were not uncommon and in the oldest animals sclerotic cells were observed in this region. There was also an increase in the numbers of neuronal satellite glia. (Figs. 3 and 4).

Hippocampus

Inconstant changes were present in the hippocampus. In the youngest animals chromatolysis and hydropic changes similar to those in the cortex were seen in both the granule and ganglion cells. In older animals sclerotic changes in the ganglion cells were not uncommon and were sometimes accompanied by a light astrocytic proliferation just beneath the granular layer.

Cerebellum

The normal variation in the histological appearance of the neurons in this division of the brain is such that it is difficult to ascribe pathological significance to any but the most severe changes. In the youngest animals, vacuolation and apparent chromatolysis of both Purkinje cells and the neurons of the central nuclei were occasionally seen. Support for the idea that cerebellar pathology might be present in swayback was obtained from 3 cases between 1 and 3 weeks of age in which there was heterotopia of Purkinjé cells (Fig. 5). Also a few older lambs showed necrosis of Purkinjé and type II Golgi cells and/or neurons in the dentate nucleus

(Fig. 6). The former changes were small in extent and found mainly in the paramedian and ansiform lobes. Thinning of the granular layer, with depletion of cells, was observed in several cases but was usually confined to folia of the paramedian lobe (Fig. 7).

Brain Stem

The extended study of this region of the central nervous system has provided little further information. The red and vestibular nuclei have been found excellent sites for the demonstration of neuronal degenerations, frankly necrotic neurons being found usually in at least one of three serial sections in animals less than 20 weeks of age. It is not possible to state from this study, however, that these nuclei are always affected in swayback. The large neurons of the reticular formation also showed degenerative forms in the majority of cases. In addition, occasional necrotic cells have been found in the lateral cuneate nucleus and in the inferior olive. The facial and spinal trigeminal nuclei less regularly contained degenerate nerve cells. The dorsal motor vagus is apparently spared.

In the oldest groups examined degenerate neurons were observed less frequently. This may have been due to the fact that after undergoing hyaline necrosis or granular disintegration such cells appear to rupture and lyse away to blend into the ground substance (Table 1).

Spinal Cord

It was from this division of the central nervous system that the best evidence for a transitional pathology was obtained. Table 1 clearly reveals differences between the various age groups.

Neuronal changes identical with those observed in the brain stem nuclei were noted at all levels of the cord. Usually they were most numerous in the cervical and lumbo-sacral enlargements and most prominent amongst the large motor cells of the ventral horns. Similar changes were also seen in the cells of the ventral grey commissure, the lateral horn and the cells of Clark's column. Less frequently the spindle-shaped cells of the substantia gelatinosa had the appearance of severe central chromatolysis.

Whilst cellular degenerations were observed in the cord in almost all the animals studied, they were most numerous in animals 2 to 12 weeks of age. In the youngest animals frankly degenerate cells were relatively infrequent, but swollen, rather basophilic neurons with an abraded appearance were very common. In the oldest lambs degenerate neurons were also infrequent. Since effete cells were observed to lyse and blend with the ground substance with little glial response, it seems reasonable to suppose that necrotic cells would have been more numerous in these older cases at an earlier age.

Nerve fibre changes were constantly observed in that part of

the lateral funiculus adjacent to the dorsal root. Less marked changes of the same type were present in the sulco-marginal funiculus and not infrequently also at the periphery of the lateral funiculus between these two regions. More rarely the dorsal funiculi also were affected. The type and intensity of the pathological changes found in the long fibres of the spinal cord varied considerably between individuals and between age groups. However, there did appear to be a direct relationship between the severity of these lesions and the clinical condition of the individual, and thus by selection of approximately equivalent clinical cases it was considered that valid comparisons might be made.

In the youngest animals a proportion of fibres in the affected regions stained bright red with Sudan IV. A fairly good correlation was obtained on adjacent blocks using Glees' method for degenerating myelin, though the localisation was not so precise since more fibres gave a positive reaction. In some cases Marchi positive fibres were present in the dorsal funiculus.

In lambs less than 2 weeks of age it was extremely rare to find evidence of demyelination in the affected areas using the iron or lithium haematoxylin methods. At this age evidence of reparative gliosis was not obtained (Table 1). Substantially greater quantities of neutral fat, disposed within the myelin sheaths or as free globules in the interstitial tissues of the affected areas, were evident in lambs 2 to 8 weeks of age. In a proportion of these appropriately stained frozen sections revealed actual demyelination (Fig. 8).

At about 2 weeks of age the earliest signs of glial proliferation could be observed in gold sublimate preparations. With advancing age the demyelination and glial reaction in the affected areas of the cord became more pronounced, whilst the amounts of neutral fat were progressively reduced and disposed more in the interstitial tissues than in the fibres themselves. A few gitter cells appeared and transported the fat to perivascular spaces, but small amounts were still present in the oldest animal examined. In no case was collagenous transformation of the glial scar observed.

Blood Vessels

A particular appearance of blood vessel walls was commonly observed in cases of swayback. The vessels concerned were some of the smaller arterioles in the white matter of the cerebral hemispheres, the diencephalon and the grey matter of the spinal cord. The intima was thickened and slightly hyaline and the lumen of the vessel reduced. No abnormal infiltrations or degenerations have yet been detected which might account for this appearance (Fig. 9).

DISCUSSION

At the outset it must be admitted that the material in this study imposes serious limitations on the interpretation of the pathology. The basic clinical criteria are crude and apply to the individual

only at the time it was killed, no consideration being taken of the actual or possible course of the disease. Even using a basic pool of nearly 200 personally observed cases, there were insufficient numbers of animals of similar clinical status to allow for more rigid control of the material before random selection into each of the 9 age groups. Thus selection has tended to load the older groups with cases in which the clinical signs did not appear until some time after birth. In such cases lesions of the cerebral white matter were absent (Table 1) and there is no knowledge as to whether lesions elsewhere in the central nervous system precede the onset of clinical signs. On the other hand, the time-spacing of the groups is such that unless the pathological changes in the brain stem and spinal cord of delayed cases differed qualitatively from those in congenital cases the effect on the overall picture would be slight. In addition, the quantitative assessment of the lesions was sufficiently coarse to obscure further any slight differences. Thus what appears theoretically to be a deficiency of the material and methods may not be so, if the results are regarded as showing trends, rather than absolute values.

In a previous paper (Barlow *et al.*, 1960) it was stated that cases showing cerebral cavitation or gelatinous lesions were all affected at birth, and that such lesions were not found in animals which had survived more than 3 weeks unless they had been hand fed. In this extended study, however, a well-circumscribed cavity was observed in an animal which had lived under natural conditions for 6 weeks.

Whilst Spais *et al.* (1961) have described laminar necrosis in the frontal, parieto-angular, temporal and occipital regions of the cortex of some of their cases, similar severe changes have not been observed in the present work. Changes resembling chromatolysis, vacuolation and necrosis have been seen in individual cells of these regions in younger animals, which taken together with more extensive hyperchromasia, satellitosis and sclerosis observed in the same regions of the oldest cases, may now be regarded as pathological changes and not artifacts. Whether they occur as a primary effect, or secondary to changes in the subjacent white matter, or as a result of a generalised disturbance of intra-cranial pressure or metabolism is not known.

The appearance of the lesions in the cerebral white matter is essentially the same as that described in earlier papers, and also by Spais *et al.* (1961), Schulz and Behrens (1960) and Innes and Shearer (1940) though evidence of perivascular liquefactive necrosis was lacking. It was again apparent (Table 1) that the presence of such lesions is not essential for clinical ataxia, though their absence may have some bearing on the survival of the individual. If, as the evidence suggests, meningeal thickenings in older lambs are the result of oedema at an earlier stage, and chronic cell changes are the end product of a degenerative process, it may be that cerebral

damage occurs in swayback even though lesions of the white matter are not detectable.

The lesions in the hippocampus and cerebellar cortex reported in this paper were of the same type as those occurring in the cerebral cortex. They were an inconstant feature of this series of cases and of such minor extent that it seems unlikely that they play an important part in the disease. It is, however, with respect to the brain stem and spinal cord changes that the most striking variations with age have been found. Granular disintegration of the neurofibrillary apparatus and hyaline necrosis were particularly noticeable amongst the large multipolar neurons and there appears to be a predeliction for the red and vestibular nuclei, the reticular formation and the ventral horns of the spinal cord. Whilst damaged cells in these areas were not uncommon in newborn animals, there was a progressive increase in animals up to 8 weeks of age, following which there appeared to be a gradual decrease in numbers which could be accounted for by lysis of effete cells. In animals 2 to 8 weeks of age similar changes were also observed in other types of cells, including the cells of Clark's column, thus confirming a finding of the first writer on swayback (Stewart, 1932).

Because of the diffuse distribution of affected fibres in the brain stem, the transitional pathology of the white matter of the long tracts was most readily followed in the spinal cord. The earliest changes recognised were in medullary sheaths of the dorsal part of the lateral funiculi and the sulco-marginal funiculi, which showed the staining qualities of neutral fat. These and other fibres were also Marchi positive and more weakly sudanophil. In slightly older animals, however, the number of truly fatty fibres was greater, free fat globules were present in the interstitium, and the earliest signs of demyelination were apparent with the Spielmeyer or Smith-Quigley methods. Later the demyelinated areas became more clearly defined ; free fatty globules were more numerous and there was some evidence of their transport to local perivascular spaces by macrophages. At this stage an increased density of glial fibres became apparent in the affected regions of the cord. With the passage of time there was further removal of the fatty products and replacement by fine glial fibres, so that in the oldest animal examined only traces of fat were present in clearly demyelinated glial scars. McDonald (1942) reported subsequent fibrosis in these areas, but this has not been confirmed within the limits of the present study.

These findings suggest that there may be an active progression of the morbid process of swayback during the early part of post-natal life. Though the cord is myelinated in all areas at birth, the first few weeks of life involve a continued rapid growth with consequent continuation of active myelin and glia formation. Failure of the mechanism of myelin fabrication, or myelin degeneration might both equally well account for the pathological changes seen. Thus

the present study cannot contribute further to the discussion of pathogenesis.

The observations show parallel variations in the numbers of affected fibres and cells in the brain stem and cord. In view of the limitations of the methods employed the inference that both may be involved concomitantly cannot be regarded very strongly. Changes in either component of the neurone might result in secondary changes in the other, a situation requiring a much more sophisticated approach than the one employed at present. It is clear that routine histological examination of paraffin sections might not reveal changes in the cord of some very young or very old cases of swayback. Degenerate nerve cells in such cases may be few and widely scattered, and early or late changes in the white matter may be difficult to appreciate by the techniques available. This may provide a possible explanation for recent statements by Innes and Saunders (1962) that cell changes were not observed elsewhere than in the red nucleus, and by Spais *et al.* (1961) that lesions were not found in either the spinal cord tracts or the anterior horns in otherwise apparently similar material.

CONCLUSIONS

A description of the pathology of swayback has been compiled from the examination of 190 cases. Twenty-seven cases in 9 age groups selected at random have been compared. The term transitional pathology is used to include both the progress and regress of the lesions.

It is suggested that the morbid process of swayback may continue after birth and be particularly evident in the spinal cord. Confirmation has been found for the conclusions of others in respect of lesions in the cerebral cortex and Clark's column. Lesions in the hippocampus and cerebellar cortex have been described. A possible explanation for the failure of other workers to demonstrate spinal cord pathology in swayback has been suggested. Features of value to the diagnostic pathologist are emphasised.

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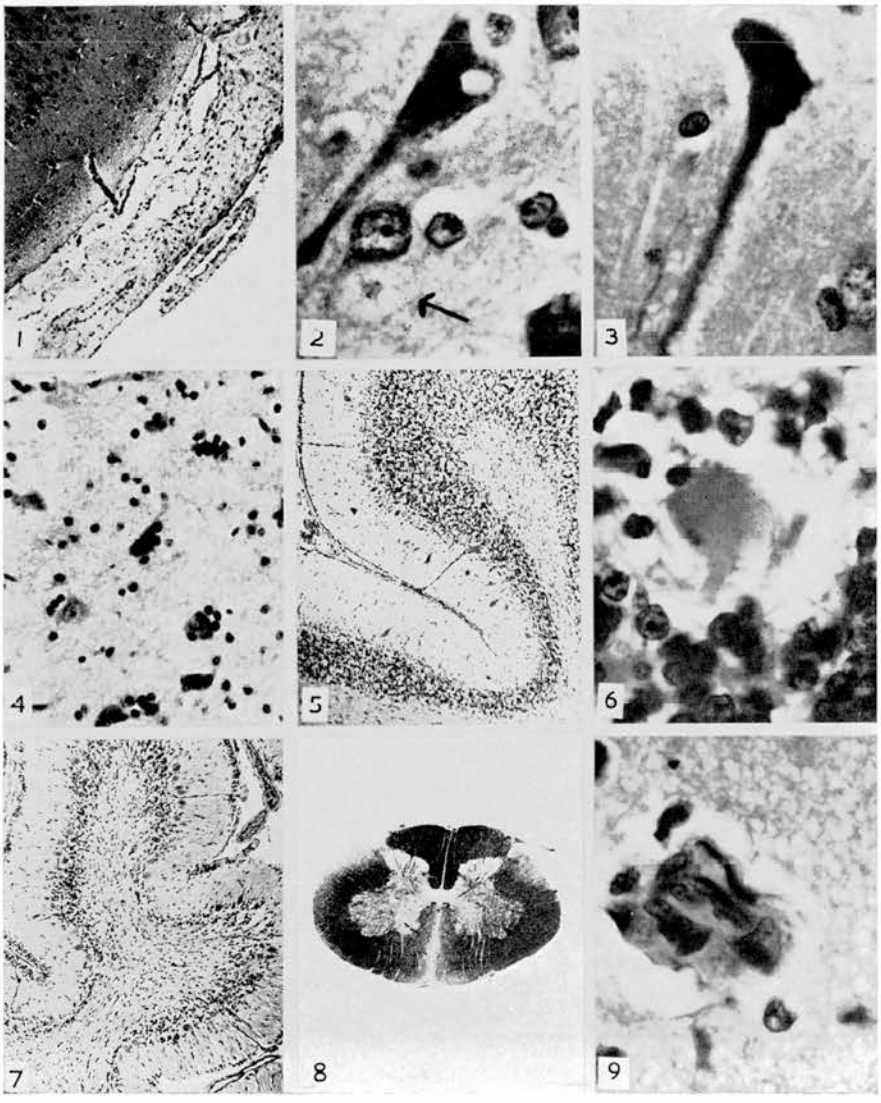
LEGENDS TO ILLUSTRATIONS

Fig. 1. Meningeal fibrosis over the surface of a cerebral convolution in a 9-month-old case of swayback. Note the eosinophilic material in the top right hand corner H. & E. × 75.
Fig. 2. Vacuolation of the cytoplasm of cortical neurons in 24-hour-old case of swayback. The arrow indicates a severely distended watery nucleus. H. & E. × 800.
Fig. 3. A sclerotic neuron in the occipital cortex of an 18-week-old case of swayback. H. & E. × 800.
Fig. 4. Satellitosis in the cortex of an 18-week old case of swayback. H. & E. × 190.
Fig. 5. Heterotopia of Purkinje cells. Cerebellum from case of swayback, 2 to 3 weeks old. H. & E. × 40.
Fig. 6. Purkinje cell from 18-week-old case of swayback showing hyaline necrosis. H. & E. × 980.
Fig. 7. Thinning and rarefaction of the granular layer of the cerebellum. Same case as Fig. 5. H. & E. × 42.
Fig. 8. Demyelination in the spinal cord of case of swayback 18 weeks old. Smith-Quigley × 3.
Fig. 9. Small arteriole in the thalamus of a case of swayback 4 weeks old, showing thickening of the wall and narrowing of the lumen. H. & E. × 880.



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II. Histochemical Localisation of Cytochrome Oxidase Activity in the Central Nervous System

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II Histochemical Localisation of Cytochrome Oxidase Activity in the Central Nervous System

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INTRODUCTION

The possibility that cytochrome oxidase is a copper containing enzyme was first considered by Keilin and Hartree (1938) and was confirmed 20 years later by Wainio, Wende, and Shimp (1958). Gallagher, Judah and Rees (1956) demonstrated that even moderate deficiencies of copper resulted in a reduction of succinoxidase and cytochrome oxidase activity in rat tissues. They showed that the reduction of succinoxidase activity was the result of actual depletion of cytochrome oxidase and suggested that this might be due to failure to synthesise haemα, the prosthetic group of the latter enzyme. Anderson and Tove (1958) showed that copper is directly involved in the synthesis of haem.

These findings stimulated Howell and Davison (1959) to investigate the cytochrome oxidase activity of lambs affected with swayback, a myelinopathic condition associated with a low copper status of the affected lamb and its mother (Bennetts and Chapman, 1937). They found a significant reduction in the copper content and cytochrome oxidase activity of both brain and liver in cases of swayback.

It therefore seemed of interest to examine the sites of cytochrome oxidase activity within the central nervous system (C.N.S.) of normal and swayback lambs to determine whether the distribution and degree of activity bore any relation to the pathology of the condition.

MATERIALS AND METHODS

The material of the study was made up of 12 severe cases of swayback varying in age from 24 hours to 20 weeks, and 9 normal animals between birth and 13 weeks of age. In the swayback group half were congenital cases, four of which showed macroscopic cerebral lesions, and half were cases in which clinical signs did not appear until the lambs were 6 to 8 weeks of age. The latter are referred to as delayed cases. The congenital cases were killed and examined within 24 hours of arrival at the laboratory but the delayed cases were all acquired simultaneously from one farm and were kept indoors on a low copper ration and deionised water until required for examination.

All the lambs were killed by decapitation through the atlanto-occipital space. The C.N.S. was removed as rapidly as possible and portions were cut out and placed on tinfoil over solid carbon dioxide. Three pieces were used for histochemical examination, namely, a slice of the cerebrum from the parietal region adjacent to the hippocampus, a slice of midbrain passing through the red nucleus and a transverse section of the spinal cord in the cervical region. These are areas in

which the lesions of swayback are most frequently found (Barlow, 1963).

In 9 swayback cases and 5 normal lambs a slice of cerebrum adjacent to the first block was taken for enzyme assay by manometric methods, the cortex being dissected as free of white matter as possible before freezing on solid carbon dioxide. Material was also taken for other histochemical and cytological studies, the remainder of the C.N.S. being preserved in 10 per cent. formol-saline for histological examination.

Blood and liver were collected for the estimation of copper by methods previously described (Barlow, Purves, Butler and Macintyre, 1960).

The blocks for histochemical study were cut fresh-frozen at 10μ using the cold knife, and sections were mounted directly on glass slides and stored briefly over solid carbon dioxide in a vacuum flask. At least four sections were taken from each block. One was fixed with formaldehyde in the 60°C oven and stained with haematoxylin and eosin. The remaining sections were removed from the solid carbon dioxide and placed for 2 to 3 minutes in a current of air in the cold room (4°C) to improve the adhesion of the section to the slide. They were then placed in the incubating medium and incubated at 37°C for the standard time of one hour.

The histochemical method used for demonstrating cytochrome oxidase activity was that of Burstone as described by Pearse (1961) using N-phenyl-p-phenylene diamine, and 1-hydroxy-2 napthoic acid. It was found that preparations mounted in glycerine jelly or other aqueous media deteriorated very rapidly, so the sections were examined immediately, the findings recorded and standard areas photographed for subsequent reference.

Succinoxidase was assayed manometrically by the method of Gallagher *et al.* (1956) and cytochrome oxidase by the technique of Schneider and Potter (1943). Copper was estimated spectrochemically in blood and liver with dithizone (Butler and Newman, 1956).

RESULTS

The results are summarised in Table 1. Using Burstone's method the sites of cytochrome oxidase activity are revealed by the deposition of a red-brown finely particulate precipitate. Activity varied from nil to very dense precipitates. Between these extremes 8 grades were defined subjectively in order to summarise the results. There was more variation between the different structures in one anatomical location than between similar structures at different anatomical levels. Thus the degree of activity in nerve cells, myelinated and unmyelinated fibres, have been recorded without reference to the site.

In the control lambs, cytochrome oxidase activity was mainly localised in the nerve cells and in unmyelinated portions of the fibres running in the grey matter. In the youngest animals, however, the arcuate fibres in the immediate subcortex were delineated by a scanty distribution of fine particles. The fibres in the region of red nucleus, the stratum lemnisci, the medial geniculate body and the dorsal horn of the spinal cord showed a dark red-brown largely non-particulate staining. Elsewhere the white matter showed a

TABLE I.

	No.	BREED	AGE	Cerebral Lesions	CYTOCHROME OXIDASE: Cells	CYTOCHROME OXIDASE: Grey Matter Fibres	CYTOCHROME OXIDASE: White Matter	Cerebral Cortex: * A	Cerebral Cortex: * B	BLOOD Cu µgms/100ml.	LIVER Cu p.p.m
CONGENITAL SWAYBACK	CD.22.	Chev.	24 hrs.	—				Not Done	Not Done	22·0	5·9
	CD.23.	B.F.	24 hrs.	Mac. G.				Not Done	Not Done	Not Done	5·14
	CD.24.	B.F.	24 hrs.	Mac. C.				20·6	36	17·7	4·65
	EE.5.	G.F.	24 hrs.	Mic. G.				Not Done	Not Done	Not Done	6·16
	CD.25.	G.F.	4wks.	Mic. C.				20	33	42·9	8·5
	CD.27.	B.F.	4wks.	—				22	70	79·1	8·27
DELAYED SWAYBACK	CD.30.	G.F.	10wks.	—				Nil	Nil	58·7	8·66
	CD.31.	G.F.	10wks.	—				16·1	37	49·8	5·65
	CD.39.	G.F.	12wks.	—				22·5	Nil	55·3	7·73
	CD.42.	G.F.	14wks.	—				22·4	78	50·9	29·9
	CD.43.	G.F.	16wks.	—				16·8	59	91·6	47·9
	CD.44.	G.F.	20wks.	—				24·7	123	40·7	26·4
CONTROLS.	EE.61.	Chev.	20hrs.	/				8	237	41·8	175·1
	EE.3.	G.F.x.	2wks.	/				Not Done	Not Done	Not Done	Not Done
	EE.22.	B.F.	2wks.	/				25·8	221	90·7	381
	EE.16.	G.F.x.	5wks.	/				Not Done	Not Done	67·8	193·3
	CD.37.	H.B.	9wks.	/				17·1	142	65·6	39·3
	CD.41.	H.B.	9wks.	/				19·2	141	66·2	171·0
	EE.18.	G.F.x.	9wks.	/				Not Done	Not Done	73·8	308·8
	CD.34.	H.B.	10wks.	/				15·1	108	53·3	17·6
	CD.32.	H.B.	13wks.	/				9·1	55	71·5	70·5

Cytochrome Oxidase activity- from ☐ very small numbers of particles within cells or along fibres to ■ heavy dense

*A = Succinate
B = Ascorbate } QO_2 µl O_2/hr/mgm

rather variable light straw-coloured diffuse staining. This was least noticeable in the white matter of the spinal cord and most evident in the centrum semiovale. Otherwise the microscopic appearances varied little between animals or between sections from the same block.

The cells of the ganglion layer of the cortex were more densely packed with granules than the more superficial neurons. In general there was no appreciable difference in particle content of cells of the same type, though occasionally a single cell was seen which contained fewer granules. Though similar in type, the cells of the ventral horn of the spinal cord were more densely packed with

particles than those of the red nucleus (Fig. 1). Occasionally a pericellular precipitate was observed in areas of strongest activity. Granules were not observed in astrocytes, but the fine particles seen in the arcuate fibres of the youngest animal may have been situated within oligodendroglial cells. Microglia did not contain granules, nor was any activity observed in the meninges or blood vessel walls.

In the swayback animals the results were more variable. There was considerable difference between individuals in both the quantity and distribution of the particles. In all except the oldest lamb there was a marked reduction in the amount of precipitate compared with the normal (Figs. 2, 3 and 4). This reduction was most clearly seen in the cells of the cerebral cortex, the multipolar cells of the red nucleus and the ventral horns of the spinal cord. In all the congenital cases and the two youngest of the delayed cases the depletion was so great that only a few cortical neurons were visible in cytochrome oxidase preparations. Cells at subcortical levels also showed variations in the strength of reaction, but in all the congenital cases and those delayed cases up to 12 weeks of age, the majority of multipolar nerve cells in the areas examined showed a very severe diminution in the amount of intracellular precipitate. In some there was a complete absence of cytochrome oxidase activity and examination of adjacent sections stained with haematoxylin and eosin revealed that a proportion of these were, in fact, necrotic. The smaller neurons of the brain stem and spinal cord showed less variation, being more uniformly filled with particles.

The fibres of the cerebral cortex, midbrain nuclei and grey matter of spinal cord also showed less activity in the swayback lambs. In about half the lambs under 12 weeks of age the particles were so reduced in number that their distribution appeared to be random rather than along the course of the unmyelinated portions of the nerve fibres. In the remainder of the lambs, apart from the oldest one, fibres could be recognised and traced, but the number of particles marking them was much fewer than in the normal series.

The cytochrome oxidase picture in the cerebral white matter only differed significantly from normal in those congenital cases with lesions in this region. In such cases in addition to precipitate along the course of the arcuate fibres, chains of small particles were present along some of those fibres remaining in gelatinous lesions or at the periphery of cavities. In the gelatinous lesions of one congenital case (CD23) the capillary endothelial cells contained appreciable quantities of precipitate. A similar deposit was found in blood vessel walls and histiocytes of the pia-arachnoid in two of the delayed cases (CD39 and CD43).

The quantitative estimations of succinoxidase and cytochrome oxidase given in Table 1 are consistent with the histochemical assessment of the cytochrome oxidase activity in the cells and

grey matter fibres in so far as both indicate a significant difference between the swayback and control lambs. This difference was most marked in congenital and those delayed cases under 12 weeks of age.

The figures given for the blood and liver copper levels of swayback lambs are similar to those published previously (Barlow *et al*, 1960). With the exception of CD27 which was hand-reared on the farm of origin, and CD43 which was a delayed case, 16 weeks old, all the swayback animals had blood copper values <60 ug./100 ml. With one exception the control animals' blood copper values were above this figure.

DISCUSSION

The results of the present study support the findings of Howell and Davison (1959) in that a marked reduction in cytochrome oxidase activity occurs in swayback. The present work, however, goes further and shows that alteration in the degree of activity occurs in those sites in which the lesions of the disease appear. The sensitivity of Burstone's method to demonstrate sites of low cytochrome oxidase activity and the freedom of the method from artifact have not been investigated, but the reproducibility of the results in normal animals would indicate that it is quite satisfactory within the scope of the present work.

Whereas in the normal animal there was strong activity in the nerve cells and the unmyelinated fibres of the cerebral cortex, the grey matter of the spinal cord and the brain stem nuclei, in swayback there was an overall depletion in these regions which was most severe in the neurons. The large nerve cells of the brain stem and spinal cord were particularly depleted. These are cells amongst which degenerative forms are found with increasing frequency during the early weeks of life (Barlow, 1963). Depletion of cytochrome oxidase might be either a cause or a result of this degenerative process. The raised metabolic demands made upon these cells by extra-uterine life would increase the effects of cytochrome oxidase deficiency, and thus if the deficiency were of a primary nature a ready explanation of the nerve cell lesions is available. Such a simple explanation is unlikely, however, since in copper deficient rats there may also be decreased phospholipid synthesis (Gallagher *et al.*, 1956) which affects the integrity of the cell by mitochondrial "ageing".

The pattern of cytochrome oxidase activity within the nerve fibres is of interest in that a sharp distinction was apparent between myelinated and nonmyelinated fibres in the normal animal. Romanes (1947) found that the arcuate fibres of the sheep were among the last to myelinate, and that the process commenced close to term and continued into post-natal life. The moderate and decreasing amounts of cytochrome oxidase activity in the arcuate fibres of normal lambs up to five weeks of age suggests that

Burstone's method may provide a sensitive marker of sites of active medullation. The occurrence of activity in the lesions of the cerebral white matter of cases of swayback is thus of great interest. It might indicate a delayed completion of medullation, or a heightened metabolic activity in demyelination, or it might merely reflect a situation more akin to that of unmyelinated cortical fibres.

The occurrence of a red-brown particulate precipitate in the blood vessel walls and meninges is unexplained. Reference to adjacent sections stained with haematoxylin and eosin indicated that those vessels showing cytochrome oxidase activity were among those small arterioles which show a faintly hyaline thickening of the intima (Barlow, 1962). Meningeal precipitates were sometimes confused with melanin deposits in early preparations and there was no morphological evidence that they occurred in areas of fibroblastic activity. The red-brown non-particulate staining in some areas of normal animals was regarded as diffusion of enzyme or the products of reaction as it was only observed in regions of highest activity.

CONCLUSIONS

The observation of previous authors that cytochrome oxidase activity is reduced in the central nervous system in swayback has been confirmed. In addition, it has been demonstrated that the most severe reduction in activity occurs in those groups of nerve cells which show the morphological lesions of the disease. Limited but abnormal cytochrome oxidase activity has been found in the vicinity of the white matter lesions of the cerebrum. The possible significance of the findings in the pathogenesis of swayback is discussed.

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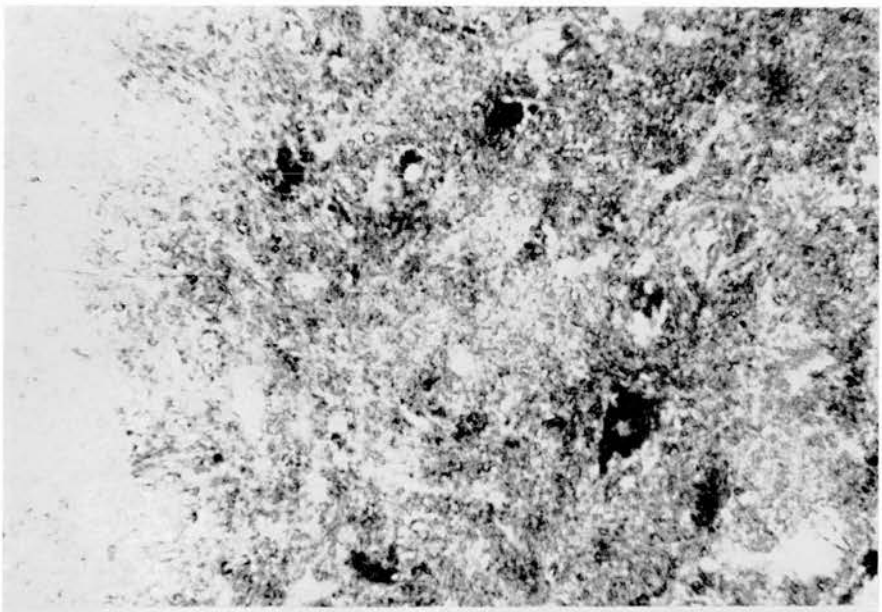

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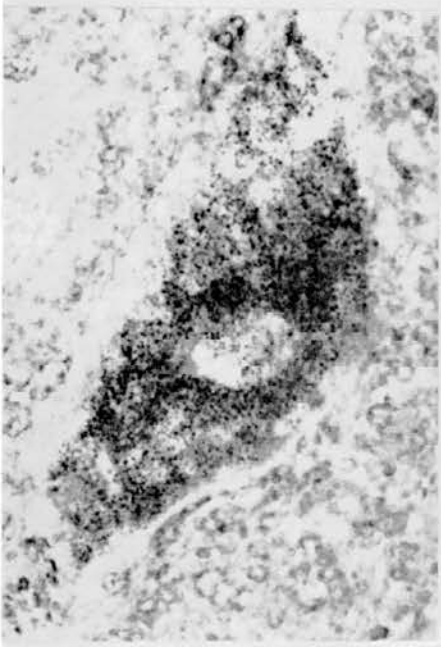

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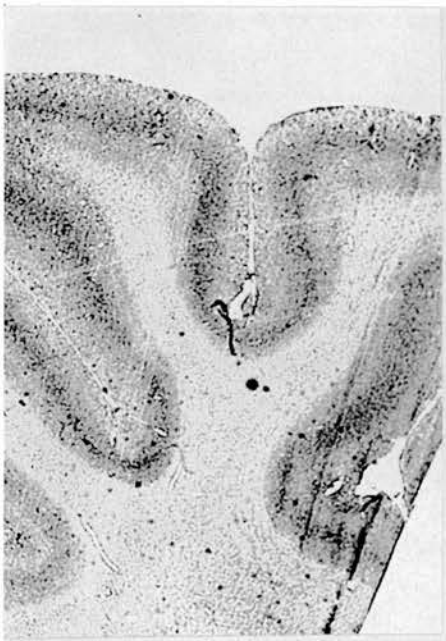

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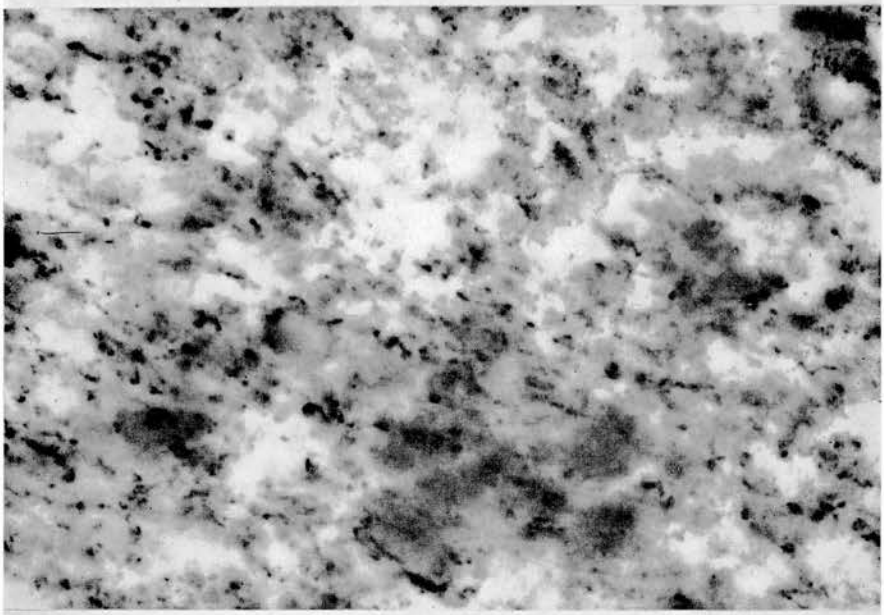

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LEGENDS TO ILLUSTRATIONS

Fig. 1. Ventral horn, first cervical segment of spinal cord, normal lamb, showing distribution of cytochrome oxidase activity in the grey matter. The white matter is on the left. Burstone's method, × 180.

Fig. 2. Nerve cells in red nucleus. Note the difference in cytochrome oxidase activity. Burstone's method (a) normal × 720 (b) swayback × 750.

Fig. 3. Parietal cortex showing distribution of cytochrome oxidase activity. Burstone's method. (a) normal × 12 (b) swayback × 9.

Fig. 4. Portion of cerebral white matter, swayback lamb. Note the cytochrome oxidase activity at the edge of the gelatinous lesion (top right corner). The chains of coarser particles are along the course of arcuate fibres. Burstone's method × 720.

3.

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FACTORS INFLUENCING THE BLOOD AND PLASMA COPPER LEVELS OF SHEEP IN SWAYBACK FLOCKS

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INTRODUCTION

During our investigation of the pathology and biochemistry of swayback in South East Scotland (Barlow, Purves, Butler and Macintyre, 1960 a and b) it was frequently observed that blood samples taken from ewes in swayback flocks during pregnancy had a lower copper content than those taken before mating and after parturition. Since our observations conflicted with the conclusion of Shearer, Innes and McDougall (1940) that the blood copper levels of such ewes "increased with the advance of pregnancy", a statement which has been regarded in Australia as an indication that molybdenum or a similar factor is involved in the aetiology of swayback (Dick, 1954), we decided to investigate the situation more intensively with the improved analytical techniques at our disposal. For comparative purposes parallel studies were carried out on ewes in a similar flock which was free from the disease. Information was also obtained on the influence of oral dosing with copper sulphate and injections of copper glycinate on the blood and plasma copper levels of sheep in affected flocks.

MATERIALS AND METHODS

Swayback flocks. During the period 1957 to 1958 the blood copper levels of groups of Blackface sheep in two swayback flocks (Farms D and E) were studied. Individual sheep were identified by ear tags or horn brands. The occurrence and diagnosis of the disease in these flocks has already been described (Barlow, *et al.*, 1960 a and b). One group of 5 gimmers and 3 groups of 8 ewes were studied on Farm D. The gimmers, aged $1\frac{1}{2}$ years in October, 1957, and one group of ewes, aged $2\frac{1}{2}$ to $4\frac{1}{2}$ years, were given no prophylactic treatment. The former were brought from a farm which was apparently free from the disease in October, 1957. Some of the ewes had been bred on the farm and others had been brought in as gimmers. The other two groups of ewes had a similar age distribution to those in the latter group and were given prophylactic treatment with copper during pregnancy. One group was given by mouth about 250 mg. of copper as the sulphate in about 50 ml. of water, in December, 1957 and January, February and March, 1958, and the other was given one subcutaneous injection of 45 mg. of copper as the glycinate ("Copper 45", supplied by

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Glaxo Laboratories Ltd.) in January, 1958. On Farm E, 3 groups of sheep were studied. One, consisting of 11 gimmers which had been brought from a swayback-free farm in October, 1957, and a second group of 14 ewes, aged $3\frac{1}{2}$ to $7\frac{1}{2}$ years, were given no prophylactic treatment. The third group consisting of 10 ewes, also aged $3\frac{1}{2}$ to $7\frac{1}{2}$ years, were given 2 injections of copper glycinate in January and March, 1958. A more detailed investigation was carried out the following year on Farm D with two fresh groups of 6 Blackface ewes, aged $2\frac{1}{2}$ to $4\frac{1}{2}$ years, one of which received an injection of "Copper 45" in January, 1959. Both whole blood and plasma copper levels were determined. The details are given in Table 1.

The management of the flocks was similar on both farms. Grazing was provided on both improved marginal and natural hill pasture and supplementary food was given during the second half of pregnancy, starting in January. This consisted of hay and crushed oats, with the addition of locust bean meal, linseed meal and flaked maize on Farm D and of concentrate nuts on Farm E. Mineral licks containing 1 to 2 p.p.m. copper were also provided.

Healthy flock. During 1958 to 1959 a parallel investigation was carried out on a Blackface flock which was known to be free from swayback (Farm X). Two groups of sheep were studied. One consisted of 10 ewes, aged $2\frac{1}{2}$ to $5\frac{1}{2}$ years, which lambed in April, 1959, and the other of 5 hoggs, aged 6 months in October, 1958, which were not mated. Both groups grazed natural hill pasture and were given no supplementary food or copper. Copper estimations were carried out on plasma as well as whole blood.

Analytical methods. Blood samples were collected at intervals during the year from the jugular vein with a stainless steel needle and polythene bottle assembly which was specially designed to minimise contamination by adventitious traces of copper (Butler, 1962). Heparin was used as an anticoagulant. Plasma was separated by centrifugation in Pyrex tubes with polythene stoppers using a Wifug Type XI centrifuge which, apart from the motor and electrical contacts, had no copper or high copper alloys in its construction. The method used for the determination of copper and the precautions taken to avoid contamination have been described and discussed in previous publications (Butler and Newman, 1956; Barlow *et al.* 1960b).

RESULTS

Untreated Sheep

The results obtained for the various groups of sheep are shown graphically in Figs. 1 to 6. It can be seen that the blood and plasma copper values for all the untreated groups of ewes on the swayback farms show a similar pattern, viz. a depression during pregnancy followed by a rise after parturition. An analysis of variance showed that these changes are statistically significant (Tables 2 to 4). This rise after parturition is not, however, apparent in the values for the gimmers (Fig. 3). At the time they were brought on to the farms in October their blood copper values were within or close to the normal range (mean values 69·6 and 67·4 μg./100 ml.) and by the following May this level had fallen below the normal range (mean values 48·1 and 30·3 μg./100 ml. respectively). During the remainder of the

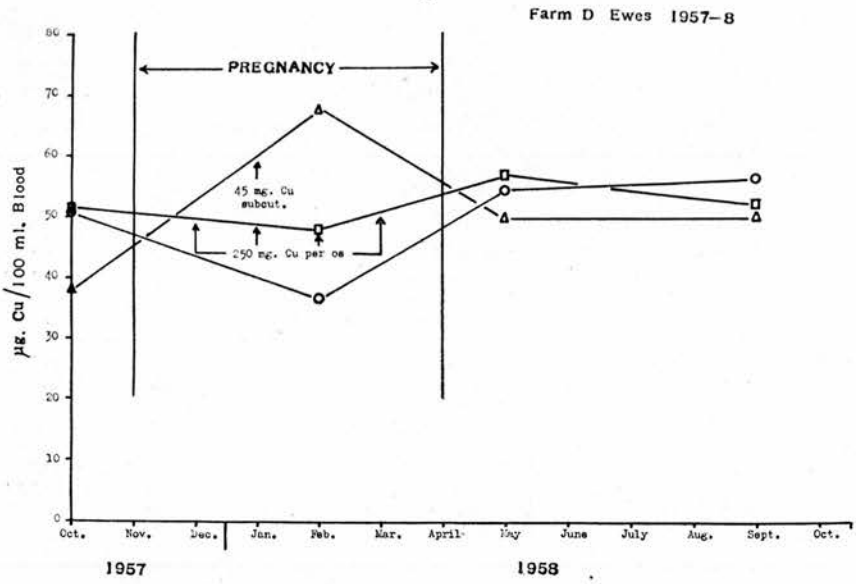
TABLE 1

DETAILS OF SHEEP AND TREATMENT

Farm and year	*Treatment*	*No. of sheep*	*Age in years* *
D (swayback) 1957–8	None	8	$2\frac{1}{2}$ – $4\frac{1}{2}$
	250 mg. Cu by mouth in Dec., Jan., Feb. and March	8	$2\frac{1}{2}$ – $4\frac{1}{2}$
	45 mg. Cu subcut. in Jan.	8	$2\frac{1}{2}$ – $4\frac{1}{2}$
	None	5	$1\frac{1}{2}$
D (swayback) 1958–9	None	6	$2\frac{1}{2}$ – $4\frac{1}{2}$
	45 mg. Cu subcut. in Jan.	6	$2\frac{1}{2}$ – $4\frac{1}{2}$
E (swayback) 1957–8	None	14	$3\frac{1}{2}$ – $7\frac{1}{2}$
	45 mg. Cu subcut. in Jan. and March	10	$3\frac{1}{2}$ – $7\frac{1}{2}$
	None	11	$1\frac{1}{2}$
X (normal) 1958–9	None	10	$2\frac{1}{2}$ – $5\frac{1}{2}$
	None	5	$\frac{1}{2}$

* The age given is that at the beginning of the investigation.

Fig. 1.



Mean blood copper levels of ewes on swayback farm D 1957-58.
O, no treatment with copper:
□, copper given by mouth:
△, copper glycinate injected subcut.

TABLE 2

ANALYSIS OF VARIANCE OF BLOOD COPPER LEVELS ON SWAYBACK FARM D 1957–8

Description	*Source of variance*	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
Untreated ewes	Sheep	6,469	7	924·1	12·99***
	Samples	1,907	3	635·7	8·94***
	Interaction	1,493	21	71·1	—
	Total	9,869	31	—	—
Untreated gimmers (Feb.–Oct.)	Sheep	3,040	4	760·0	8·44**
	Samples	64·9	3	21·6	0·24
	Interaction	1,080	12	90·0	—
	Total	4,185	19	—	—
Ewes given 4 × 250 mg. Cu by mouth	Sheep	5,984	7	854·9	7·19***
	Samples	339	3	113·0	0·95
	Interaction	2,500	21	119·0	—
	Total	8,823	31	—	—
Ewes injected once subcutaneously with copper glycinate (45 mg. Cu)	Sheep	1,849	7	264·1	2·50*
	Samples	3,544	3	1,181·0	11·17***
	Interaction	2,219	21	105·7	—
	Total	7,612	31	—	—

The levels of significance of the F-ratio are indicated thus:—
* P = 0·05 — 0·01
** P = 0·01 — 0·001
*** P = <0·001

TABLE 3

ANALYSIS OF VARIANCE OF BLOOD COPPER LEVELS ON SWAYBACK FARM E 1957–8

Description	*Source of variance*	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
Untreated ewes	Sheep	17,493	13	1,346	18·78***
	Samples	3,040	4	760	10·60***
	Interaction	3,728	52	71·7	—
	Total	24,261	69	—	—
Untreated gimmers (May–Oct.)	Sheep	2,017	10	201·7	3·41**
	Samples	131·7	3	43·9	0·74
	Interaction	1,774	30	59·1	—
	Total	3,923	43	—	—
Ewes injected twice subcutaneously with copper glycinate (45 mg. Cu)	Sheep	1,189	9	132·1	2·31*
	Samples	3,565	4	891·3	15·61***
	Interaction	2,056	36	57·1	—
	Total	6,810	49	—	—

The levels of significance of the F-ratio are indicated thus:—
* P = 0·05 — 0·01
** P = 0·01 — 0·001
*** P = <0·001

period of observation, i.e. until October, these levels showed no significant change (Tables 2 and 3), those for the gimmers on Farm D remaining above those for the group on Farm E ($P = < \cdot 001$). This difference is also shown by the ewes and is consistent with the differences which have been found in the copper content of the herbage on the two farms (Barlow *et al*, 1960a).

The blood and plasma copper values of the ewes on the swayback-free Farm X followed a similar pattern to those of the ewes on the swayback farms and were also generally below the normal range

TABLE 4

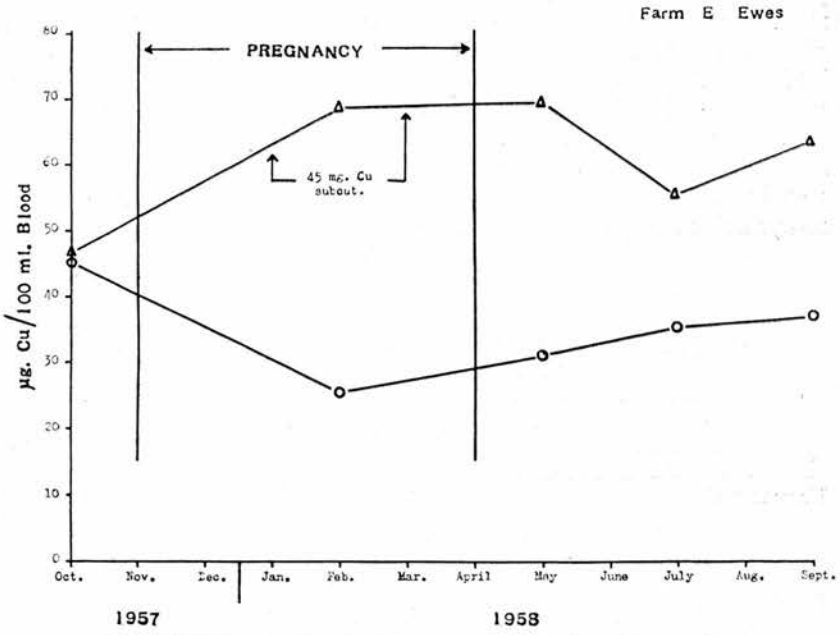
ANALYSIS OF VARIANCE OF BLOOD AND PLASMA COPPER LEVELS ON SWAYBACK FARM D 1958–9

Description	*Source of variance*	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
Untreated ewes	Sheep	10,071	5	2,014	42·28***
	Samples	4,274	8	534·3	11·21***
	Interaction	1,905	40	47·6	—
Whole blood	Total	16,250	53	—	—
Ewes injected once subcutaneously with copper glycinate (45 mg. Cu)	Sheep	3,695	5	739	5·04**
	Samples	9,672	7	1,382	9·43***
	Interaction	5,126	35	146·5	—
Whole blood	Total	18,493	47	—	—
Untreated ewes	Sheep	8,348	5	1,670	14·45***
	Samples	6,522	6	1,087	9·41***
	Interaction	3,466	30	115·5	—
Plasma	Total	18,336	41	—	—
Ewes injected once subcutaneously with copper glycinate (45 mg. Cu)	Sheep	1,850	5	370	1·20
	Samples	15,543	6	2,591	8·40***
	Interaction	9,255	30	308·5	—
Plasma	Total	26,648	41	—	—

The levels of significance of the F-ratio are indicated thus:—
** P = 0·01 — 0·001
*** P = <0·001

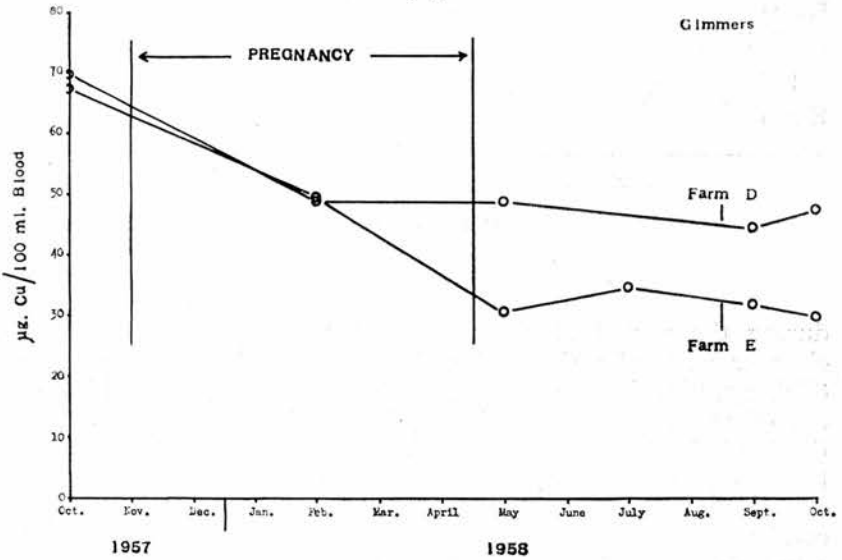
during the period of observation (Table 5, Fig. 5). Significant changes were also shown by the untreated hoggs on this farm (Table 5, Fig. 6), but they differed from the ewes in that their blood and plasma copper levels did not show a rise until August whereas the levels in the ewes had risen by July. An analysis of variance (Tables 2 to 5) showed that the copper levels of individual sheep differed significantly and that these differences were generally consistent throughout the year. The graphs of mean values (Figs. 1 to 6) illustrate the changes shown by the graphs for the individual sheep.

Fig. 2.



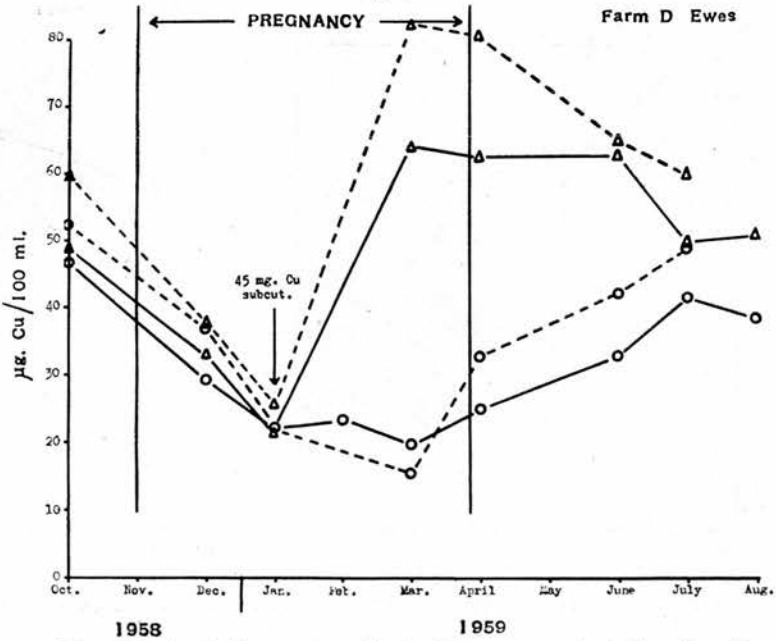
Mean blood copper levels of ewes on swayback farm E 1957-58.
O, no treatment with copper:
△, copper glycinate injected subcut.

Fig. 3.



Mean blood copper levels of untreated gimmers on swayback farms D and E, 1957-58.

Fig. 4.



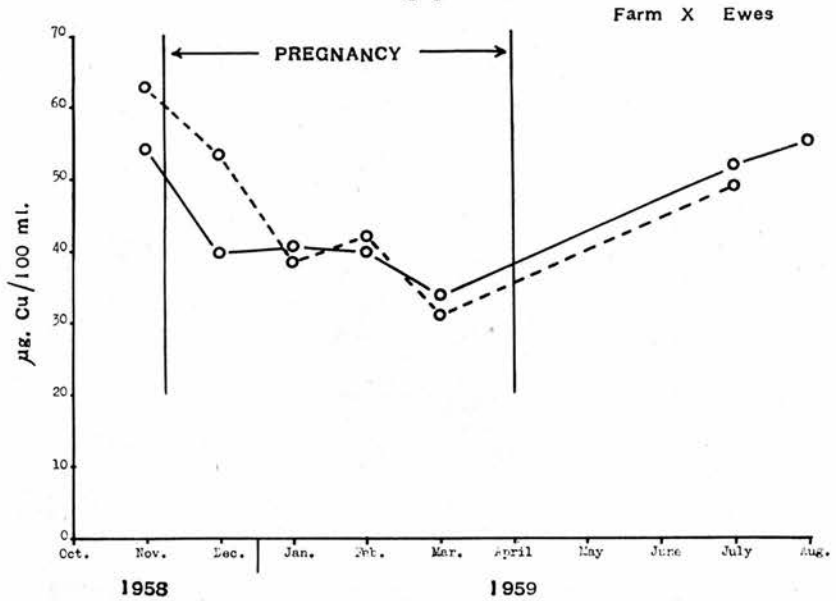
Mean blood and plasma copper levels of ewes on swayback farm D 1958-59.
O—O, blood levels of untreated sheep:
O---O, plasma levels of untreated sheep:
△—△, blood levels of sheep injected subcut. with copper glycinate:
△---△, plasma levels of sheep injected subcut. with copper glycinate.

TABLE 5
ANALYSIS OF VARIANCE OF BLOOD AND PLASMA COPPER LEVELS ON SWAYBACK-FREE FARM X 1958–9

Description	*Source of variance*	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
Untreated ewes	Sheep	8,045	9	893·9	7·68***
	Samples	4,332	6	720·3	6·19***
	Interaction	6,286	54	116·4	—
Whole blood	Total	18,653	69	—	—
Untreated hoggs	Sheep	4,799	4	1,200·0	11·6***
	Samples	2,646	6	441·0	4·28**
	Interaction	2,473	24	103·0	—
Whole blood	Total	9,918	34	—	—
Untreated ewes	Sheep	7,061	9	784·6	9·63***
	Samples	6,542	5	1,308·0	16·05***
	Interaction	3,666	45	81·5	—
Plasma	Total	17,269	59	—	—
Untreated hoggs	Sheep	7,034	4	1,759·0	13·61***
	Samples	2,662	5	532·4	4·12**
	Interaction	2,584	20	129·2	—
Plasma	Total	12,280	29	—	—

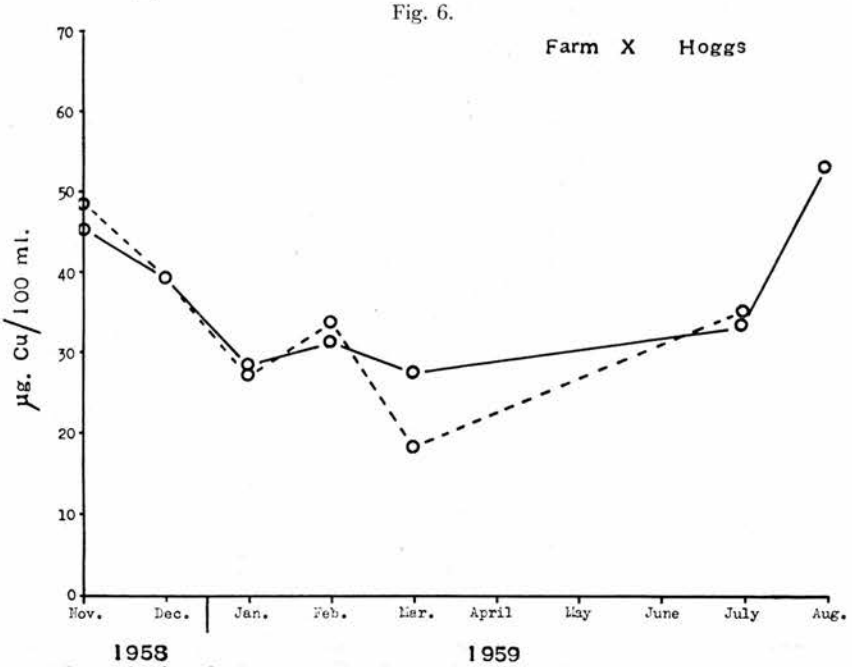
The levels of significance of the F-ratio are indicated thus:—
** P = 0·01 — 0·001
*** P = <0·001

Fig. 5.



Mean blood and plasma copper levels of untreated ewes on swayback-free farm X, 1958-59.
O—O, blood levels:
O---O, plasma levels.

Fig. 6.



Mean blood and plasma copper levels of untreated hoggs on swayback-free farm X, 1958-59.
O—O, blood levels:
O---O, plasma levels.

Oral Dosing with Copper

The group of ewes on Farm D which were given four doses of 1 g. $CuSO_4 . 5H_2O$ by mouth during pregnancy showed no significant changes in their blood copper levels (Table 2, Fig. 1) and it may be concluded that this treatment prevented the decline during pregnancy that was apparent in the untreated group.

Subcutaneous Injections of Copper Glycinate

Marked rises in blood and plasma copper levels to within the normal range were observed after subcutaneous injections of 45 mg. of copper as glycinate. In 1958, on Farm D a significant rise was detected a month after a single injection ($P = < \cdot 001$), but 3 months later the levels had fallen to become similar to those in the untreated group (Fig. 1). A more prolonged effect was observed the following year with a different group of ewes and was still apparent 5 months after the injection (Fig. 4). A month later, in July, there was no significant difference between the levels in the injected and untreated groups. On Farm E two injections were given in 1958, in January and March, and their effect was still apparent when the sheep were sampled for the last time in September (Fig. 2). It can be seen from the analysis of variance (Tables 2 to 4) that this treatment also reduced the individual variation in the copper levels. The incidence of swayback in untreated sheep in 1958 and 1959 was too low to enable any assessment of the prophylactic value of this treatment to be made.

DISCUSSION

The above observations on the seasonal variation in the blood copper levels of untreated ewes in swayback flocks are not in agreement with the conclusion of Shearer *et al* (1940) that in these flocks the levels increase with the advance of pregnancy. All the groups of ewes that we studied showed a fall during pregnancy and a rise after parturition. However, an examination of the results published by Shearer *et al* (1940) shows that their conclusion is not in fact valid. They took blood samples from 4 untreated groups of ewes on a farm in Derbyshire in November before tupping, in January and again in April after lambing. In two of these groups the values for the November and January samples are not significantly different, but after lambing both groups show a significant increase ($P = < 0 \cdot 01$). The same pattern is shown by two other groups which had access to licks containing a small amount of copper (0·3 per cent.). These results therefore agree with ours in so far as they show a rise in blood copper levels after parturition. It should be pointed out that in Australia the misleading statement made by these authors has been taken as evidence that molybdenum or a similar factor is involved in the aetiology of swayback (Dick, 1954). This interpretation was based on the widely held belief that pregnancy has no influence on the blood copper levels of sheep (Underwood, 1956) and

the observation that the administration of molybdate causes an elevation of these levels (Marston, 1951).

Blood copper values at different times of the year for ewes in a swayback flock in Derbyshire have also been published by Allcroft, Clegg and Uvarov (1959). Their results are similar to those obtained in our investigation in that they show a fall during the first half of pregnancy, but they differ in that they indicate a rise in February before the end of pregnancy. Our more detailed investigation on Farm D in 1958 and 1959 showed clearly that there was no significant rise until after parturition. The reason for this difference is not known. Both investigations showed that oral doses or injections of copper will prevent the fall during pregnancy. It is obvious from our results for ewes in a swayback free flock that these changes are not confined to swayback flocks. These results also illustrate the fact that a comparable degree of hypocupraemia can occur in healthy flocks, which in our experience is not uncommon.

A different pattern was shown by the two groups of gimmers which had recently been introduced on to the swayback farms. Their blood copper levels fell rapidly from the normal range during pregnancy, but did not rise again after parturition. For the next six months they showed no significant change and were similar to those in the older ewes on the same farm. It is probable that this difference in behaviour is due to differences in their initial copper status.

Among the factors responsible for these seasonal changes the dietary intake of copper and the physiological disturbances caused by pregnancy and parturition are probably of considerable importance. The intake of copper by sheep at pasture has not been studied in Britain, but on the type of farm under consideration it is likely that it will decrease during the winter owing to the diminution in the amount of herbage available. It is probable that this factor played an important part in producing the changes observed in the unmated hoggs on the swayback-free farm. On both the swayback farms studied the analysis of the pasture had indicated a deficiency of copper i.e. levels below 5 p.p.m. DW, and it is interesting to note that the differences in the blood copper levels of comparable groups of sheep on the two farms are consistent with the difference observed in the copper content of the pasture. The copper content of the pasture on the swayback-free farm has not been studied.

Although it has been generally assumed that pregnancy and parturition have no influence on the blood copper levels of sheep (Underwood, 1956) this does not appear to be true under all circumstances. Our own studies of pregnant and barren sheep on a constant intake of copper have shown that the blood and plasma levels fall during pregnancy and rise again after parturition (Butler, 1963) thus following a similar pattern to that described in this paper. These changes probably reflect to some extent the demands of the foetus on the copper reserves of the mother. Thus it is likely that both pregnancy and a diminishing intake of copper operate together in

producing the fall in blood copper levels observed in sheep at pasture. Apart from these factors it is clear that individual characteristics of the sheep also determine the copper content of the blood. Previous authors (Eden, 1941) have reported wide variations in the blood copper levels of different sheep sampled at the same time and comparable random fluctuations in the same sheep at different times. Our results, like those of McDougall (1947), show that these individual differences were in fact consistent throughout the year. Furthermore the seasonal changes were regular rather than random.

Parallel changes were observed in the copper content of heparinised plasma which was generally similar to that of whole blood. When the results for the 150 samples obtained from untreated sheep are compared it can be seen that there is in fact no significant overall difference between the copper content of whole blood and plasma. The copper content of the red cells in terms of weight per volume must therefore have been similar. The same conclusion was drawn by Eden and Green (1939) and McCosker (1961) from their results for 11 and 9 sheep respectively. There is little information elsewhere in the literature on this distribution and in most instances the reliability of the data is doubtful because of technical and analytical defects and the use of powerful chelating agents, such as oxalate, as anticoagulants which might disturb the normal equilibrium.

CONCLUSIONS

Seasonal variations in the copper content of the blood of hypocupraemic ewes in two swayback flocks and one healthy flock followed a consistent pattern. There was a marked fall during the first half of pregnancy and a generally rapid return to the pre-mating levels after parturition. Gimmers with a normal initial copper status which had recently been introduced on to the swayback farms also showed a fall during the first half of pregnancy, but afterwards maintained constant levels which were comparable with those of older ewes on the same farm. The plasma copper levels showed parallel changes and were not significantly different from those in whole blood over the entire period of investigation.

A decrease in the intake of copper during the winter and the physiological disturbances associated with pregnancy probably play an important part in the production of these changes. The difference observed in the blood copper levels of the two swayback flocks was consistent with the difference which had been found in the copper content of the pasture.

It was found that oral dosing with copper sulphate and subcutaneous injections of copper glycinate, previously found to be effective in reducing the incidence of swayback, also prevented the decline in the blood and plasma levels during pregnancy.

In each group of sheep studied there were significant and consistent differences between the blood copper levels of individual animals. These differences tended to diminish when the levels were

raised to within the normal range by the injection of copper glycinate.

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COPPER DEFICIENCY IN RELATION TO SWAYBACK IN SHEEP

1. Effect of Molybdate and Sulphate Supplements During Pregnancy

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COPPER DEFICIENCY IN RELATION TO SWAYBACK IN SHEEP

I. Effect of Molybdate and Sulphate Supplements During Pregnancy

By

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INTRODUCTION

Research into the cause and pathogenesis of swayback has been hindered by the difficulties inherent in the reproduction of the disease under experimental conditions. Several years ago, swayback occurred at Weybridge in lambs from ewes which had been fed hay from a swayback farm during pregnancy and it was concluded that the causative factor was present in the hay (Green, 1951). However, this conclusion does not appear to be justified since the ewes themselves were taken from a swayback flock after mating, and subsequent work has shown that this method of reproducing the disease is not sufficiently reliable for experimental purposes (Butler and Nisbet, unpublished).

Recently, Mills and Fell (1960) reported the occurrence of swayback with microscopic lesions in three out of four lambs whose mothers had been maintained on high intakes of molybdate and sulphate for the last three months of pregnancy. They repeated the experiment the following year and obtained similar results (Fell, Williams and Mills, 1961). Since the incidence of the disease was high this method showed considerable promise, even though it appeared to have little relevance to field conditions. We therefore decided to investigate it further. This paper describes an unsuccessful attempt to reproduce swayback by this method and draws attention to factors which may be particularly important in this respect.

MATERIALS AND METHODS

Sheep and diet. About 2 months after mating with a Blackface ram, 8 Blackface ewes, about 7 years old, were divided into two groups of 4 so as to give similar pre-mating blood copper values (20 to 50 μg./100 ml.). About 1½ years previously they had been brought from an unimproved hill farm on which the sheep had a low copper status but were free from swayback. Since then they had been housed at the Institute in pens with concrete floors from which all accessible copper and brass fittings had been removed, and maintained on a low copper diet which supplied 2 to 3 mg. of copper daily.

A similar diet was used as the basic diet in this experiment. The daily ration consisted of 680 g. of hay and 450 g. oats to which 8 g. of a vitamin-mineral mixture were added. The hay and oats were obtained from farms which, so far as could be ascertained, were free from swayback.

Table 1 shows the concentration of various mineral and trace elements present in these foods, from which it was calculated that they supplied 2·6 mg. copper, 0·6 mg. molybdenum and 10·7 g. sulphate daily. The composition of the vitamin mineral mixture was as follows: 500 g. calcium orthophosphate (B.D.H. precipitated), 200 g. sodium chloride (B.D.H. Analar), 100 g. Rovimix E (Roche Products Ltd., containing 10 per cent dl-α-tocopheryl acetate), 4 g. Rovimix A-50 x D_3 (Roche Products Ltd., containing 50,000 i.u. Vit. A and 12,500 i.u. Vit. D_3 per g.)

In addition, supplements of 1 mg. cobalt and 115 mg. iodine were given by mouth each week as aqueous solutions of Analar $CoCl_2$ 6 H_2O and KI respectively. Approximately 1 gallon of deionised water containing negligible amounts of copper and other elements was offered to each sheep daily.

The sheep in Group 1 were given 50 mg. of molybdenum daily by mouth in the form of 20 ml. of an aqueous solution of Analar ammonium molybdate, and Analar hydrated sodium sulphate was added to their mineral vitamin mixture so as to increase the sulphate intake by 10 g. per day. The molybdenum supplement was similar to that employed by Mills and Fell (1960) while the sulphate intake was twice that of their sheep. These supplements were given to three of the sheep for the last 12 weeks of pregnancy and for 2 weeks after parturition. The remaining animal received them for the last 9 weeks of pregnancy and for 5 weeks afterwards.

These ewes gave birth to 7 lambs. Two of these, a pair of twins, were weak at birth and had difficulty in rising. They were slaughtered for examination when they were 4 to 5 weeks old because they were in poor condition due to contagious pustular dermatitis. The remainder were killed at the age of 7 weeks. Five lambs were born in the control group (Group 2). One died soon after birth as a result of a volvulus; a pair of twins were slaughtered at the age of 4 weeks when their mother developed tetany and died, and the other two were slaughtered when they were 7 weeks old.

In view of the previous association of muscular dystrophy in lambs with this type of basic diet (Nisbet, Butler and MacIntyre, 1959) all the lambs were given a Vitamin E supplement (0·6 g. Rovimix E suspended in water) by mouth on alternate days beginning when they were 2 days old.

Copper analysis. Blood samples were taken for copper analysis from the lambs and their mothers just before the former were killed by decapitation. The apparatus used for collecting the samples has been described elsewhere (Butler, 1962). After death the entire ventral lobe of the liver was removed with stainless steel instruments and placed in a Pyrex dish. The preparation of the samples for analysis, the precautions taken to avoid contamination and the analytical method used have been described and discussed in previous publications (Butler and Newman, 1956; Barlow, Purves, Butler and MacIntyre, 1960b).

Histological procedures. The entire brain and spinal cord were removed for detailed histological examination. Primary fixation was in 10 per cent formol-saline. Standard blocks were post-fixed in saturated mercuric chloride solution and embedded in paraffin. Adjacent blocks were taken for frozen section work. The staining methods applied were those described in an earlier paper (Barlow *et al.*, 1960b).

RESULTS

None of the lambs showed any sign of ataxia and no significant lesions indicative of swayback were found in the central nervous system. In two lambs from dosed ewes (114 and 108) occasional chromatolytic cells were observed in the ventral horns of the spinal cord or in the lateral vestibular nucleus. These changes were not accompanied by obvious lesions in nerve fibres and as one lamb (108) was severely affected by contagious pustular dermatitis at the time of death no significance was attached to their presence. In foetal

TABLE 1

TRACE AND MINERAL ELEMENT CONTENT OF HAY AND OATS

	*p.p.m.**		% *					
	Cu	*Mo*	SO_4	*Mg*	*Ca*	*K*	*Na*	*P*
Hay	2·58	0·55	1·16	0·148	0·70	1·36	0·11	0·210
Oats	1·98	0·51	0·63	0·123	<0·05	0·50	0·007	0·319

*These values are for the hay and oats in the condition in which they were fed to sheep.

TABLE 2

COPPER CONTENT OF LIVER AND BLOOD OF EXPERIMENTAL AND SWAYBACK LAMBS

Mean values are given in brackets

Group	*Treatment*	*Lamb No.*	*Age in weeks*	*Liver Cu p.p.m. D.W.*	*Blood Cu. µg./100 ml.*	
					Lambs	*Ewes*
1	Ewes given 50 mg. Mo and 10 g. SO_4 daily during pregnancy	102	7	12·1	27·6	73·3
		103	7	11·8	38·4	
		106	7	9·1	44·2	65·6
		107	7	12·9	68·7	
		108	4 – 5	6·6	28·0	58·0
		109	4 – 5	9·1	—	
		115	7	14·6	30·4	74·7
2	None (Controls)	104	1 day	84·4	—	35·5
		105	7	12·8	48·5	49·1
		110	4	8·4	64·2	43·8
		111	4	16·5	72·4	
		114	7	13·3	49·3	22·2
12 natural cases of swayback			4 – 7	1·8 – 10·1 (6·1)	11·1–42·9 (24·2)	—
6 experimental cases of swayback (Fell, Williams and Mills, 1961)			1 – 14	0·2 – 4·6 (2·6)	—	—
					—	—

material from apparently normal sheep, swollen cells with little or no Nissl substance and extruded or marginated nuclei are not uncommon in certain situations at certain periods of gestation (Barlow, 1959). Such cells are not normally seen after birth, but their presence in the late stage foetus suggests that too much weight should not be placed on the finding of very small numbers of apparently chromatolytic neurones in young lambs.

The values obtained for the copper content of liver and blood are shown in Table 2 in comparison with those for natural cases of swayback of the same age and the cases produced experimentally by Fell, Williams and Mills (1961). It can be seen that the copper status of our two groups of lambs was very similar and was low as judged by the criteria discussed previously (Barlow *et al.*, 1960b). The blood copper values of the ewes were, however, higher in Group 1, which had received the molybdenum and sulphate supplements, and had risen above the premating values, whereas the levels in the control group showed no significant difference. This effect of molybdate in raising blood copper levels has been noted by previous authors (Marston, 1952; Dick, 1954). The treatment had no obvious effect on the health and physical appearance of the ewes.

DISCUSSION

In order to develop this method of inducing swayback to a satisfactory state for work on the pathogenesis of the disease it is necessary to identify the factors responsible for the failure of our experiment and the success of those carried out at The Rowett Institute (Mills and Fell, 1960; Fell, Williams and Mills, 1961). Unfortunately, complete descriptions of these experiments have not yet been published.

One explanation of the failure of our experiment which might be put forward is that the copper status of the lambs had not been reduced to sufficiently low levels. It can be seen from Table 2 that the liver copper values reported by Fell, Williams and Mills for their experimental cases which showed ataxia are all below 5 p.p.m. D.W. and are much lower than those obtained for our experimental lambs, the lowest of which is 6·6 p.p.m. However, several of the latter are close to those we have found so far in natural cases of swayback of the same age and 4 (3 dosed; and 1 control) are actually within this range of values (1·8—10·1 p.p.m. D.W.).

The major differences between the experiments concern the breed of the sheep and the nature of the basic diet and these may be particularly important. Scottish Blackfaces with an initially low copper status were used in our experiment whereas Mills and Fell (1960) used Cheviots, of unspecified initial copper status, whose lambs have a higher growth potential and may, in consequence, be more susceptible to the factors causing swayback. Our basic diet consisted of hay and oats from swayback-free farms with a vitamin-

mineral supplement and supplied about 2·6 mg. copper per day, whereas Mills and Fell used grass cubes from an unspecified source which supplied twice this amount of copper. The total sulphate intake of our supplemented ewes (20·7 g./day) was about twice that of those in their experiments. Thus, so far as the copper and sulphate intakes are concerned, our diet might be expected to be more effective in reducing the copper status of the lambs.

The period of supplementation in our experiment was a month shorter than that employed by Mills and Fell in their first experiment. It was, however, similar to that in their second experiment which apparently produced a milder manifestation of the disease. In the brief description which has been given of this experiment (Fell, Williams, and Mills, 1961) it is stated that 3 lambs whose mothers received only the sulphate supplement also showed mild chromatolysis of neurones in the red nucleus and the impression is given that these were regarded as very mild cases of swayback. The copper status of all the lambs in both groups was low as judged by the copper content of their livers. An untreated control group was not included in either experiment.

A considerable amount of interest is therefore attached to the basic diet of grass cubes used by Mills and his colleagues and to the possibility that it contained factors which were involved in the reduction of the copper status and the productions of lesions in the central nervous system. This aspect of the experiment has recently been magnified in importance by the occurrence of swayback in kids from goats which had been fed an experimental diet of hay and a mixture of bean meal, oats, maize and decorticated ground nuts (Barlow, Robertson, Owen and Proudfoot, 1962). Further work is obviously needed to identify the significant factors in these experiments and to determine their relevance, if any, to conditions in the field. Pasture analysis has suggested that the molybdenum intake of ewes in affected flocks may be considerably less than that of the sheep in the experiments discussed above and is probably insignificant so far as the limitation of copper storage is concerned (Allcroft and Lewis, 1957; Barlow *et al.*, 1960a). However, it should be borne in mind that their actual intake of these elements and also that of sulphate has not yet been measured.

CONCLUSIONS

The administration of large amounts of molybdate and sulphate (50 mg. Mo and 10 g. SO_4 daily) to pregnant Blackface ewes, with a low copper status and maintained on a low copper diet, did not produce swayback in their lambs.

This treatment raised the blood copper levels of the ewes, but had no obvious influence on the liver copper levels of the lambs, several of which were similar to those found in naturally occurring cases of delayed swayback of the same age.

It is suggested that differences in the nature of the basic diet and the breed of the sheep may be responsible for the failure of our experiment to produce swayback and the success of those carried out elsewhere.

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COPPER DEFICIENCY IN RELATION TO SWAYBACK IN SHEEP

II. Effect of Dosing Young Lambs with Molybdate and Sulphate

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COPPER DEFICIENCY IN RELATION TO SWAYBACK IN SHEEP

II. Effect of Dosing Young Lambs with Molybdate and Sulphate

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INTRODUCTION

In the first article of this series (Butler and Barlow, 1963) a description was given of an attempt to produce swayback in lambs by the administration of large amounts of molybdate and sulphate to their mothers during and after pregnancy. These lambs had access to the sulphate supplement which was mixed with the basic diet, but had no opportunity to ingest the molybdate supplement except through their mothers' milk. In view of the failure of this experiment and the success of those carried out elsewhere (Mills and Fell, 1960; Fell, Williams and Mills, 1961) it was decided to investigate the effect of dosing young lambs with large amounts of these substances beginning soon after birth. It was thought that this second experiment might be relevant, so far as time of onset is concerned, to the delayed type of swayback which often does not make its appearance until the lambs are several weeks old. Although it has sometimes been assumed that the initial lesions of this form of the disease are produced in foetal life this has not yet been proved.

Lambs with a low initial copper status were used in this experiment and the effect of the treatment on their clinical condition and the copper content of their blood and liver was studied. After death the central nervous system was examined histologically. Assays of cytochrome oxidase activity were also carried out on selected portions of the brain since Howell and Davison (1959) had reported that it is unusually low in swayback lambs. This finding has since been confirmed by Mills and Williams (1962) and Barlow (1963 b).

MATERIALS AND METHODS

Sheep, Diet and Treatment.

The lambs came from a group of North Country Cheviot gimmers which had been maintained on a low copper diet, containing 2 to 3 p.p.m. copper, from about a month before mating with a ram of the same breed, and had previously been reared on a farm which so far as could be ascertained was free from swayback. Their management and diet were similar to that of untreated sheep in previous experiments (Butler and Barlow, 1963).

When the lambs were born they were allocated to the treated and control groups so as to give 5 lambs in each group, each being paired with

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a lamb of similar weight in the other group. In view of the previous association of muscular dystrophy with this type of diet (Nisbet, Butler and MacIntyre, 1959), all the lambs were injected subcutaneously with an aqueous solution of sodium selenate containing 1 mg. selenium every 10 days from birth.

The lambs in the treated group were drenched daily with an aqueous solution of Analar sodium sulphate and ammonium molybdate, the aim being to give the maximum amounts that they would tolerate. The initial doses, which were given a day after birth, contained 2.5 mg. molybdenum and 0·5 g. sulphate in 5 ml. and were diluted with an equal volume of cows' milk. The lambs were examined daily and the dose was increased twofold and then fourfold when it was felt that the animal could tolerate it. The total amounts given to each lamb are shown in Table 1.

One lamb in the control group (207) died of starvation at the age of $5\frac{1}{2}$ weeks due to contagious pustular dermatitis and the occurrence of mastitis in its mother. The remainder were killed for examination at ages varying from 7 to 12 weeks (Table 1).

Histological Procedures.

After removal of standard blocks of fresh tissue for cytochrome oxidase assay the entire central nervous system was fixed in 10 per cent. formol saline. Blocks were then processed and examined for lesions as described previously (Barlow, Purves, Butler and MacIntyre, 1960). Adjacent blocks were retained for frozen sections as required.

Copper Analyses

Blood samples were taken for copper analysis when the lambs were a day old and at three weekly intervals thereafter. After death the entire ventral lobe of the liver was also taken for this purpose. The techniques used for the collection, preparation and analysis of these samples have been described elsewhere (Butler and Newman, 1956; Barlow, *et al.*, 1960; Butler, 1962).

Assay of Cytochrome Oxidase Activity in Brain

Histochemical. Standard blocks of fresh tissue were taken from the occipital lobe and hippocampus, the mid-brain at the level of the red nucleus and transversely through the spinal cord at the level of C2. Sections were cut at 10μ, mounted on glass slides and stored briefly on solid carbon dioxide. The localisation of cytochrome oxidase was demonstrated by the method of Burstone (Pearse, 1961) and its activity was evaluated as described in a previous paper (Barlow, 1963b).

Manometric. A portion of occipital cortex adjacent to the area used for histochemical assay and separated as cleanly as was practicable from the underlying white matter, was used for manometric assay. This tissue was chilled immediately after removal from the animal and homogenised in a glass vessel with a Perspex pestle driven by an electric motor (Aldridge, Emery and Street, 1960). The cytochrome oxidase activity was determined by the method of Schneider and Potter (1943) using freshly prepared solutions of cytochrome C (Sigma Chemical Co.) and sodium ascorbate (Roche Products Ltd.).

RESULTS

Clinical

Clinical abnormalities first appeared when the lambs were between 3 and 8 weeks of age. They were entirely locomotor and, at first, were only observed after exercise. There was slight stiffness and inco-ordination of the hind limbs but no ataxia. In lamb 210 there was also slight unnatural lateral mobility of the hock joints and lameness of the right hind limb which appeared to have its origin in the region of the hip. This lamb became recumbent and was killed at 7 weeks of age. None of the control lambs was similarly affected and treatment did not influence growth, the mean weight gains for the first 7 weeks of life being 20·3 lb. for the experimental group and 20·5 lb. for the control group of lambs.

TABLE 1

LIVER COPPER VALUES AND ACTIVITY OF CYTOCHROME OXIDASE IN BRAINS OF LAMBS

N.A. indicates that no assay was carried out

Treatment	*Lamb No.*	*Age (wks.)*	*mg. Mo received*	*g. SO_4 received*	*Cu in liver, (p.p.m. D.W.)*	*Cytochrome oxidase activity in brain*	
						Q_{O_2} *	*Histo-chemical assay*
Molybdate and sulphate by mouth	202	11	538	108	14·4	204	+++
	204	9	440	88	26·7	91	++++
	205	9	445	89	14·2	150	++++
	208	9	463	93	26·7	0	++++
	210	7	375	85	21·8	85	N.A.
None	201	12	—	—	72·0	55	++++
	203	10	—	—	17·5	108	++++
	206	9	—	—	39·3	142	++++
	207	5½	—	—	33·2	N.A.	N.A.
	209	9	—	—	68·0	141	++++
Cases of delayed swayback† (own data)				Range	3·5 — 106	0 — 107	—
				Mean ± S.E.	18·1 ± 5·1	41 ± 17	—
Cases of delayed swayback aged 1 to 3 months (Mills and Williams, 1962)				Range	2 — 64	31 — 79	—
				Mean ± S.E	12·4 ± 1·1	59 ± 4·5	—

* Q_{O_2} = µl. of O_2/mg. dry wt./hr.

† The liver copper values shown are for 22 lambs aged 6 to 12 weeks and the cytochrome oxidase activities are for 6 lambs aged 4 to 12 weeks.

Histological

With the exception of one lamb (208) no changes were found which could be identified with swayback or any specific pathological process. However, in this lamb, which was one of the dosed group, small localised areas of meningeal thickening were present over the cerebrum, and there was chromatolysis and prominent satellitosis of some of the cells of the ganglion layer of the hippocampus. In addition, a single "ghost" neurone was found in the spinal cord. These minor changes have been reported recently in swayback (Barlow, 1963a), but the definitive lesions of the disease i.e. hyaline necrosis of multipolar nerve cells and degenerative changes of long fibres in the spinal cord were not observed.

TABLE 2

BLOOD COPPER VALUES OF LAMBS

Results expressed as μg. Cu/100 ml. whole blood

Treatment	*Lamb No.*	*Age*			
		1 day	*3 wks.*	*6 wks.*	*9 wks.*
Molybdate and sulphate by mouth	202	56·3	62·9	70·2	41·3
	204	30·9	32·2	35·7	30·2
	205	39·3	40·4	48·9	37·6
	208	66·2	66·7	71·1	66·7
	210	57·8	60·0	60·9	56·6†
	Mean	50·1	52·4	57·4	46·5
None	201	65·2	70·7	71·5	73·1
	203	59·0	64·4	53·3	57·1
	206	40·9	65·6	71·6	64·4
	207	45·3	49·1	—	—
	209	60·0	66·2	66·2	62·2
	Mean (excluding No. 207)	56·3	66·7	65·7	64·7

† This lamb was killed at the age of 7 weeks, and the figure shown for 9 weeks was calculated by Yates' method for missing values.

Biochemical

The values for the copper content of the blood determined at three-week intervals are given in Table 2. After omitting those for lamb 207 which died at $5\frac{1}{2}$ weeks these figures were subjected to an analysis of variance as shown in Table 3, from which it was concluded that the dosed group showed significant variations between themselves and with time. It was also found that these variations were not statistically significant in the undosed control group and that the difference in the behaviour of the two groups was very significant.

TABLE 3

ANALYSIS OF VARIANCE OF BLOOD COPPER VALUES

Source of variance	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*†
		Dosed group		
Lambs	3,291·75	4	822·94	37·42***
Time	311·74	3	103·91	4·73*
Residual	263·90	12	21·99	—
Total	3,867·39	19	—	—
		Controls		
Lambs	337·80	3	112·60	2·43N.S.
Time	274·23	3	91·41	1·97N.S.
Residual	417·01	9	46·33	—
Total	1,029·04	15	—	—
		Both groups		
Time	383·00	3	127·7	0·83N.S.
Groups	1,225·66	1	1,225·7	7·97**
Interaction	202·97	3	67·7	0·44N.S.
Residual	4,310·45	28	153·9	—
Total	6,122·08	35	—	—

† The levels of significance of the F-ratio are indicated thus:-
N.S. = Not significant (P = >0·05)
* = Significant (P = 0·05 — 0·01)
** = Very significant (P = 0·01—0·001)
*** = Highly significant (P = <0·001)

However, although the difference between the initial mean values for the two groups was not significant by Students "t" test, it was felt, in view of the small number of animals involved, that it might be real and that a correction should be made for it in assessing the effect of treatment. Since the effects of individual variation, time and treatment cannot be separated by a simple analysis of variance, a non-orthogonal analysis was carried out with the aid of a Sirius computer. This showed that dosing with molybdate and sulphate produced a significant depression of the copper content of the blood after 9 weeks (P = 0·05 — 0·01), the mean differences between the two groups being $-6{\cdot}8 \pm 5{\cdot}1$, $-1{\cdot}5 \pm 5{\cdot}3$ and $-11{\cdot}8 \pm 5{\cdot}5$ μg. Cu/100 ml. at 3, 6 and 9 weeks respectively. About half the values for the dosed group and a third of those for the controls were below the lower limit of normality (i.e. <60μg./100 ml.).

The results shown in Table 1 suggest that this treatment had a similar effect on the concentration of copper in the liver, assuming that the values for the two groups were similar at the beginning of the experiment. However, although there is a difference of 27·2 p.p.m. D.W. between the mean values the statistical significance of this is doubtful, owing to the much greater variance of the results for the control animals (P = 0·01 — 0·001). Under these circumstances the "t" test must be modified (Fisher and Yates, 1948) and this

results in a loss of sensitivity. All the values are indicative of a low copper status.

The subjective histochemical assay of the activity of cytochrome oxidase in the various sections of the brain showed no appreciable difference between the dosed and control lambs and both the activity and distribution of this enzyme appeared to be normal in all of them. However, the manometric method failed to detect any activity in one lamb (208) from the dosed group and the same result was obtained when the estimation was repeated. Since the histochemical assay did not reveal this abnormality it is possible that it was due to the presence of an inhibiting factor which was localised in the tissue and released when it was homogenised for the manometric determination. It is interesting to note that minor localised lesions similar to those associated with swayback were found in this lamb. Apart from this result the two groups show no significant differences.

DISCUSSION

The primary object of the experiment discussed above was to determine whether dosing lambs with large amounts of molybdate and sulphate would produce the delayed form of swayback. Although all the lambs developed mild locomotor disturbances none showed the characteristic histological lesions of swayback though one showed slight changes of a type which have been associated with this disease. There is thus some indication that this treatment produced a mild locomotor disturbance, but there is insufficient evidence to prove that it was swayback.

The interpretation of the values obtained for the activity of cytochrome oxidase in the brain presents similar difficulties and gives support to this conclusion. Mills and Williams (1962) found activities ranging from 31 to 79 Q_{O_2} in cases of delayed swayback 1 to 3 months old and from 74 to 126 Q_{O_2} in normal lambs of the same age, the mean values being 59 and 95 respectively. It can be seen from Table 1 that on this basis one lamb in each group would be regarded as having an abnormally low activity, one value of 55 being within the swayback range and the other at zero. The values we have obtained so far for cases of delayed swayback of the same age are, however, more scattered than those reported by the above authors and range from zero to 107 Q_{O_2} with a mean value of 41 (Table 1). We have found an even higher value of 123 in a swayback lamb 5 months old. It is not known whether this difference between our results and those reported by Mills and Williams reflects individual variation or a difference in the site at which the samples were taken. In comparison with our own data three of the five lambs in the dosed group and one in the control group had values within the swayback range. The fact that only one lamb showed histological lesions in the central nervous system and that this was the one in which no cytochrome oxidase activity could be detected manometrically may be of considerable significance.

No abnormalities were revealed by the histochemical assay of the activity of this enzyme, but it should be pointed out that this depends on the subjective evaluation of selected sections of tissue and, therefore, cannot be expected to be as quantitative or as sensitive as the manometric method. Mills and Williams (1962) suggested that the latter might be useful for diagnostic purposes since it can be carried out more quickly than the estimation of copper or the histological examination, but it is obvious that much more information on normal values and those associated with other diseases of the central nervous system is needed before the diagnostic value of this assay can be properly assessed.

The copper status of all the lambs was low due to the low dietary intake of copper and as was noted above there was some evidence that dosing with molybdate and sulphate reduced it still further but only to a small extent. In the dosed lambs the blood and liver copper levels were very similar to those found in natural cases of delayed swayback of the same age.

CONCLUSIONS

The oral administration of large amounts of molybdate and sulphate to young lambs with a low initial copper status produced a slight stiffness and inco-ordination of the hind limbs. Slight non-specific lesions of a type associated with swayback were found in the central nervous system of one lamb, but the definitive lesions of this disease were not present.

This treatment also caused a slight reduction in the copper content of the blood. The levels of copper in the blood and liver and some of the values obtained for the activity of cytochrome oxidase in the brain were as low as those found in natural cases of delayed swayback of the same age.

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Combating Swayback in Lambs

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WHILST not in the first rank of important sheep diseases swayback does cause severe and dramatic losses in lambs. During recent years technical developments in a number of fields have made possible much better control of the condition, despite the fact that knowledge of the fundamental processes involved is still very incomplete. Before proceeding to describe these control methods it might be helpful to outline briefly the conditions under which swayback occurs and how it may be recognised.

Much of the investigation work from which the following information has been derived was carried out in south-east Scotland and the ensuing remarks are strictly applicable only to this area, though they may also have some general relevance.

Swayback occurs predominantly on high arable and marginal farms and there is some evidence to suggest that farms with a general southerly aspect are most liable to it. All breeds of sheep can be affected: in south-east Scotland susceptible farms are generally producing Greyface lambs from Blackface ewes crossed with Border Leicester tups, so the incidence of the disease seems to be greatest in this class of stock. The larger and faster growing lambs of other breeds are, however, also affected.

Losses from swayback have increased in post-war years, severe outbreaks occurring on farms where previously the disease was unknown. It is almost always associated with measures to increase the fertility and productivity of the land. Crop rotations or the reseeding of permanent pastures, or heavy dressings of lime are the usual precursors of an outbreak of swayback.

Effect of Weather

The incidence and severity of swayback vary very much from year to year and are greatest in those years in which the biggest losses from hypomagnesaemic tetany occur. The weather conditions which favour the development of both conditions would thus appear to have much in common. As far as swayback is concerned, the months of February and March, when the ewes are in the last two months of pregnancy, are the most important times and proper preventative treatment instituted then can virtually eliminate the disease.

What are the conditions which favour a high incidence of swayback? They are not easy to define in terms of the average figures for weather data published by the Meteorological Office: but mild wet weather with plenty of sunshine in February and a forecast of similar weather in March should put the farmer on his guard. A short spell of snow later on into March may not influence the situation much but a longer period of cold weather with continuing snow in January and February is likely to be followed by a low incidence of swayback. The two years 1962–63 and 1963–64 illustrate this well. The former was a very hard winter and the incidence of swayback was negligible, but the following winter was mild and open, and swayback was a major problem over wide

areas of Scotland. The mild winter of 1956–57 preceded the previous high incidence for swayback. From experiences of the last 40 years or so it seems that bad years of swayback often occur in pairs at intervals of 7 to 10 years. If these observations can be used to indicate future trends, those who have experienced the disease must not relax their vigilance in the light of two or three seasons' freedom from swayback.

The disease itself takes two forms, one 'congenital', in which the signs are apparent at birth, and the other in which the onset of clinical disease is delayed for up to 6–8 weeks after birth. In both types the signs are similar, but they are generally milder in the 'delayed' form. They range from complete paralysis of the new-born lamb to a swaying, staggering gait affecting particularly the hind limbs but which may be so mild as to be noticeable only when the flock has been driven some distance. These signs are associated with defects within the central nervous system. A number of other conditions can cause similar symptoms and so it is very important that the diagnosis is made by your veterinary surgeon with laboratory facilities to assist him. Inappropriate treatment based on faulty 'do-it-yourself' diagnosis can have disastrous results.

The Rôle of Copper

Whilst little is known about the fundamental mechanisms of the development of swayback in the lamb, it has long been known that the affected lambs and their mothers are deficient in the trace element copper. The situation is complicated by two facts—firstly, that not all copper-deficient ewes give birth to swayback lambs; and secondly, that copper deficiency in sheep, and swayback, may occur where the pasture contains apparently adequate amounts of copper. These facts notwithstanding, the administration of adequate supplementary copper to pregnant ewes on susceptible farms will prevent the disease in lambs. Even on farms where the disease has been recognised for the first time amongst the early lambs, dosing of those ewes still to lamb will effect a worthwhile degree of control of the disease.

Treatment of affected lambs is less satisfactory. In the majority of congenital cases the damage to the nervous system is so extensive as to be irreparable. Treatment of the milder delayed cases, however, will to some extent halt the progress of the disease. Dosing of apparently normal lambs on such farms is well worthwhile as it will stop the condition developing in them. The practicalities of gathering at this time of year of course do provide further hazards for the lambs, and great difficulties for the shepherd. Consultation with a veterinary surgeon with his extensive local knowledge of the developing situation will again help the farmer to assess the relative risks of treating the lambs or leaving them alone.

As sheep are very susceptible to poisoning from copper, great care must be used in its administration and it should be given only where a diagnosis of swayback has been firmly established. The forms in which copper may be administered are many and varied.

Four Treatments

The applicability of the various methods is dependent upon the importance of the sheep enterprise to the farmer, the labour available, the degree to

which the farm is liable to swayback and how far dosing can be integrated with other procedures. It is wise therefore to consider the merits and demerits of each method.

Copper sulphate solution by mouth. A solution of crystalline copper sulphate is prepared so that it will contain 1·0 grammes of copper sulphate in the convenient dose of 2 oz. of water. This drench must be given to all the pregnant ewes every three to four weeks from the beginning of February until lambing. It is highly effective and has the advantage that the individual sheep are known to have received the dose. Repeated gathering allows their condition to be easily assessed and also permits the treatment of other conditions such as foot rot. The cost per head for material is negligible, but the work is laborious and if abortions are to be avoided handling must be gentle as lambing time approaches.

Solutions of copper sulphate in water can also be used for the prevention and treatment of the disease in the lambs themselves. For young lambs the dose must not exceed 0·25 grammes of copper sulphate and it should be diluted in several ounces of water or milk to avoid acute stomach upsets. Particular care must be taken with lambs that are not known to have sucked as their delicate stomach walls are completely unprotected from the astringent effects of the solution. In lambs 5–6 weeks of age which are taking substantial amounts of grass or weigh 40–50 lb., the dose may be increased to 0·5 grammes. Dosing should be repeated every three to four weeks until the lambs are three months old. There is little likelihood of new cases appearing after this time.

Injections. Ethical pharmaceutical companies make a variety of organic copper compounds suitable for injection. Depending on the type, a single dose is given subcutaneously or intramuscularly about 6–10 weeks before lambing. These injections are very satisfactory in controlling swayback, the cost varies from 1s. to 1s. 6d. per head, and they have the advantage that the sheep need only be handled once during pregnancy. The disadvantage is that in 3–5 per cent. of sheep, 'cold abscesses' develop at the site of injection and this may subsequently reduce the value of the carcass or skin. Experience has indicated that copper injections are at present unsuitable for administration to lambs.

Mineral mixtures. Mineral mixtures with a high copper content may be used if supplementary feeding is practised. They are satisfactory if the makers' instructions are carefully followed. The disadvantage is that some ewes may not take box feeding whilst others may take too much. Thus the incidence of some cases of swayback or the less likely possibility of an occasional sheep dying of chronic copper poisoning may discredit a perfectly reputable product in the eyes of the farmer.

Mineral Licks. Copper-fortified mineral licks share the advantages and disadvantages of mineral mixtures. With both it is important to choose the product of a really reputable firm, which declares the analysis of its product on the container. 1,500 parts per million of copper for a mineral mixture, and 1,500–2,500 parts per million of copper for a lick are necessary, yet examples of anti-swayback licks and mixtures containing less than 200 p.p.m. have been found too frequently.

Having reached a decision about the form of copper supplementation to use, the farmer must be careful to avoid the administration of further amounts of copper in worm drenches or supplementary feeds, since the dangers of copper poisoning cannot be over-emphasised.

Conclusions

Swayback is a problem of increasing importance on high arable and marginal farms in south-east Scotland. Farms with a southerly aspect raising Greyface lambs may be particularly liable to the disease which appears to be related to liming and pasture improvements. If the weather in February is sunny and mild and open and the forecast for March is good then there is a real likelihood of serious outbreaks of swayback, and preventative measures should be instituted without delay on farms where the disease is known. Several years' freedom from swayback should not lead one to believe that the danger has gone forever as past experience has indicated that the condition reaches serious proportions only once or twice in a decade.

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MEASUREMENT OF NERVE CELL DAMAGE IN THE SPINAL CORD OF LAMBS AFFECTED WITH SWAYBACK

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INTRODUCTION

Barlow, Purves, Butler and Macintyre (1960) have enumerated the diagnostic criteria of swayback as (1) ataxia of the newborn or young lamb; (2) cavitation or gelatinous lesions of the cerebral white matter and/or a characteristic picture of chromatolysis, neurone necrosis, and myelin degeneration in the brain stem and spinal cord; (3) low copper status. The findings of the majority of authors agree with these criteria. However, Spais, Palsson, and van Bogaert (1961) and Innes and Saunders (1962) have concluded that widespread neuronal lesions are absent in swayback and the latter even suggested that their presence indicates another disease process.

In view of this discord it is important for the diagnostic pathologist to have some numerical index of nerve cell damage, and precise and easily located sites in which damaged cells may be expected. Whereas the red and vestibular nuclei are almost always found to contain necrotic cells (Barlow *et al.*, 1960; Barlow, 1963) their limits are not readily determined from external landmarks. The spinal cord contains circumscribed areas of grey matter which can be related to external landmarks and thus appears to be good material in which to make an attempt to assess quantitatively the cellular changes in swayback.

Since assessments of the amount of damage by actual counting of cells are rare in neuropathology a method had to be evolved which would take account of the different sources of variation. In view of the considerable amount of labour involved the work was designed also to investigate the rate of progress and cumulative effects of the disease process, in addition to information of direct diagnostic value.

MATERIALS AND METHODS

The swayback lamb material was drawn from a collection of 200 clinically, pathologically and biochemically verified cases. The normal lamb material was collected for the purpose of the present study and comprised a series of 20 Blackface lambs from a farm with no history of swayback. The lambs were aged 24 hours to 14 months. Twelve lambs covered the first 4 months and 8 lambs the remaining 10 months, thus giving better coverage of the period of most rapid growth.

The spinal cords were removed in toto and the meninges opened dorsally and ventrally before placing in 10 per cent. formol saline. After 24 hours fixation transverse blocks (A) 3 mm. thick were cut from 4 levels viz. 1st, 6th, 14th and 24th nerve roots in the freed cord. Assuming all the sheep to have 13 thoracic and 6 lumbar vertebrae, these correspond to the

regions C_2, C_7, T_7 and L_4, since C_1 is left attached to the medulla oblongata when the head is removed by section through the atlanto-occipital space. In the normal series a second similar block (B) immediately behind (A) was taken from each site and kept separate. The blocks were returned to fixative for one month. They were then processed through an alcohol-benzene series and embedded in paraffin. Sections were cut at 7μ and 3 alternate sections from a series mounted together on a glass slide and stained with haematoxylin and eosin.

All the counting was done on one monocular microscope at a fixed final magnification of X300. The field stop of the microscope was made square and fitted with a 0·5 mm. graticule. The whole area of central grey matter was traversed and only those nerve cells of sufficient size to touch at least 3 sides of a graticule square were scored. By confining attention to cells whose maximum dimension was greater than 25μ the difficulties of both pathological assessment of small cells and the total amount of work were reduced. It was inevitable, however, that a few long spindly cells e.g. in the substantia gelatinosa Rolandi, in which no assessment of pathological change could be made, would be scored.

Chromatolysis and nerve cell necrosis (Barlow *et al.*, 1960; Barlow, 1963) were the criteria of damage. The appearance of normal and damaged cells in haematoxylin and eosin stained sections was defined in the following terms so that an observer untrained in pathology and hence more free of subjective bias, could be used to do the counting:-

Normal cells may be rounded, elongated or stellate. Except at the point of exit of the axon (at one of the apices of the cell) the cytoplasm contains coarse or fine light blue or slightly pink particles (Nissl's granules). The nerve cell nucleus is large, central, rounded and clear. It contains a dark nucleolus and variable numbers of chromatin particles. The nuclear membrane is smooth and fine. Some normal cells may be very faintly staining, but when they are seen their structures are defined and not "ghost-like" as in the case of lysing cells.

Diseased cells for the purpose of this study may be swollen or shrivelled. The cytoplasm has a glassy pink colour and if it contains any blue particles these are situated towards the periphery of the cell (chromatolysis). The nucleus may be absent or appear as a small solid dark body situated eccentrically or at the margin of the cell. The nucleolus and chromatin particles may be indistinguishable and the nuclear membrane thickened and crenated. In some cases the nucleus is disrupted and appears as a loose focus of dark staining fine particles. A cell whose cytoplasm is a uniform glassy pink and contains neither blue particles not any evidence of a nucleus is necrotic.

RESULTS

Development of Method of Counting

As the counting of neurones is time consuming, especially in regions like cervical and lumbar enlargements where the number of cells is large, it is essential to assess the relative importance of the various sources of error in order to get the greatest accuracy for a given amount of work. The sources of variation in a comparison of the number of healthy and diseased neurones in a section of a particular area of the spinal cord from swayback and from normal lambs

are as follows: variation between lambs, variation between blocks taken from the same region, variation between sections taken from the same block, variation between operators and unconscious systematic variation in the interpretation of the criteria laid down for healthy and diseased neurones. It was decided to do all the counts with the same operator, so that, although the actual counts would be partly subjective and would vary with the operator, the comparisons would be valid.

To assess the variation between sections from the same block, two 7μ sections (28 to 35μ apart) from the 2nd cervical segment were counted on 13 separate occasions. Sections 1 and 2 were mounted on the same slide, the usual practice in the laboratory. The operator was unaware of the experimental design and the identity of any of the slides, each slide being labelled differently on each occasion and mixed with a number of other unknown slides of both the swayback and normal series. The results of these counts are given in Table 1. where it can be seen that there was little difference between sections, the mean for the total number of neurones in sections 1 and 2 being 47·6 and 47·3 respectively. There was a significant correlation ($r = 0{\cdot}725$ $P<0{\cdot}01$) between sections counted on the same occasion, indicating bias of the operator once the first section was counted. The variation between repeat counts of the total neurones in a section was relatively small, the standard deviation and coefficient of variation for section 1 were 6·0 and 12·6 per cent. respectively. On these sections from the cervical region of a normal lamb there were neurones, however, which came within the criteria for diseased but not necrotic cells. The numbers were small and, apart from the first occasion, there was little difference in the counts of diseased cells. It was, therefore, decided because of the bias and because of the small variation between sections from the same block that only one section from each block would be counted. It must be pointed out that the variation between sections from the same block may be greater for other regions of the spinal cord.

TABLE 1

REPEAT COUNTS OF DISEASED AND TOTAL NEURONES

Two sections from the same block taken from the 2nd cervical segment of the spinal cord of a normal lamb

		Counts												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Section I	Total	56	46	64	44	49	48	46	46	43	45	45	43	44
	Diseased	15	0	6	3	3	2	3	3	3	2	3	2	3
Section II	Total	55	48	57	44	47	45	48	49	47	44	44	45	42
	Diseased	48	0	3	4	5	3	4	4	4	3	3	4	3

To examine the variation in counts between blocks from the same region of the spinal cord, the total numbers of neurones in a single section taken from each of two blocks, A and B, from the region of 2nd cervical nerve roots of 20 normal lambs were counted and the results are given in Table 2. Statistical analysis of the results showed that there was, firstly, no systematic difference between blocks A and B, and secondly, no effect of age on the number of neurones per section. Ignoring the age of the lamb, the difference between sheep was significant at the 5 per cent. level. Estimates of the standard deviation (S.D.) of variation between sheep and between blocks from the same sheep were 13·3 and 15·0 neurones/section respectively, both much greater than the S.D. of 6 for the reproducibility of counts on different occasions. The difference between blocks from the same sheep cannot, therefore, be accounted for by operator errors. From the above considerations, it was thought better to increase the number of sheep and confine the counting to a single count of the neurones on

TABLE 2

COUNTS OF TOTAL NUMBER OF NEURONES

Single sections from 2 blocks from the cervical regions of normal lambs of different ages

Age	*Sheep No.*	*Block A*	*Block B*	*Age*	*Sheep No.*	*Block A*	*Block B*
1 day	1	57	64	4 months	11	85	72
	2	101	106		12	84	70
7 days	3	50	56	6 months	13	61	99
	4	69	56		14	89	90
	5	76	63		15	48	52
1 month	6	122	75	10 months	16	79	59
	7	69	80		17	67	52
2 months	8	75	62	12 months	18	51	54
	9	122	75		19	116	94
3 months	10	57	86	14 months	20	80	87

one section from one block from each region of the spinal cord, rather than counting sections of more than one block from each region. In addition, much of the swayback material had been obtained prior to this investigation and only one block had been taken from each of the 4 regions of the spinal cord.

Changes in the interpretation of the criteria for diseased and healthy neurones by the observer, leading to a bias in the counts, were assessed by counting appropriate standard sections at intervals throughout the period of counting, the identity of the standard section being unknown to the operator. At no time was there evidence for any changes with time in the interpretation of the criteria. The data, however, allowed estimates of operator error to be calculated for the total and diseased neurones of the 4 regions of the spinal cord of normal and swayback lambs. The standard sections

TABLE 3

REPEATABILITY OF COUNTS OF DISEASED AND TOTAL NEURONES

Neurones in (numbers/section) from 4 regions of the spinal cord of normal and swayback lambs

		Cervical *	*Cervical enlargement**	*Thoracic**	*Lumbar enlargement**
Swayback	Total	68·9 ± 17·0	281·1 ± 35·2	60·6 ± 7·52	223·0 ± 17·2
	Diseased	11·7 ± 6·92	20·8 ± 7·73	8·29 ± 6·02	13·6 ± 5·13
Normal	Total	92·6 ± 9·13	268·0 ± 23·8	87·3 ± 15·8	192·0 ± 15·5

* Mean + standard deviation.

were counted on 7 occasions and the data obtained are summarised in Table 3. Only the data for total cells of the sections from the normal lamb are given in the table, since the number of "diseased" cells were so small that the variability of their counts was meaningless. The repeatability in the case of total neurones, as measured by the S.D., tended to decrease with the number of cells per section. Thus the cervical enlargement had the greatest number of cells and the greatest variation of the regions. The repeatability of counts of total cells within a region tended to be the same for normal and swayback lambs, the coefficient of variation for each region ranged from 7 to 25 per cent. in both groups. For diseased cells, the repeatability of counts tended to be the same for all regions, but the S.D's. were smaller and the coefficient of variation greater than the corresponding values for total neurones.

Necrotic Cells

The numbers of necrotic cells per section found in any of the regions of the spinal cords of affected lambs examined was small, except for 3 lambs which will be dealt with separately. The mean number of necrotic cells per section found in each of the regions in each age group are given in Table 4. In general, the numbers of necrotic cells, expressed in absolute terms and as a percentage of diseased cells (including necrotic cells) were greater in the two enlargements than in the other two regions and in the older lambs. Lambs less than 1 week of age had the smallest number of necrotic cells in all regions.

The numbers of necrotic and diseased cells found in the regions of the spinal cords from the 3 lambs with abnormally high numbers of necrotic cells are given in Table 5. In the lumbar enlargement, for example, the percentage of diseased cells which were necrotic ranged from about 40 to 60 per cent. All regions, however, were not affected to the same degree. The value (9·1 per cent.) for the cervical region of the oldest lamb (> 16 weeks) was similar to the mean 8·9 per cent.

TABLE 4

MEAN NUMBER/SECTION OF NECROTIC (N) AND DISEASED (D) NEURONES

4 regions of the spinal cord of swayback lambs

Ages (weeks)	*Cervical*		*Cervical enlmt.*		*Thoracic*		*Lumbar enlmt.*	
	N	*D*	*N*	*D*	*N*	*D*	*N*	*D*
0—1*	0·33	13·6	1·42	27·1	0·25	11·8	1·17	23·5
	(12)	(13)	(12)	(13)	(12)	(13)	(12)	(13)
1–4*	0·75	18·2	2·75	30·9	0·25	13·1	3·08	26·1
	(13)	(14)	(13)	(14)	(13)	(14)	(13)	(14)
5—8	0·57	16·4	3·86	33·7	0·71	13·0	4·00	26·9
	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
9—16	0·86	12·0	2·57	26·4	0·29	9·6	2·29	22·7
	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
Over 16	1·08	12·2	1·77	27·1	0·62	8·4	1·69	20·9
	(13)	(14)	(13)	(14)	(13)	(14)	(13)	(14)

( ) Number of sheep in group

* In 0—1 week and 1—4 week old groups 8 and 2 lambs, respectively, showed defects of cerebral myelin. Necrotic cells were not recorded in the case of one such individual in each group.

found for the same region from the other swayback lambs of the same age, whereas the corresponding values for the cervical enlargement were 73·2 and 6·5 per cent. respectively. Reference to the protocols of these 3 animals, however, failed to reveal any common features, and whilst the figures are extremely interesting it is not possible to advance any explanation for them.

Diseased Cells

The mean numbers of diseased cells per section found for each of the 4 regions of the spinal cord of swayback lambs of different ages are given in Table 4. They differed significantly ($P < 0.001$) between regions and between age groups. Since the changes in the mean counts of diseased neurones with age of the affected lamb were similar for each of the regions, the numbers were pooled for all regions within the same age group and the mean number per section calculated. The values for the 0 to 1, 1 to 4, 5 to 8, 9 to 16 and over 16 weeks age group were 19·0, 22·1, 22·5, 17·7 and 17·1 diseased cells per section, respectively. Thus the degree of pathological damage varied in a systematic manner, a maximum being shown in affected lambs killed at 5 to 8 weeks of age.

To examine the difference between regions, the numbers of damaged cells in each region were pooled over all age groups and the mean for each region calculated. The values for the cervical, cervical enlargement, thoracic and lumbar enlargement were 14·6, 28·8, 11·1 and 23·8 diseased cells per section, respectively. The damage in both enlargements was much greater than in the other two regions.

TABLE 5

NUMBER PER SECTION OF NECROTIC (N) AND DISEASED (D) NEURONES

Regions of the spinal cord from the 3 swayback lambs with relatively high numbers of necrotic neurones

Age (weeks)	*Cervical*			*Cervical enlargement*			*Thoracic*			*Lumbar enlargement*		
	N	*D*	$\frac{N}{D} \times 100$	*N*	*D*	$\frac{N}{D} \times 100$	*N*	*D*	$\frac{N}{D} \times 100$	*N*	*D*	$\frac{N}{D} \times 100$
0—1	16	21	76·2	43	54	79·6	10	19	52·6	20	45	44·4
1—4	0	29	0·0	9	46	19·6	—	—	—	19	45	42·2
Over 16	1	11	9·1	22	30	73·2	2	9	22·2	16	27	59·2

Total Cells

The total number of neurones in a single section from the 4 regions of the spinal cord of 20 normal lambs, whose ages ranged from 1 day to 14 months, are given in Table 6. There was no observable effect of age on the total number of neurones in any of the 4 regions examined. For this reason the results were pooled; the mean counts with their S.D's. for the cervical, cervical enlargement, thoracic and lumbar enlargement were respectively, 72·1 ± 16·7, 250·2 ± 39·8, 63·3 ± 18·1 and 202·3 ± 52·2.

TABLE 6

VARIATION IN TOTAL NUMBER OF NEURONES

Neurones (number/section) in regions of spinal cord from normal lambs of different ages

Age	*Sheep No.*	*Cervical*	*Cervical enlargement*	*Thoracic*	*Lumbar enlargement*
1 day	1	71	181	79	136
	2	93	293	65	190
7 days	3	39	290	44	310
	4	63	220	81	113
1 month	5	68	248	63	194
	6	98	279	72	271
2 months	7	92	278	74	227
	8	78	205	53	191
3 months	9	69	265	71	289
	10	67	261	110	200
4 months	11	77	254	68	238
	12	70	277	37	181
6 months	13	88	307	55	245
	14	53	212	43	189
10 months	15	72	245	55	222
	16	97	231	56	177
12 months	17	41	276	44	188
	18	76	246	92	148
14 months	19	75	283	53	216
	20	56	193	52	122

An estimate of the cumulative damage of swayback was obtained by comparing total cells in sections taken from the spinal cord of swayback and normal lambs of the same age. It was not feasible to count all available sections from swayback lambs; counts were made of the total cells in sections from 4 regions of the cord of lambs of one age group (>16 weeks) and from the cervical region of lambs of differing age. The mean number and range of neurones, found in the 4 sections of the spinal cord from swayback and normal lambs aged more than 16 weeks, are given in Table 7. Apart from the cervical enlargement, there were no observable effects of swayback on the total number of neurones in the different regions of the spinal cord. The reduction in number of neurones in the cervical enlargement

TABLE 7

NUMBER PER SECTION AND RANGE OF TOTAL NEURONES

4 regions of the spinal cord of swayback and normal lambs aged more than 16 weeks

	No. of lambs	*Cervical*	*Cervical enlargement*	*Thoracic*	*Lumbar enlargement*
Swayback	14	76·1	224·0	66·0	199
		47—109	111—384	34—101	96—254
Normal	13	79·5	259	64·3	202
		4—113	180—359	37—102	148—266

TABLE 8

NUMBER PER SECTION AND RANGE OF TOTAL NEURONES

Cervical region of the spinal cord of swayback and normal lambs of differing age

Age (weeks)	*No. of lambs*	*Swayback*	*No. of lambs*	*Normal*
0—1	13	78·7 36—144	4	66·0 50—101
1—4	14	77·0 50—100	2	99·0 76—122
5—8	7	72·6 54—93	2	72·0 69—75
9—16	7	65·2 40—87	2	89·5 57—122
>16	14	74·1 45—115	13	73·9 48—116

from 259·2 in the normal to 224·0 neurones per section in the swayback lambs was significant at the 5 per cent. level. The ranges in each region of normal and swayback lambs were similar and very wide. The finding that the percentage of total cells which were diseased was similar for all regions of the spinal cord (cervical 19·5 — 3·3 to 48·9; cervical enlargement 16·1 — 8·3 to 26·7; thoracic 19·5 — 6·0 to 31·6; lumbar enlargement 17·2 — 9·5 to 32·1), indicated that, firstly, the disease process affected all regions to an equal extent and secondly, the increased numbers of diseased cells found in the cervical and lumbar enlargements were a simple reflection of the greater total number of neurones. The mean values for the cervical, cervical enlargement, thoracic and lumbar enlargement were 19·5, 16·1, 19·6 and 17·2 per cent. respectively.

The mean number and range of total neurones found in sections from the cervical region of the spinal cord of swayback and normal lambs of differing ages are given in Table 8. Statistical analysis of the results showed no significant effect of either the age of the lamb or swayback on the total number of neurones. It is interesting to note that the cervical region in the oldest group of lambs was counted twice independently with very similar results (See Tables 7 and 8).

DISCUSSION

This paper is concerned with the frequency of chromatolysis in and necrosis of, nerve cells in the spinal cords of sheep affected with swayback. Since Spais *et al.* (1961) have declined to interpret intermediary forms of degeneration it is important to determine whether the frequency of cell necrosis alone is sufficient to have any diagnostic usefulness.

The data presented shows large variations between sheep and between regions of the same sheep. In the 55 cases examined no necrotic cells were seen in single sections from 4 regions of 4 sheep, whilst in 3 animals very large numbers of necrotic cells were found. The data do not provide any explanation for this variation. There was no obvious region of choice. The cervical enlargement which had the greatest number of necrotic cells per section showed no necrotic cells in 20 per cent of cases. Thus if necrosis is the only criterion of cell damage it is evident that there is some justification for the conclusions of Spais *et al.* (1961).

The validity of the relevant diagnostic criterion of Barlow *et al.* (1960) rests, therefore, on the recognition of pre-necrotic forms. In the present work care has been taken to control the sources of variation and produce an objective piece of research. However, the criteria for scoring cells are themselves the result of subjective experience and may not be endorsed by all pathologists. Using them an observer with no previous experience of pathology had some difficulty in classifying cells as normal or diseased at first, but after examination of 10 slides of mixed swayback and normal spinal cord sections this operator was able to obtain reproducible results. Throughout, small

numbers of neurones in normal animals were classified as diseased, but not necrotic cells, which may be why Spais *et al.* (1961) were unwilling to interpret minor changes. The application of the same criteria of cell recognition to both groups of animals, however, gave such highly significant and reproducible differences that doubts regarding their absolute pathological validity do not affect the final analysis.

The finding that the percentage of total cells which were diseased was the same for all regions is of considerable practical and theoretical importance, suggesting that the disease has a diffuse effect, possibly on particular cell types, but does not discriminate between particular functional units. Since the cervical enlargement has the greatest number of cells, it follows that diseased cells will be encountered most frequently at this level. The mean figure for all age groups of the series is 29·4 diseased cells per section of the cervical enlargement. Thus, where the material which the pathologist can examine is limited, it is important that a block from the cervical enlargement of the cord should be selected.

The finding of maximum numbers of diseased and necrotic cells in the 5 to 8 week old group confirms a previous more subjective analysis (Barlow, 1963). Yet, despite the fact that on average 18 per cent. of the total cells were diseased, in general there was no evidence for a reduction in the number of cells remaining in older animals. This can be interpreted as indicating that the progession of the lesion is slow. In other words, after initial damage cells may take some weeks to become necrotic, but having died they remain in the tissues a further few weeks. Alternatively diseased cells may recover or be "replaced" by hypertrophy of small cells ($<25\mu$ diameter).

However, greater numbers of necrotic cells occurred, both in absolute terms and as a percentage of diseased cells, in the 2 enlargements and in older animals. In lambs more than 16 weeks of age there was also a significant reduction in the number of cells surviving in the cervical enlargement. These facts show that whilst the initial effects of the disease may be diffuse, there are other local factors which influence the ultimate fate of the cell. Their nature is speculative, but they may be related in some way to limb movements.

The failure to demonstrate any effect of age upon the number of cells in a given region of normal animals is interesting and surprising since the weight of the cord increases 10-fold between birth and maturity (Golle, 1958). It suggests that increase in length and girth of the spinal cord is accompanied by (1) comparable increases in nerve cell volume; (2) an increase in the total number of countable cells in the spinal cord through development of neurones too small to count in the newborn lamb, with the result that the number of nerve cells per section remain relatively constant; or (3) development of tissues other than nerve cells, the numbers of which remain relatively constant in a given section due to slight dispersion of cells within the grey columns.

The present work contains 2 sources of bias, namely the severity of the disease and whether the lamb was affected clinically at birth or subsequently. It would have been most interesting to investigate congenital and delayed cases of swayback separately, but it is virtually impossible to make comparable age-balanced groups to do this. Thus it was inevitable that the younger age groups were predominantly congenital and perhaps more severe cases, whilst the older groups contained a higher proportion of delayed and milder cases. This bias may be responsible for the changes attributed to age.

CONCLUSIONS

A quantitative technique has been developed for the investigation of nerve cell changes in the spinal cords of lambs affected with swayback. The criticisms of some workers regarding such changes have been evaluated.

It has been shown that the incidence of necrotic cells varies greatly between sheep and between regions of the same sheep. The incidence of less severe changes is more uniform, a mean value of 18 per cent. of the total cells being obtained for all regions of the oldest group of lambs.

The cervical enlargement is the region of the cord with most diagnostic usefulness in that it is liable to contain the greatest number of necrotic and diseased cells. Even in this region necrotic cells any be absent from 20 per cent. of cases. There was a significant reduction in the total cells in this region in swayback lambs over 16 weeks of age. It has been suggested that whilst the initial cellular damage is very widespread the fate of the damaged cell may be influenced by local factors.

In normal lambs the number of nerve cells within a given region appeared to be random and significant alterations with age were not detected.

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8.

Structural Changes of the Central Nervous System in Swayback (Enzootic Ataxia) of Lambs*

I. Light Microscopy Using Phosphatases as Organelle Markers

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Swayback is a copper deficiency-dependent ataxia of newborn and young lambs (Barlow et al., 1960). In some cases there are cystic or gelatinous transformations of the cerebral white matter which have been ascribed to a process of demyelination analogous to Schilder's disease (Innes and Shearer, 1940), to perivascular softening and necrosis — "spongy transformation" (Behrens and Schulz, 1959; Spais et al., 1961), or to neurodysgenesis (Barlow et al., 1960).

Barlow et al. (1960) showed that these lesions were not essential to the condition but occurred in less than half of 79 cases which they studied. They defined the criteria for swayback as chromatolysis and necrosis of large multipolar nerve cells in the brain stem and spinal cord, together with degeneration of fibres in the long tracts. These changes may or may not be accompanied by deficiency of cerebral myelin. Subsequent work (Barlow, 1963a; Barlow et al., 1964) extended the knowledge of the brain stem and cord changes and showed the progress of the lesions. It was found that the lowered cytochrome oxidase activity of the nervous system in swayback originally reported by Howell and Davison (1959) was most prominent in the large nerve cells (Barlow, 1963b). However, the pathogenesis of the neuronal lesion remains obscure. Chromatolysis in swayback resembles that seen in axonal reaction after section of a nerve. The cytological changes of axonal reaction have been studied extensively by histochemical methods (Barron and Tuncbay, 1964; Novikoff and Essner, 1962; Novikoff and Goldfischer, 1961). The present study was made to determine whether similar changes in organelles occur in swayback.

Materials and Methods

18 lambs (Border Leicester X Blackface, & Cheviot X Dorset) with clinical signs of swayback were used. They came from 6 farms in Scotland and varied in age from 1—69 days. The diagnosis of swayback was confirmed by pathological and biochemical examination of each case but only nine lambs showed gelatinous lesions or cystic cavitation of the cerebral white matter. The mean level of copper in liver was 8.2 ppm dry weight (range 2.6—14.6).

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Six clinically normal lambs (Border Leicester X Blackface, Scottish Halfbred X Blackface) originating from 2 farms with no history of swayback served as controls. The mean level of copper in liver was 72 ppm dry weight (range 4.2—226).

All the animals were killed by decapitation through the atlanto-occipital space. Except where perfusions were carried out for electron microscopy (Cancilla and Barlow, 1966 a, b), the entire central nervous system was rapidly removed. Transverse blocks 3—4 mm thick were cut from the parietal region, the mesencephalon, the medulla with adjacent cerebellum at the level of the middle peduncle, and the cervical spinal cord. A longitudinal block of cervical cord was also taken immediately cranial to the transverse slice from the cervical enlargement. These tissues were fixed in cold Baker's formalin (10% formalin with 1% calcium chloride) and processed by Gomori's acid phosphatase (A.P.) technique according to the method of Holt (1961). Similar 12—15 μ frozen sections were used to demonstrate the Golgi apparatus using the thiamine pyrophosphatase method (T.P.P.) of Novikoff and Goldfischer (1961). In some instances adjacent sections were stained with A.P., T.P.P., and hematoxylin and eosin, respectively.

Results

In normal neurones lysosomes were distributed diffusely throughout the cytoplasm and processes; the staining was intense but not uniform (Fig. 1). The Golgi apparatus formed a complex of vesicular and threadlike structures in the perinuclear and midzonal regions of the cytoplasm and extended for a short distance into the dendrites (Fig. 2). In none of the cases of swayback was nerve cell degeneration recognized in the forebrain. Acid phosphatase-rich macrophages were not found in abnormal numbers in any of the 9 cases with gross disease of cerebral myelin.

Central chromatolysis with swelling of the cell body and margination of the nucleus was found in the nerve cells of selected areas of the brain stem or spinal cord in 17 of the 18 swayback lambs and had proceeded to actual necrosis in 11. The changes in the organelles of these nerve cells within the red and vestibular nuclei, reticular formation and ventral horns revealed by the phosphatase techniques were of relatively late occurrence in the degenerative process. Observation of a strict sequence of changes in the organelles was not possible, though a general trend was deduced from examination of cases of increasing age.

In swollen nerve cells with marginated nuclei and only a residual rind of Nissl substance no significant alterations in the number, size or distribution of lysosomes was evident (Fig. 3 and 4). In the last stages of cell death the lysosomes disappeared (Fig. 5). In these cells the Golgi apparatus, however, showed one of three distinct changes: it contained large vesicles and was displaced to the periphery of the cell (Figs. 6—8), it was reduced to a few fragments scattered throughout the cytoplasm (Figs. 9 and 10), or it was condensed into a central agglomeration (Figs. 11 and 12). Ultimately, there was complete dissolution of this organelle.

Both A.P. and T.P.P. reactions were sensitive markers of gliosis in the degenerating spinal tracts. The former lightly stained the glial nuclei, whilst the latter delineated the processes of the fibrous astrocytes.

Discussion

Since lysosomes and Golgi apparatus are intimately associated with cell function, it was surprising that morphological changes in these organelles were evident only in the later stages of the chromatolysis associated with swayback. In axonal reaction in the rat, Novikoff and Essner (1962) observed thinning of the Golgi

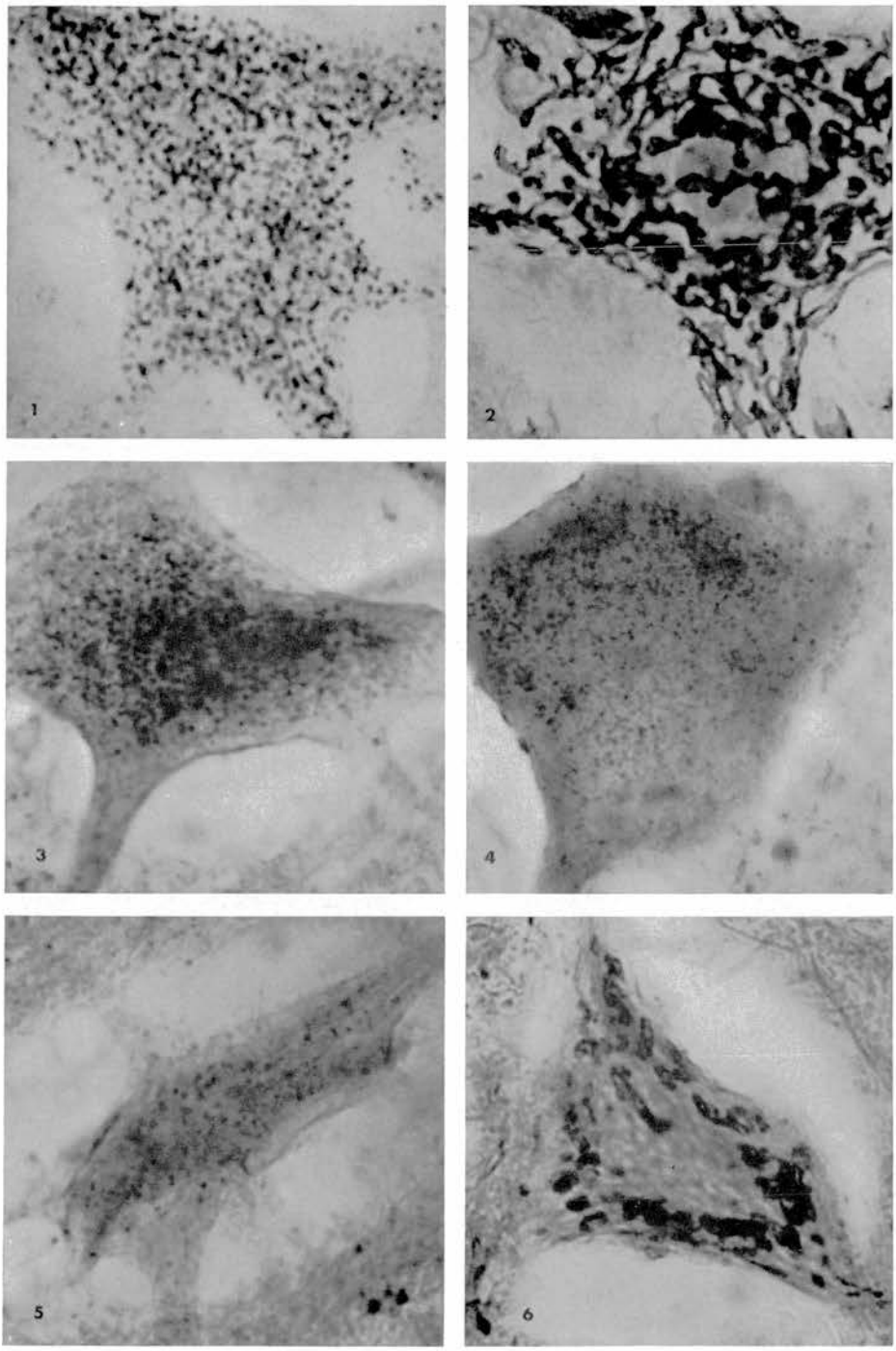

Fig. 1. Normal distribution of lysosomes. Acid Phosphatase. ×400

Fig. 2. Normal distribution of the Golgi apparatus. Thiamine Pyrophosphatase. ×400

Fig. 3, 4, 5. Successive stages in the dissolution of lysosomes. Acid Phosphatase. ×400

Fig. 6. Peripheral margination and swelling of the Golgi apparatus in an altered neurone. Thiamine Pyrophosphatase. ×400

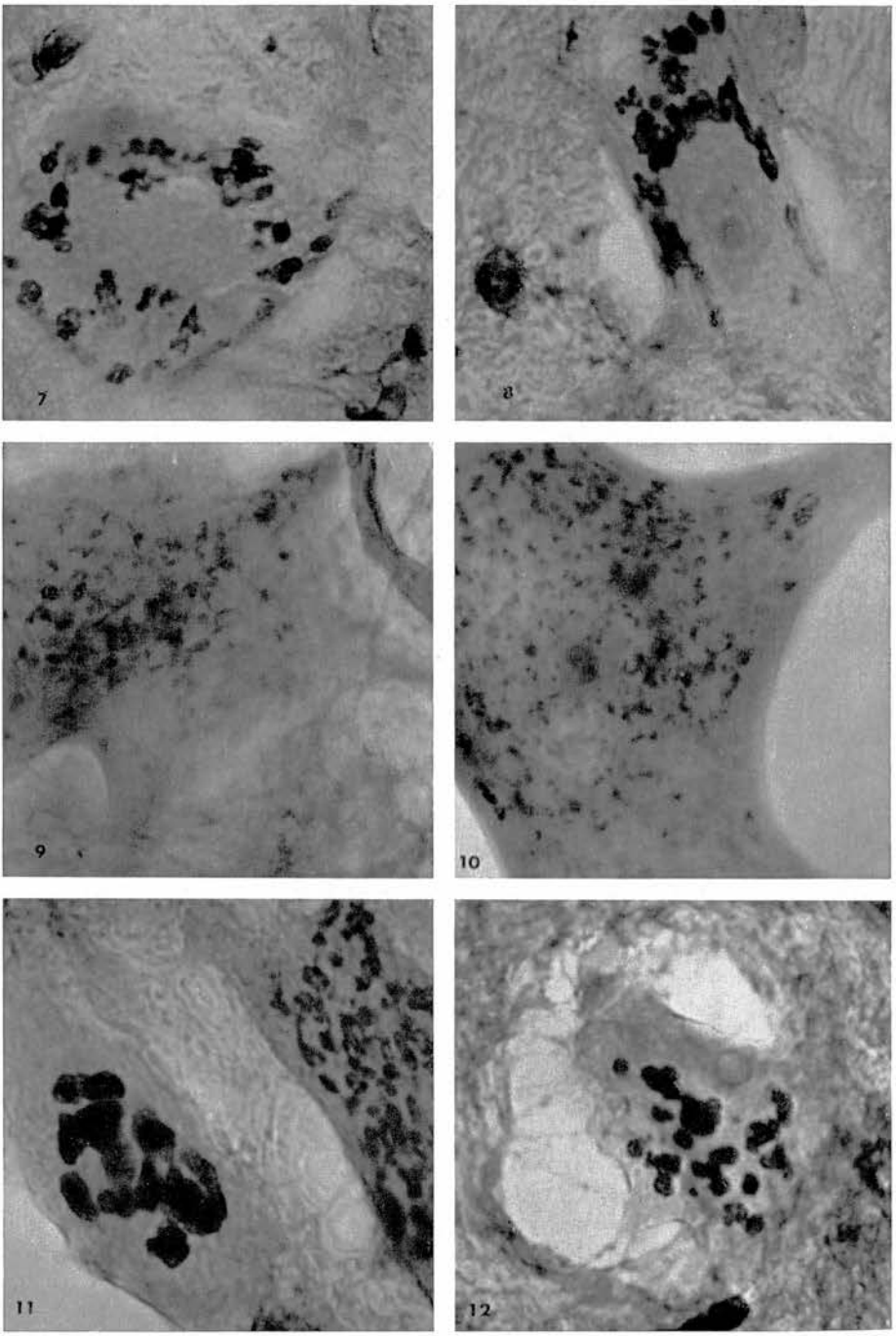

Fig. 7. Margination and fragmentation of Golgi apparatus in a chromatolytic cell with eccentric nucleus. Thiamine Pyrophosphatase. ×400

Fig. 8. Same as Fig. 7. The nucleus is central. ×400

Fig. 9 and 10. Diffuse fragmentation of Golgi apparatus. Thiamine Pyrophosphatase. ×400

Fig. 11. Central agglomeration of Golgi apparatus. Contrast with normal neurone at right. Thiamine Pyrophosphatase. ×400

Fig. 12. Agglomeration of Golgi apparatus in a chromatolytic neurone. Note eccentric nucleus. Thiamine Pyrophosphatase. ×400

laminae within 10 days of nerve section. BARRON and TUNCBAY (1964), using a similar T.P.P. method after brachial plexectomy in the cat, confirmed NOVIKOFF and ESSNER's finding and noted migration of the Golgi network to the periphery of the cell, the "retispersion" described by PENFIELD (1920) in his silver impregnation studies of this organelle. Some of the changes observed in swayback are similar, but to infer any analogy with axonal reaction in their causation would be premature. The formation of Golgi agglomerates and the gradual disappearance of lysosomes, without an intermediate increase in their number, are striking features of swayback. These changes differ from those reported after nerve section in the rat and cat and suggest that the degenerative processes involved in swayback and those associated with axonal reaction are not similar. This view is supported by a consideration of the cerebral lesion in which there is extensive axonal destruction unaccompanied by changes in the organelles of their cortical neurones. Since species differences and external metabolic factors may play a part, a study of axonal reaction in the copper deficient lamb would have to be done in order to determine its role in the pathogenesis of the lesions in swayback.

Summary

The Golgi apparatus and lysosomes of nerve cells in swayback have been studied. In the nerve cells of the brain stem and spinal cord, changes have been found which accompany the late stages of chromatolysis. In part, these alterations resemble those which occur after section of a nerve, but more striking are the gradual loss of lysosomes and the formation of Golgi agglomerates, features which differ from those described in axonal reaction.

Zusammenfassung

Golgi-Apparat und Lysosomen von Nervenzellen im ZNS an „Swayback" (enzootischer Ataxie) erkrankter Lämmer wurden untersucht. An den Nervenzellen in Hirnstamm und Rückenmark wurden Veränderungen im Zusammenhang mit späten Stadien der Chromatolyse angetroffen. Diese Veränderungen erinnern teilweise an solche nach Nervendurchtrennung, doch treten der allmähliche Verlust der Lysosomen sowie die Bildung von Golgi-Anhäufungen stärker hervor, wodurch sich Abweichungen gegenüber den Veränderungen bei Axon-Reaktionen ergeben.

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9.

Structural Changes of the Central Nervous System in Swayback (Enzootic Ataxia) of Lambs

II. Electron Microscopy of the Lower Motor Neuron*

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Alterations of ganglion cells have been described as a characteristic finding in brain stem nuclei and anterior horn cells of the spinal cord in swayback (Barlow et al., 1960). Previous studies have demonstrated central chromatolysis, nuclear eccentricity, hyalinization of the perikaryon and transformations of lysosomes and Golgi substances (Barlow et al., 1960; Barlow and Cancilla, 1966). This report extends the study of these cellular variations by describing the fine structural features of the diseased cells.

Materials and Methods

Tissue from lambs with clinically typical swayback was obtained at 2, 4, 21 and 69 days after birth. The animals were acquired in Scotland from two different farms. The breeding was Blackface × Cheviot or Blackface × Dorset. The clinical diagnosis was subsequently confirmed by pathological and biochemical examinations. Normal lambs from swayback-free farms served as controls.

The animals were sacrificed by decapitation. The abdomen was quickly entered and a portion of the lower abdominal aorta isolated between clamps. This region of the aorta was catheterized with use of a large caliber needle and perfused with 3% glutaraldehyde buffered to pH 7.4 with 0.067 M sodium cacodylate (Sabatini et al., 1963) to obtain a slow segmental perfusion of the spinal cord. The spinal cord was removed after delivery of 250 to 300 cc of perfusing fluid for 30—45 min. Small blocks of tissue were obtained from the ventral horn in the perfused segments and retained overnight in fresh fixative. After a brief rinse in a cacodylate-sucrose wash solution the tissue was transferred to 1% osmium tetroxide in phosphate buffer at pH 7.4 (Millonig, 1961) and post-fixed for 2 hr. It was then rapidly dehydrated in graded alcohols, changed twice in propylene oxide and embedded in Epon 812 (Luft, 1961). Overnight polymerization was obtained in an oven at 65° F. Thick sections from the epon blocks were stained with toluidine blue or paraphenylenediamine and examined with the light microscope in order to select regions for final ultrasectioning. The thin sections were mounted on uncoated grids, stained in a saturated solution of uranyl acetate in 50% ethyl alcohol and examined with an RCA EMU-3F electron microscope.

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Results

The normal neurons of the anterior horn of the spinal cord had a central, uniform, electron-lucent nucleus limited by a double membrane with numerous pores (Figs. 1 and 2). A prominent nucleolus occupied the center of the nucleus. The extensive perikaryon contained aggregates of free and membrane-associated ribosomes forming Nissl bodies; abundant, irregularly distributed Golgi substance; numerous mitochondria; scattered dense bodies and occasional multivesicular bodies. These organelles were separated by fine fibrils measuring 60—75 Å in diameter or tubules measuring approximately 150 Å in diameter. The fibrillar and tubular forms were most prominent at the periphery of the cell where organelles were inconspicuous. Typical axodendritic and axosomatic synapses were observed. The neuropil was formed by compact neural processes, their synapses, myelinated fibers, and scattered fibrillary astrocytes (Fig. 1).

The altered neurons were generally larger than adjacent normal cell bodies. Axo-somatic synapses were preserved (Fig. 3). The earliest change was a progressive loss of free and membrane-associated ribosomes which commenced in the central regions and extended peripherally until the perikaryon was completely denuded of these organelles (Figs. 3 and 4). Mitochondria, fibrils and tubules were prominent in the Nissl-free central zone and expanded neural processes (Figs. 3, 4, and 5). Frequent alteration in mitochondrial structure was evidenced by enlarged forms with a dense granular matrix and irregular condensed cristae (Figs. 3, 5, and 6). The Golgi apparatus was prominent, with both an increase and a swelling of lamellar and vesicular components (Figs. 7 und 8). There was condensation and redistribution of Golgi profiles which were separated by bundles of neurofibrils and scattered neurotubules (Figs. 7 and 8). Such "hypertrophied" Golgi apparatus was usually located in the midzone of the perikaryon although peripheral profiles were discernible. Rare membranous condensations with electron dense granular aggregates were encountered and interpreted as karyorrhexis (Fig. 9).

The formation of bundles and interwoven fascicles of fine filaments measuring 60—75 Å in diameter were prominent features (Figs. 3—9). These neurofibrils, which were present throughout the perikaryon, formed the cytoplasmic background that surrounded organelles (Figs. 6, 7, and 8). Interspersed among the fibrils were 150 Å tubules and larger branched remnants of the endoplasmic reticulum. In near effete cells the swollen perikaryon was formed almost exclusively of accumulations of fibrils.

Discussion

These ultramicroscopic findings confirm the observation that neuronal changes occur in swayback (Barlow et al., 1960; Barlow and Cancilla, 1966). Morphological comparisons with other conditions do not suggest the nature of the underlying functional disorder. Increased cell size, loss of Nissl substance and alteration of the Golgi apparatus are features common to axonal reaction (Evans and Gray, 1961; Bodian, 1964), and the lesion described here. It is tempting to implicate axonal reaction as the basis for the cellular alterations observed in this disease. The advanced changes encountered would then indicate a more intense or later phase than has been described. There are notable differences, however, in the changes in swayback and the reaction of the neuron to axonal injury. Hudson

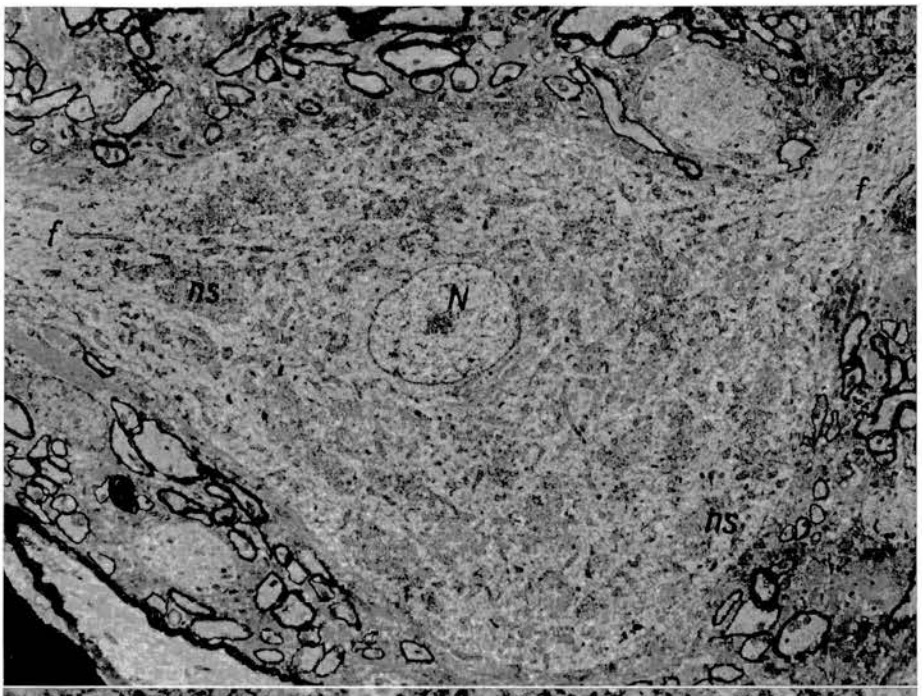

Fig. 1

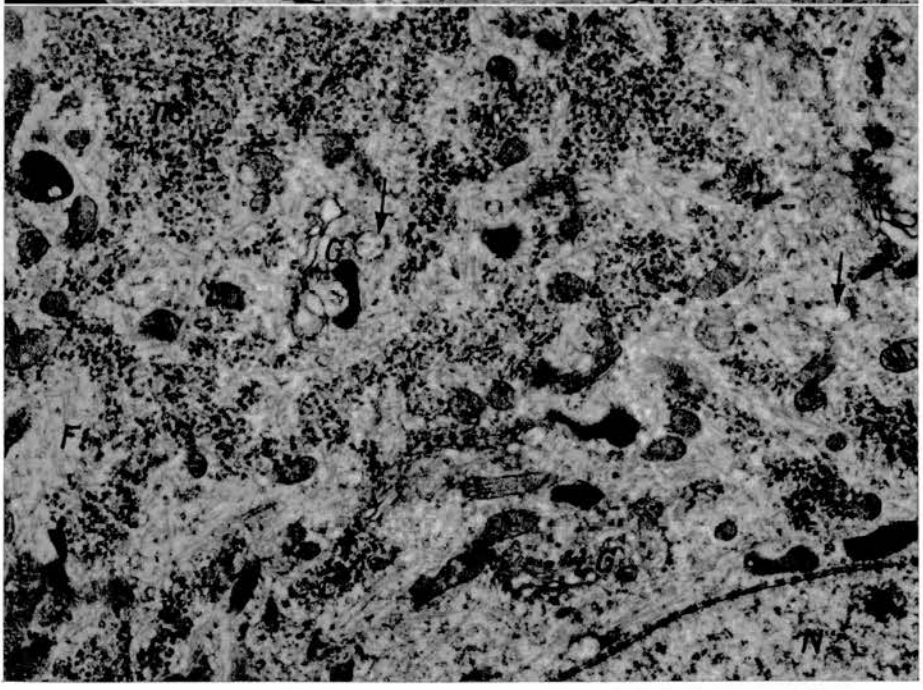

Fig. 2

Fig 1 Normal neuron with central nucleus (*N*), Nissl substance (*ns*) and fibrils (*f*) surrounded by a compact neuropil. × 2,500

Fig.2. Normal neuron with a portion of nucleus (*N*), Golgi substance (*G*), Nissl substance (*ns*), fibrils (*F*), multivesicular bodies (arrows), numerous mitochondria and irregular dense bodies. × 11,700

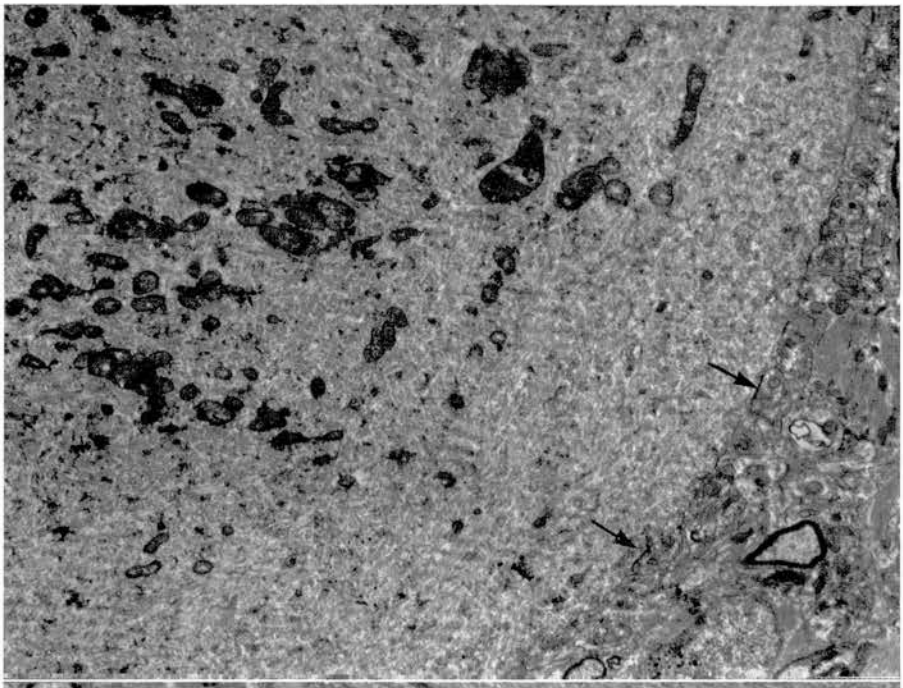

Fig. 3

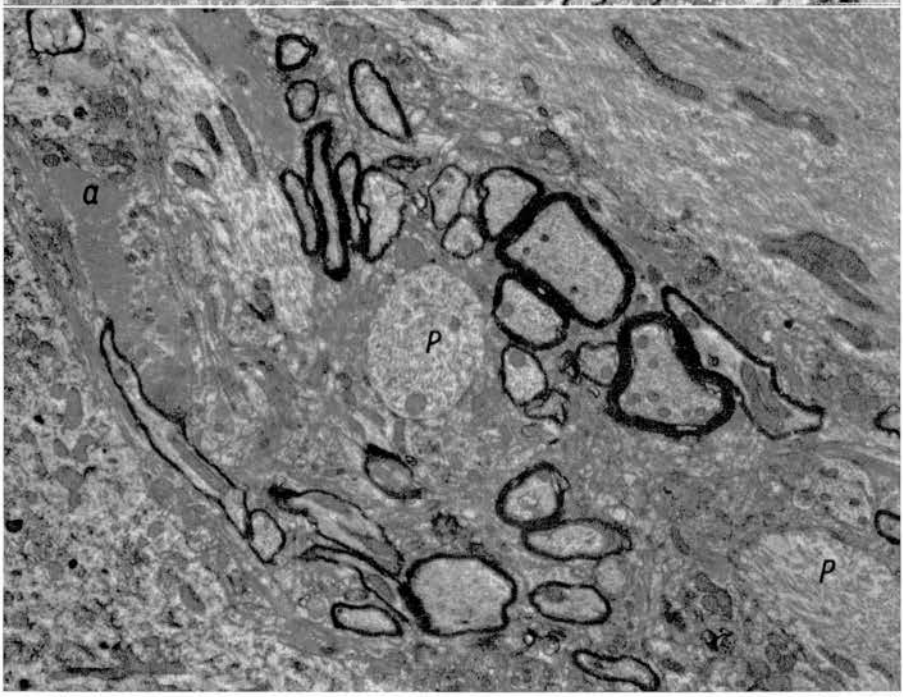

Fig. 4

Fig. 3. Altered neuron and adjacent neuropil. Axo-somatic synapses are preserved (arrows). There is complete loss of Nissl substance, aggregation of mitochondria and numerous interweaving bundles of fibrils within the cytoplasm of the neuron. × 5,000

Fig. 4. Portion of altered neuron (upper right) and normal neuron (lower left) separated by neuropil with neural processes (*p*), astrocytic processes (*a*) and myelinated fibers. Note loss of Nissl substance, prominence of fibrils and paucity of mitochondria in altered neuron. × 5,000

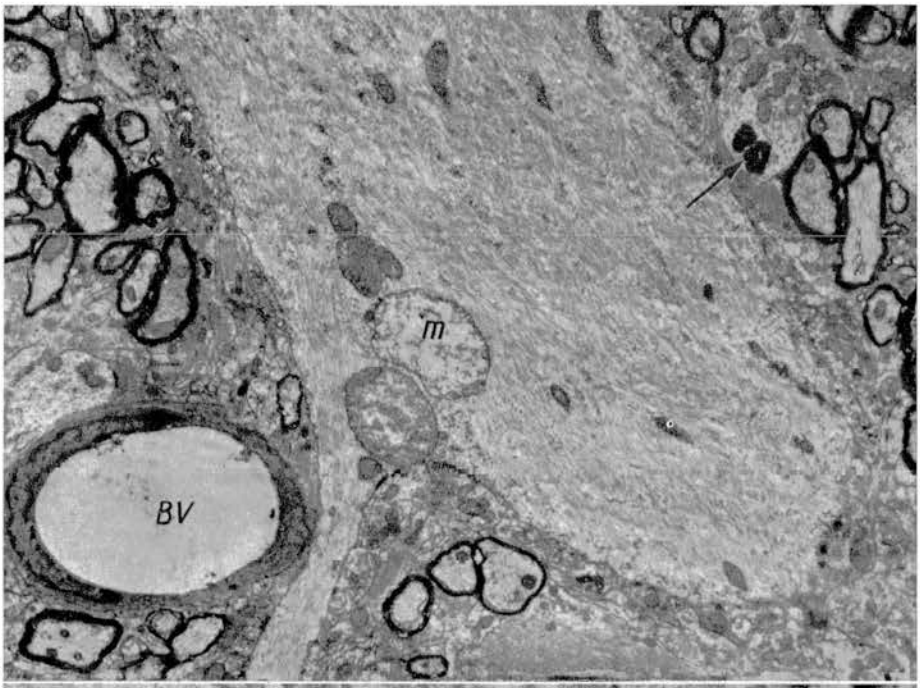

Fig. 5

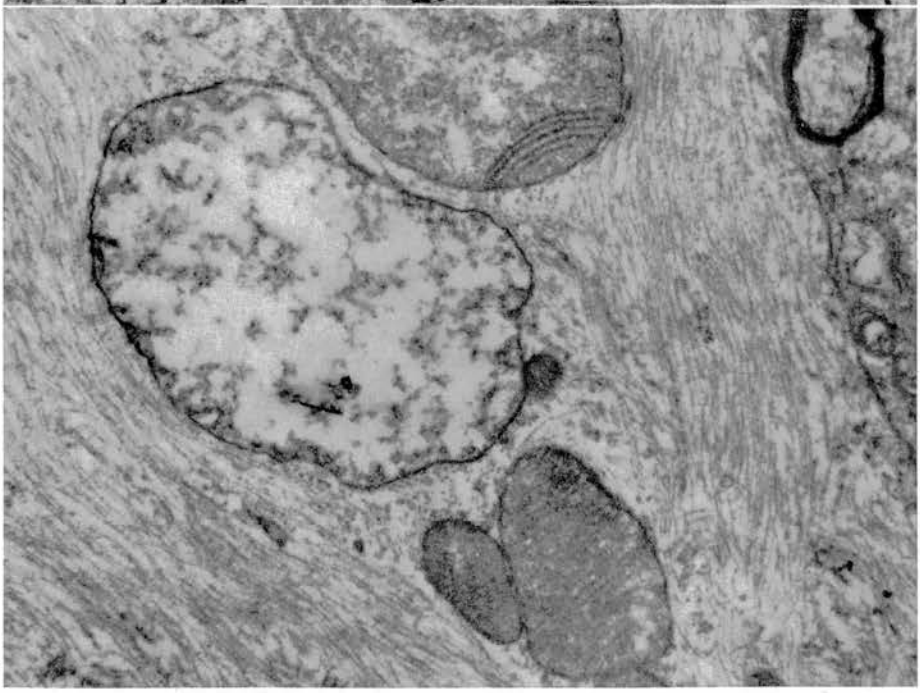

Fig. 6

Fig. 5. Process of altered neuron packed with fibrils. Enlarged mitochondria (*m*) are present in the process. Perfused blood vessel (*BV*). The arrow indicates myelin figures in a neural process. × 4,500

Fig. 6. Higher magnification of mitochondria shown in Fig. 5. There is swelling, condensation of cristae and granular change. Fascicles of fibrils surround the mitochondria. × 14,000

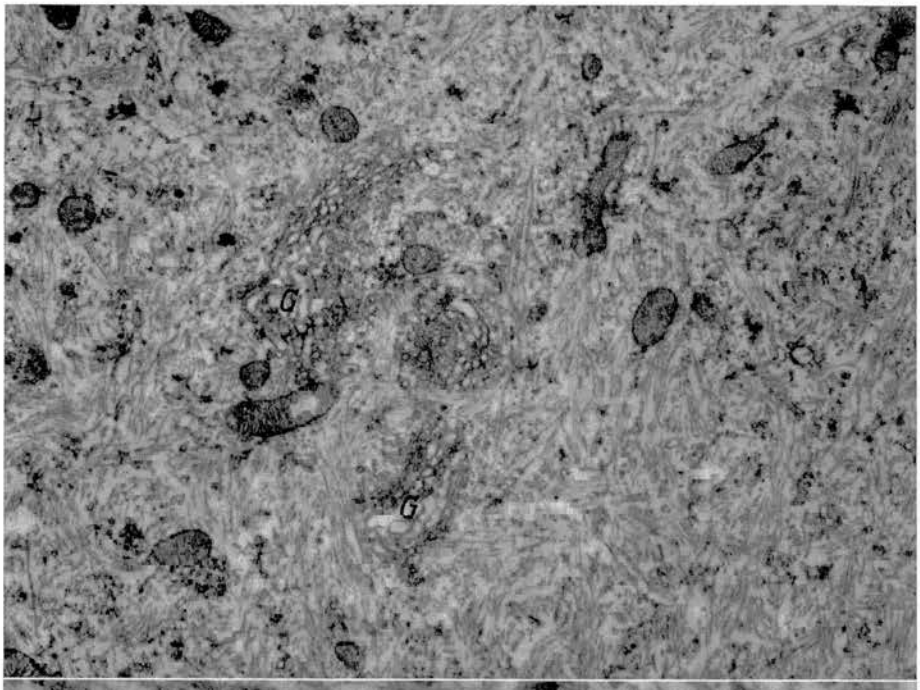

Fig. 7

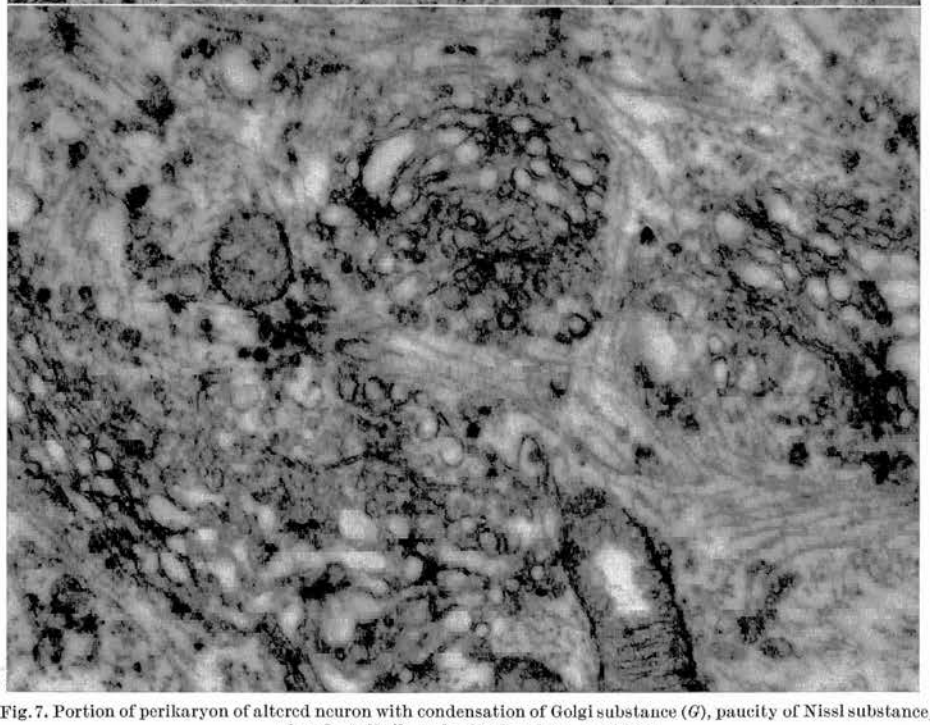

Fig. 8

Fig. 7. Portion of perikaryon of altered neuron with condensation of Golgi substance (*G*), paucity of Nissl substance, abundant fibrils and mitochondria. × 11,700

Fig. 8. Higher magnification of figure 7 with "hypertrophied" Golgi substance and fibrils. ×28,000

et al. (1961) have described a quantitative increase and a qualitative change in mitochondria associated with dense bodies in the hypoglossal nucleus of the rabbit after section of the nerve. BODIAN (1964), who was unable to confirm these findings in monkeys, had the impression that mitochondria were unchanged. The mitochondria observed in our lambs were different not only in distribution but also in morphology from those described by other authors. Swelling of mitochondria, agglutination of the cristae and the formation of an unusually dense

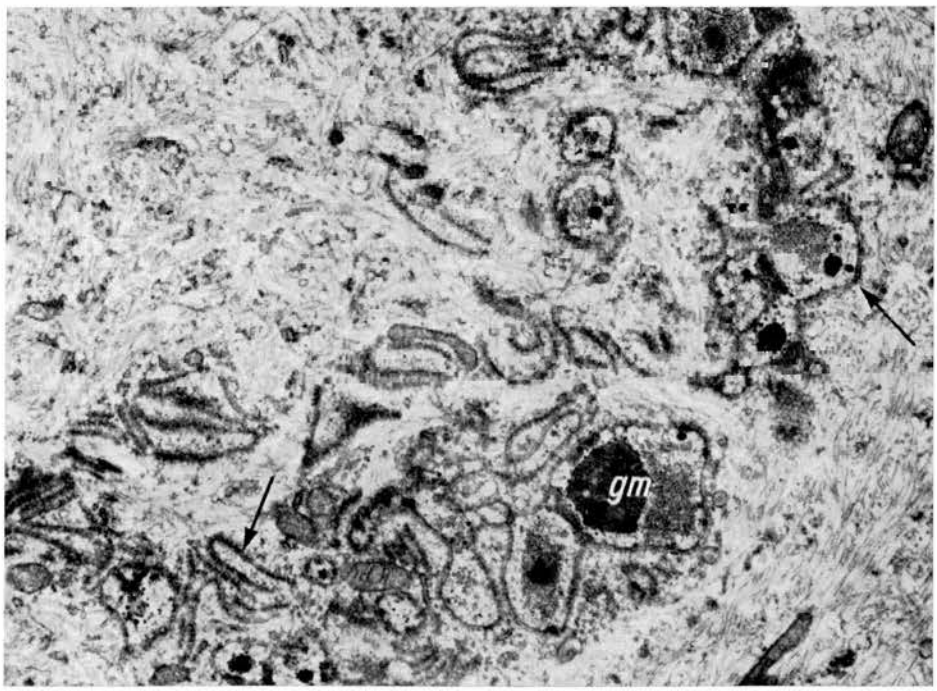

Fig. 9. There are irregular profiles with double membranes (arrows), Granular material (*gm*) and mitochondria with interwoven fibrils. × 11,000

granular matrix were prominent features and contrasted sharply with the appearance of mitochondria in adjacent normal neurons, cell processes and astrocytes. Dense bodies were rarely encountered in diseased cells. Since such structures are normally rich in acid phosphatase activity (NOVIKOFF and ESSNER, 1962; HOLT and HICKS, 1961), this observation correlates with our histochemical finding (BARLOW and CANCILLA, 1966) of diminished acid phosphatase activity in the chromatolytic neurones of swayback.

Accumulation of fibrils was the most prominent and consistent abnormality observed. There was a vast increase of fibrils morphologically indistinguishable from those encountered in a normal neuron. Such fibrillar accumulations have not been described as a result of axonal damage. The fibrils were similar to axonal aggregates in B-B′-imidodipropionitrile induced lesions of rats as described by CHOU and HARTMANN (1963/64), but axonal fibrillary changes in our material were much less marked than those in the perikaryon. The neurofibrillary changes in Alzheimer's disease have recently been studied with the electron microscope

by Terry (1963) and Kidd (1964) both of whom reported an intense proliferation of the tubular, not the fibrillar, components. This difference may account for the observation of dust-like degeneration of neurofibrils in swayback (Barlow et al., 1960) rather than neurofibrillary tangles in silver preparations as seen with the light microscope.

Summary

The ultrastructure of the nerve cell change in swayback is presented. Notable features are an increase in cell size, progressive loss of Nissl substance, alteration in form and distribution of the Golgi apparatus and a striking increase in neurofibrils within the perikaryon. Similarities and differences in the fine structural changes of other pathological states are discussed.

Zusammenfassung

Die Ultrastruktur der Veränderungen in der Nervenzelle beim „Swayback" (enzootische Ataxia) wird dargestellt. Erwähnenswert sind Zunahme des Zellumfangs, fortschreitender Schwund von Nissl-Substanz, Veränderungen der Form und der Verteilung des Golgiapparats sowie eine auffallende Vermehrung von Neurofibrillen innerhalb des Perikaryons. Ähnliche und abweichende Veränderungen der Feinstruktur verschiedener pathologischer Zustände werden besprochen.

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Structural Changes of Central Nervous System in Swayback (Enzootic Ataxia) of Lambs

III. Electron Microscopy of the Cerebral Lesions*

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Cystic and gelatinous lesions of the cerebral white matter not infrequently occur in swayback. The changes are associated with a copper deficient state in the newborn lamb and have been ascribed to demyelination (INNES and SHEARER, 1940; INNES and SAUNDERS, 1962), venous stasis with spongy transformation (BEHRENS and SCHULZ, 1959; SPAIS et al., 1961), or neurodysgenesis (BARLOW et al., 1960). The paucity of myelin breakdown products and significant cellular reaction together with the limited resolution of conventional light microscopic techniques have prevented further characterization of this lesion. The following electron microscopic study was done in an effort to identify the changes.

Materials and Methods

Three Border Leicester X Blackface lambs with swayback from different farms in Scotland were examined 12, 48 and 96 hr after birth. The animals were sacrificed by decapitation, one internal carotid artery in the neck was cannulated, and the brain fixed by perfusion with 3% glutaraldehyde buffered at pH 7.4 with 0.067 M sodium cacodylate (SABATINI et al., 1963) delivered at the rate of 3—400 cc in 30—45 min. The opposite carotid artery and both vertebral arteries were ligated. Tissue from typical cystic and gelatinous lesions (Fig. 1) and the overlying cortex was obtained, divided into small blocks, and transferred to fresh fixative. The tissue was processed for electron microscopy as previously described (CANCILLA and BARLOW, 1966) and then examined with an RCA EMU-3F electron microscope.

Results

The ultrastructural changes in the cystic and gelatinous lesions were similar. Each lesion was composed of numerous cell processes, myelinated fibers, fibrillary

Fig. 1. Coronal section of sheep brain with cystic and gelatinous lesions of swayback disease. Note the transformation of the white matter, preservation of the cortex and ventricular dilatation

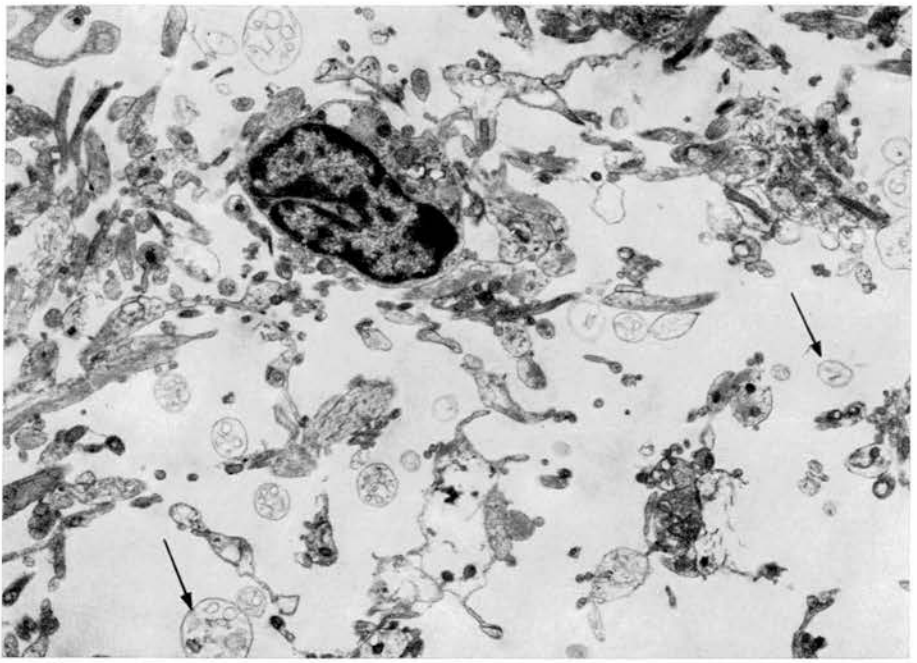

Fig. 2. Electron micrograph of a cystic lesion with numerous processes of neurons and astrocytes in an expanded extracellular space. Numerous ill-defined profiles (arrows) are present throughout. A nucleated cell of unidentified type is present. × 6,300

astrocytes and scattered histocytes in an expanded extracellular space (Fig. 2). The cell processes were of three types:

1. Neural processes that occurred as single or aggregated profiles bounded by unit membranes. They contained unaltered mitochondria and uniformly distributed tubules measuring 150—200 Å in diam. (Figs. 2 and 3).

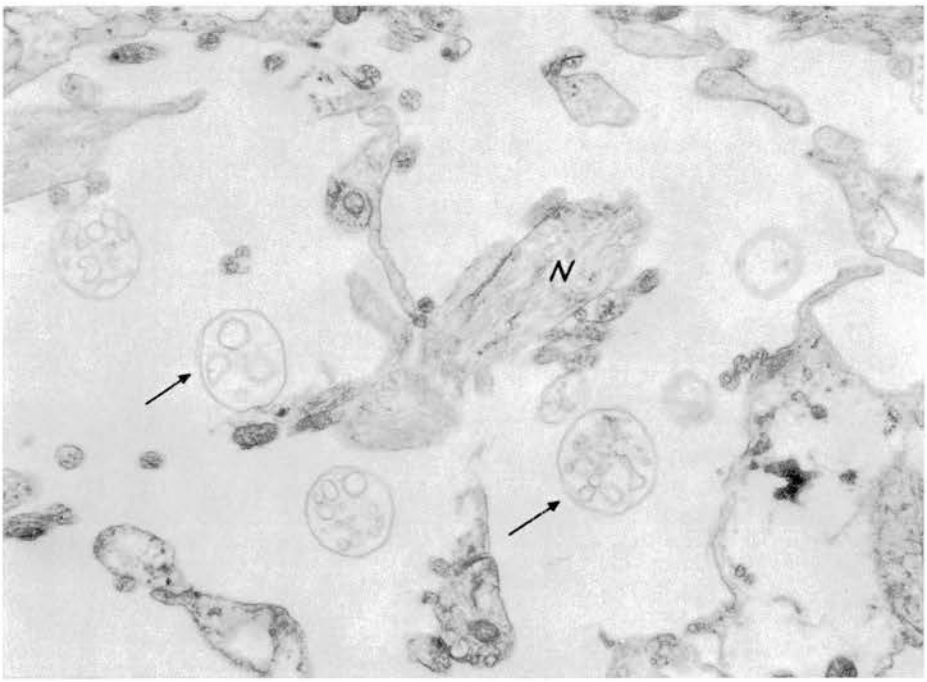

Fig. 3. Higher magnification of Fig. 2. There are vesicle filled profiles (arrows), astrocyte extensions (*A*) and neural processes (*N*) in a dilated extracellular space. ×14,000

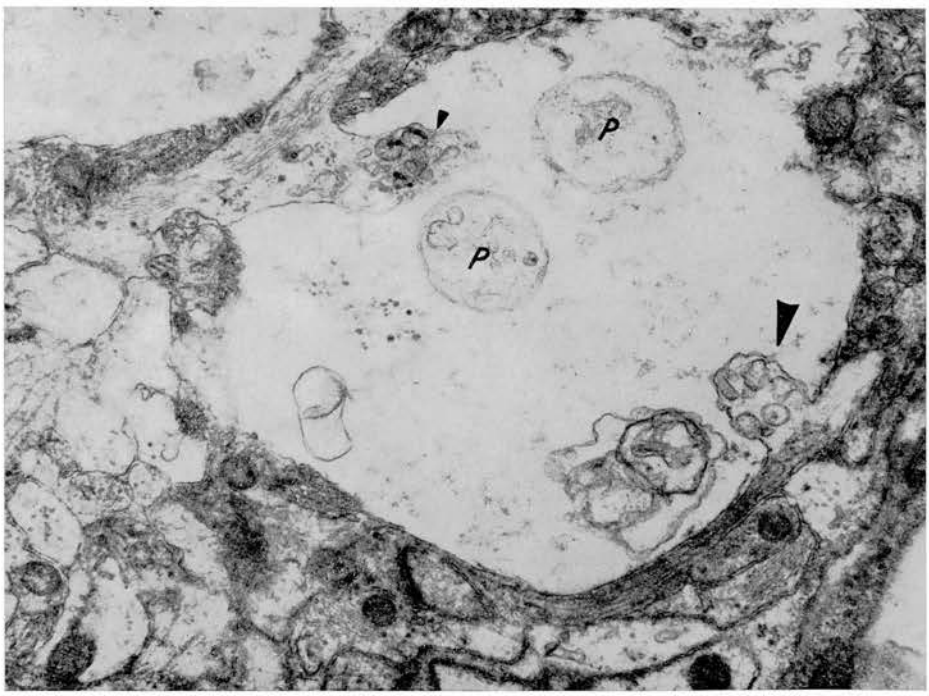

Fig. 4. Early cystic lesion with irregular, ill-defined processes (*P*) and segmental alteration of astrocyte process (small arrow) and neural process (Large arrow). × 21,000

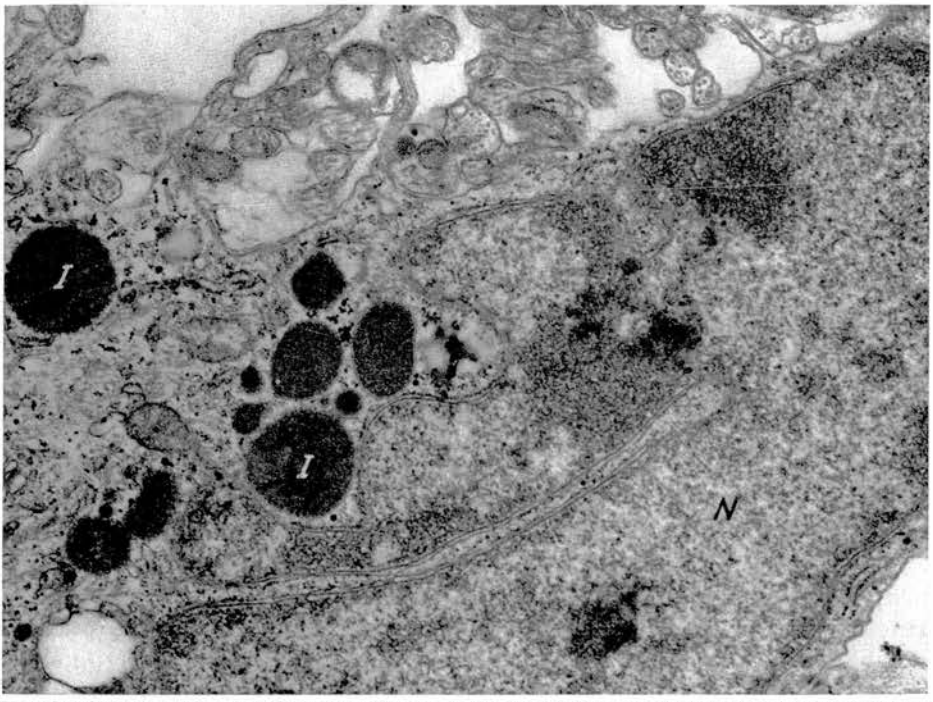

Fig. 5. Portion of an astrocyte with nucleus (*N*), dense bodies (l), fibrils (f) and glycogen granules. Several neural processes in an expanded extracellular space are in the upper portion of the micrograph. × 22,500

Fig. 6. Normal neurone and neuropil from the cortex overlying a cystic lesion. Nucleus (*N*), Golgi substance (*G*) and NISSL substance (*er*) are identified. × 6,300

2. Astrocytic processes with characteristic 75—100 Å fibrils, glycogen and unaltered organelles (Figs. 2 and 3).

3. A unique and frequent process which measured up to 1 μ in diam. and had a circular profile with a smooth or slightly irregular outline (Figs. 2 and 3). Apart from a variable number of vesicular profiles, such processes were usually devoid of contents. The limiting membranes of both the process and the vesicular profiles varied in thickness and frequently were split, ill-defined, and distorted. Continuity of vesicular profiles with the surface membrane, and transitions of mitochondria into vesicular forms were sometimes demonstrated. Endoplasmic reticulum and Golgi substance were not discernible. The processes were identified in favorably oriented sections as segments of both neural and astrocytic processes (Fig. 4).

Myelinated nerve fibers were present and appeared unchanged. Large numbers of astrocytes and their processes with abundant organelles, fibrils and glycogen were encountered (Fig. 5). Macrophages containing electron dense material were infrequently observed in the Virchow-Robin spaces. Oligodendroglia were not identified.

The neurons of the cortex overlying the cystic and gelatinous lesions were unremarkable. They contained uniformly distributed Nissl substance, abundant mitochondria, prominent profiles of the Golgi substance, scattered dense bodies and interspersed neurotubules (Fig. 6). The nucleus was round, rarely infolded, and occasionally eccentric. The neuropil was compact. It was formed by neural extensions and their synapses and astrocytic processes. Occasional cell processes were observed with changes similar to those in the cystic and gelatinous lesions.

Discussion

The occurrence of structureless profiles as the significant cellular component of the cystic and gelatinous lesions was surprising. A similar alteration has been described in astrocytes and their processes in subacute spongiform encephalopathy in humans (Marin and Vial, 1964). Although mitochondrial alterations occurred, there was no autophagic vacuole formation (Ashford and Porter, 1962; and Novikoff and Essner, 1962), only a gradual transition of mitochondria into structureless profiles. Similar vesicular outlines were also derived from the external membrane by infolding and sequestration. It seems probable that they represent a degenerative process of a focal or diffuse nature, affecting both neurites and astroglia.

The mechanism of such a degenerative process is obscure. Clearly it occurred after the completion of cortical cell migration and affected otherwise normal processes. Since affected neurites were always unmyelinated and the products of myelin degeneration were insignificant, the degeneration must have occurred prior to the formation of myelin and prevented its formation. It may be that a primary oligodendroglial defect resulted in the degeneration of developing axons or that damage to the axon inhibited the normal process of myelination. The lysis of affected processes with release of cytoplasmic contents into the extracellular space could have initiated or contributed to the gelatinous and cystic change. The frequency with which astrocytic processes were observed in the lesions suggests an attempt at repair by gliosis.

Summary

The ultrastructure of the cystic and gelatinous lesions of swayback is described. The significant changes are a segmental or diffuse alteration of neuronal and astroglial processes with the formation of ill-defined membranous profiles together with an astrogliosis. There were no notable changes in the overlying cortical ganglion cells or in myelinated fibers. Possible mechanisms by which the membranous alterations evolved are presented.

Zusammenfassung

Die Ultrastruktur der cystischen und gelatinösen Läsionen in „Swayback" (enzootische Ataxia) wird besprochen. Bemerkenswerte Erscheinungen sind eine segmentale oder diffuse Veränderung der Nerven- und Astroglia-Fortsätze durch die Bildung von unklaren Membranprofilen zusammen mit einer Astrogliose. Keine erwähnenswerten Veränderungen in den darüberliegenden corticalen Nervenzellen oder in den Markfasern wurden gefunden. Mögliche Mechanismen, durch die die Membranveränderungen hervorgerufen werden, werden dargelegt.

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Structural Changes of the Central Nervous System in Swayback (Enzootic Ataxia) of Lambs*

IV. Electron Microscopy of the White Matter of the Spinal Cord

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Summary. The alterations in the fine structure of the axon and myelin sheath in the white matter of the spinal cord of lambs affected with swayback are presented. A progressive degeneration of the axon is followed by changes in the myelin sheath. The concept of swayback as primarily a disease of the neurite is discussed in relation to previous work. The hypothesis is advanced that the neuron is unable to sustain its more distant peripheral cytoplasm during growth and that function and functional stresses may lead to irreversible changes in the nerve cell.

Zusammenfassung. Die Veränderungen in der Feinstruktur des Axons und der Myelinscheide der weißen Substanz des Rückenmarks an bei „Swayback" (enzootischer Ataxie) erkrankten Lämmern werden dargestellt. Einer fortschreitenden Degeneration des Axons folgen Veränderungen in der Myelinscheide. Die Auffassung von „Swayback" als einer ursprünglichen Erkrankung des Neuriten wird im Zusammenhang mit früheren Arbeiten besprochen. Die Hypothese wird aufgestellt, daß das Neuron infolge von Cytochromoxydaseverarmung und Kupfermangel unfähig ist, sein weiter entferntes peripheres Cytoplasma während des Wachstums zu erhalten, und daß Tätigkeit und funktionelle Belastungen zu irreversiblen Veränderungen in der Nervenzelle führen.

Key-Words: Nervous System — Swayback — White Matter — Demyelination — Spinal Cord.

Electron microscopy has been used in several studies of the peripheral and central nervous systems to define the changes that occur in experimental demyelination (Vial, 1958; Terry and Harkin, 1959; Hess, 1960; Bunge *et al.*, 1960; Webster, 1962; Nathaniel and Pease, 1963; Collins *et al*, 1964; Gonatas *et al.*, 1964, 1965; Lampert, 1965a; Lampert and Carpenter, 1965b; Lampert and Cressman, 1966). By contrast, there have been relatively few studies of the naturally-occuring disorders of myelin (Cancilla and Barlow, 1966a and b, 1968). We have been interested in swayback, a condition associated with copper deficiency that affects the nervous system of lambs, and we have reported upon the ultrastructural and histochemical abnormalities that are found in the lower motor neuron (Cancilla and Barlow, 1966a; Barlow and Cancilla, 1966) and the cerebral white matter (Cancilla and Barlow, 1966b). Degeneration of myelin in the spinal cord is a significant component of the disease (Innes and Shearer, 1940; Barlow *et al.*, 1960). The alterations in the fine structure of the lateral and ventral columns of the spinal cord are described here and compared

with those of some experimentally produced disorders of myelin. The conclusions reached are relevant to the pathogenesis of swayback.

Material and Methods

Four lambs with swayback, 1—4 days of age, were obtained from farms in Scotland. The diagnosis of swayback was made from the typical clinical findings and confirmed by gross, microscopic and chemical examinations. Two clinically normal lambs of two and six days of age served as controls. The spinal cord was fixed by perfusion with buffered glutaraldehyde as previously described (CANCILLA and BARLOW, 1966a). Blocks of tissue from the lateral and ventral columns were post-fixed in 1% osmium tetroxide in phosphate buffer and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate or lead citrate. A Siemen's Elmiscope IA was used to examine and photograph the sections.

Results

In both the normal and swayback animals, intact axons with well developed or thin myelin sheaths were observed. The axon contained the usual components of tubules, fibrils and mitochondria, and the myelin sheath had the typical inner and outer loops and repeating period.

In the swayback lambs the normal fibers were separated by numerous altered axons and myelin sheaths. The earliest abnormality recognized was a granular or vacuolar degeneration of the axoplasm (Fig. 1). The myelin sheath was intact and the lamellar pattern and period were maintained. Later stages of axonal dissolution were accompanied by distortion and collapse of the myelin sheaths and separation and fragmentation of myelin lamellae (Figs. 1 and 2). Occasionally, lipid-laden histiocytes were observed within otherwise empty myelin tubes (Fig. 3).

The lesions were not accompanied by an intense cellular reaction, though astrocytes and their processes were prominent and numerous macrophages were present. The macrophages were distended by membrane-bound vacuoles containing portions of myelin in various stages of breakdown. In some the phagocytosed material retained its normal lamellar pattern and period (Figs. 4 and 5). In others homogenization, especially of central lamellae, had occurred and was attributable to loss of the period and intraperiod lines (Fig. 6). Occasionally, a pale and homogeneous structure occupied a vacuole and often, vacuolated lipid was noted. The nucleus of the macrophage was eccentric and the small portions of cytoplasm between the phagocytic vacuoles contained a prominent Golgi zone, scattered elements of the rough endoplasmic reticulum, mitochondria and centrioles. In some, concentric myelin-like figures with a variable period were present in the cytoplasm (Fig. 5).

Discussion

The pathological manifestations of swayback have been attributed to demyelination (INNES and SHEARER, 1940) and to a neurodysgenesis involving nerve cells, myelin sheaths and glia (BARLOW *et al.*, 1960). If the myelin sheath degenerated first, then swayback is a demyelinating condition; if the axon degenerates first, then a neuronal disorder is indicated. It is important that the disease be classified correctly for proper consideration of the pathogenesis.

Although the examinations described here encompass only a brief part of the total course of the disease, it has been possible to follow a pattern of degeneration. This began with granular and vacuolar dissolution of axonal organelles,

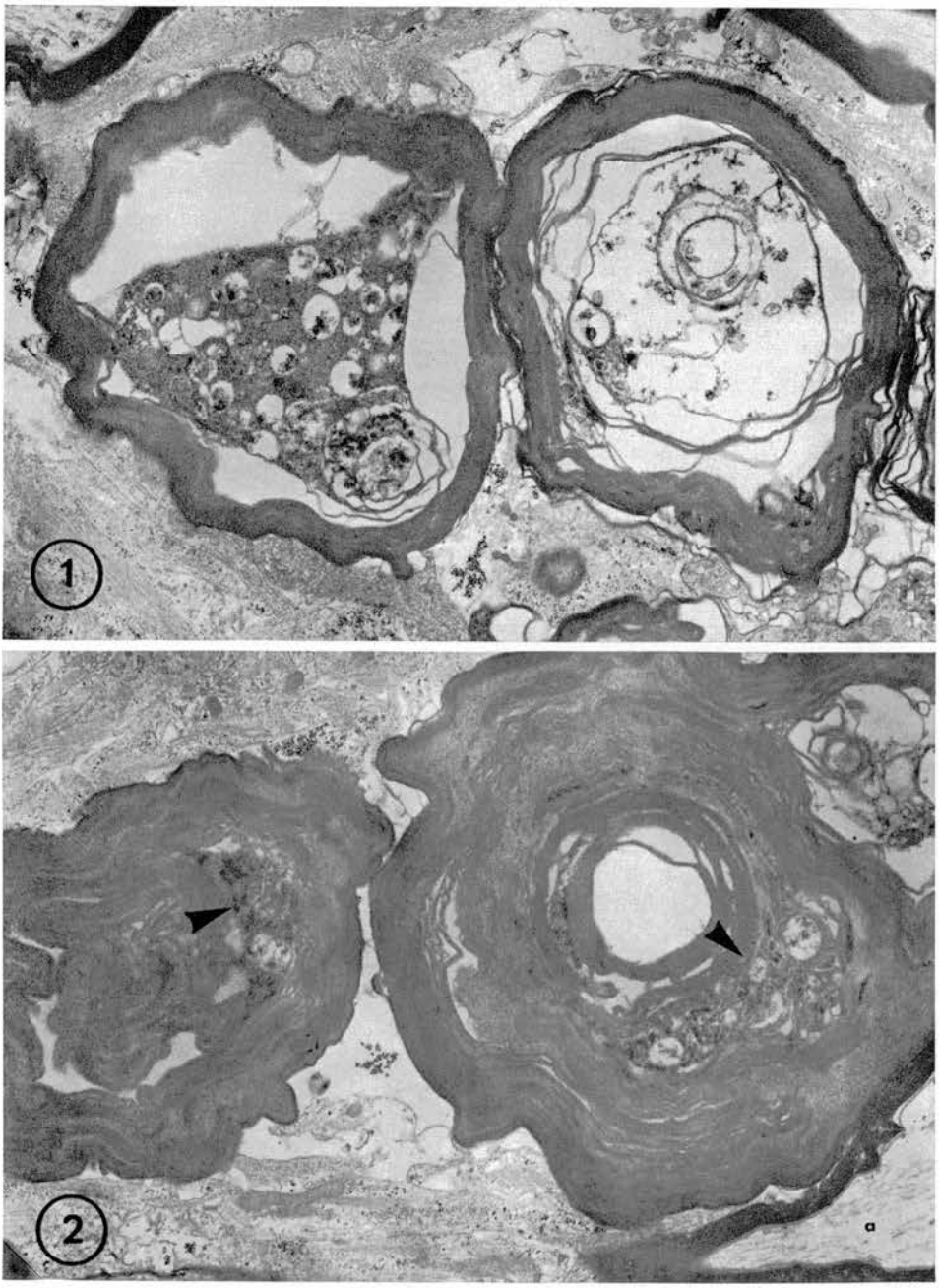

Fig. 1. Stages in the degeneration of the axon in two myelinated nerve fibers. There is a granular and vacuolar alteration of the axon on the left and almost complete loss of axon structure on the right. The myelin sheaths are intact. × 9,000

Fig. 2. There is collapse of the myelin sheath about remnants of a degenerated axon (arrows). A normal myelinated axon is labeled a. × 8,200

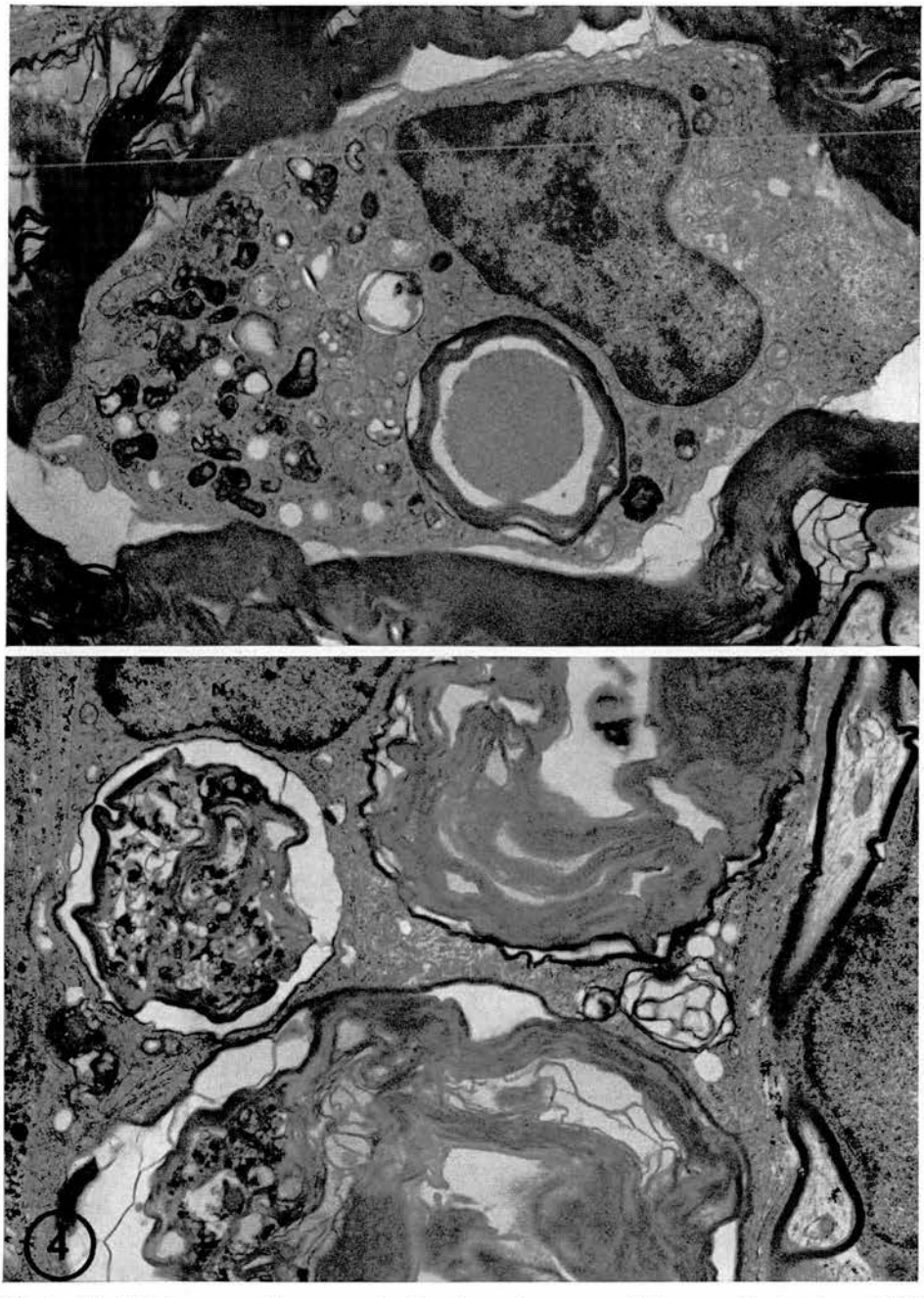

Fig. 3. A lipid-laden macrophage occupies the place of an axon within a myelin sheath × 8,000

Fig. 4. Accumulation of degenerating myelin within a macrophage. The nucleus of the macrophage (*N*) is eccentric and only small portions of the cytoplasm are visible between the phagocytic vacuoles. × 8,200

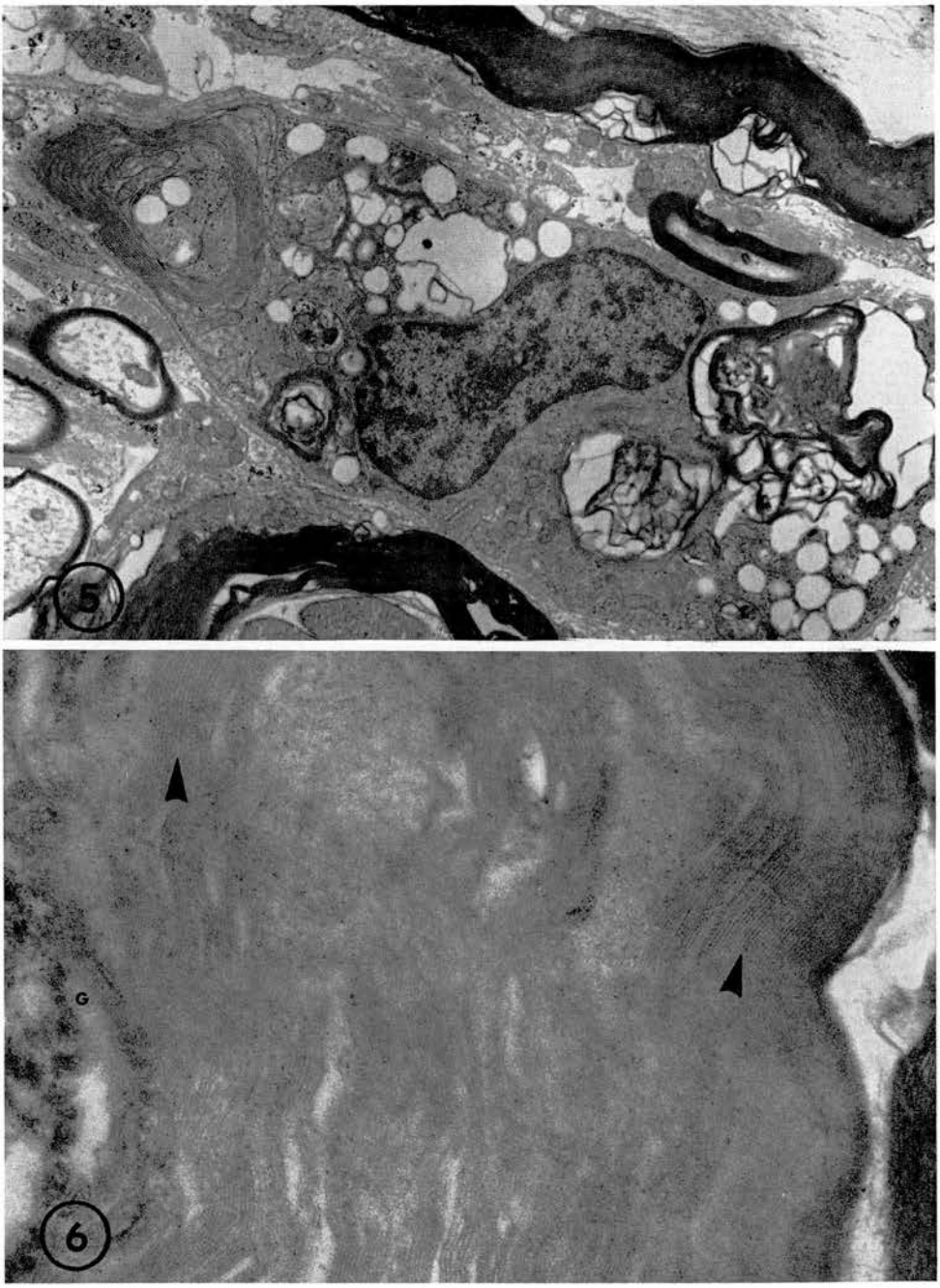

Fig. 5. Lipid-laden macrophage. Some lipid has dissolved in processing producing a vacuolated appearance. A myelin-like membranous structure is present at one pole of the cell (arrow). × 8,000

Fig. 6. Degeneration of myelin. The small arrow indicates the normal myelin period. The large arrows indicate a loss of the intraperiod line. Granular material (*G*) may be derived from the degenerating axon or myelin sheath. × 67,500

continued with disintegration of the axon, and culminated in the destruction of the myelin sheath. All these steps are identical with those described in Wallerian degeneration following axon section in the spinal cord (LAMPERT and CRESSMAN, 1966) and point to a primary neuronal disorder. This conclusion is consistent with those of our previous studies in which axonal alterations in the cerebral lesions (CANCILLA and BARLOW, 1966b) and profound neuronal changes in the ventral horns (CANCILLA and BARLOW, 1966a; BARLOW and CANCILLA, 1966) were demonstrated.

The cerebral lesion of swayback develops after neuronal migration and before myelination (CANCILLA and BARLOW, 1966b). It thus occurs during the period when the increase in brain volume is greatest and hence is associated with rapid elongation of the axons. In the spinal cord morphogenesis commences earlier than in the cerebrum (ROMANES, 1947), but growth and thus elongation of axons is continued for a considerable period into post-natal life, when most of the axons are already invested with myelin sheaths. In swayback the cerebral lesions are amyelinating while the spinal lesions are associated with Wallerian degeneration. Both are due to axonal degeneration, which suggests to us that swayback may be primarily the result of failure of the neuron to sustain its axon during periods of active growth. The nature of the limiting factor is speculative, but there may be a relationship between the severe depletion of the mitochondrial enzyme cytochrome oxidase which occurs in swayback and in copper deficiency (HOWELL and DAVISON, 1959; BARLOW, 1963a), and the loss of mitochondria at a very early stage in the process of axon degeneration.

The absence of changes in the cortical cells of origin of unmyelinated cerebral axons (CANCILLA and BARLOW, 1966b; BARLOW, 1963b) contrasts sharply with the severe degenerative changes in subcortical neurons whose axons have myelinated (CANCILLA and BARLOW, 1966a; BARLOW and CANCILLA, 1966; BARLOW, 1963b). The commencement of function has been correlated with the development of the myelin sheath (ROMANES, 1947), and it may be that the fate of the nerve cell body in swayback is dependent upon its functional state. The observations that greater numbers of necrotic neurons in the spinal cords of lambs with swayback occur in animals which have lived a few weeks than in newborn lambs, and that the proportion of necrotic cells is greatest in regions concerned with limb movements (BARLOW *et al.*, 1964) has already raised the question of a relationship between function and the fate of the nerve cells in swayback.

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Structural Changes of the Central Nervous System in Swayback (Enzootic Ataxia) of Lambs

V. Electron Microscopic Observations of the Corpus Callosum * **

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Summary. An electron microscopic study of the corpus callosum of normal and swayback lambs has revealed morphological evidence of normal myelinogenesis in both groups of animals. Axons wrapped by two cell processes were frequently encountered. Reactive changes were found in axons in the swayback lambs. The significance of these findings is discussed.

Zusammenfassung. Elektronenmikroskopische Untersuchung des Corpus callosum normaler Lämmer und von solchen mit „Swayback" (enzootische Ataxia) ergab den morphologischen Nachweis normaler Myelinentwicklung in beiden Tiergruppen. Von zwei Zellfortsätzen umgebene Axone wurden häufig beobachtet. Reaktive Veränderungen wurden in Axonen der „Swayback"-Lämmer gefunden. Die Bedeutung dieser Befunde wird besprochen.

Key-Words: Swayback of Lambs — Electron Microscopy — Corpus Callosum — Myelogenesis — Axonal Changes.

Ultrastructural changes in the lower motor neuron and the cerebral and spinal white matter of lambs with swayback have previously been described (Cancilla and Barlow, 1966a and b, 1968). From these and other studies it was suggested that swayback is not a demyelinating condition but a primary failure of the neuron to sustain its peripheral axon during periods of rapid growth, and that function and functional stresses may result in irreversible changes in the nerve cell.

Since the corpus callosum contains long fibres and is one of the last regions of the sheep's brain to myelinate (Romanes, 1947) callosal fibers of swayback lambs might be expected to show axonal alterations. The present electron microscopic study of the corpus callosum was done to test this possibility and to make observations on the formation of myelin, especially as it occurs in copper deficiency.

Material and Methods

Two normal lambs and six lambs with swayback all less than 3 weeks of age were obtained from farms in Scotland. The diagnosis of swayback was made from the typical clinical findings

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and confirmed by gross, microscopic and chemical examinations. The brain was fixed by perfusion with buffered glutaraldehyde as previously described (Cancilla and Barlow, 1966b). Blocks of tissue were obtained from the corpus callosum. They were washed in buffer, post-fixed in 1% osmium tetroxide in phosphate buffer and embedded in Epon 812. Sections one micron thick were stained with alkaline toluidine blue and examined by light microscopy for orientation. Ultrathin sections were stained with uranyl acetate or lead citrate and examined and photographed with a Siemens Elmiskop 1A electron microscope.

Results

The corpus callosum of both normal and swayback lambs was composed of closely packed axons of varying size amongst which myelinated fibers were randomly distributed. The axons contained mitochondria, tubules 200 Å in diameter, and collections of fibrils (Fig. 1). In the swayback lambs, however, a number of unmyelinated axons showed local massive accumulations of mitochondria (Fig. 2), homogeneous and laminated dense bodies, tubules and vesicles, resulting in gross distention of the intact axonal membrane (Fig. 3). These focal dilatations may have been at the distal extremity of the axon, as only single narrow transition points between the axon and the enlargement were observed (Fig. 3).

No differences between the normal and swayback lambs with respect to myelin sheaths and glia were recognised. Though typical spiral wrapping of axons and the formation of thin well-formed myelin sheaths occurred, a very common but unexpected finding in both groups of lambs was wrapping of the axon by more than one cell process (Fig. 4, 5 and 6). This usually presented as an axon enveloped by a large outer process containing an occasional mitochondrion and scattered tubules, and a thin inner process with a dense matrix generally free of organelles. Both processes were wound in the same direction and eventually one or the other reached the axon and formed an inner loop. Occasionally a dense line appeared at points of contact of the membranes of the two cell processes. The cell or cells of origin of the processes were not observed, although occasionally a process contained fibrils and glycogen and was then interpreted as astrocytic in origin (Fig. 4). Other cell processes contained laminated dense bodies in addition to mitochondria.

Discussion

The corpus callosum of the fetal sheep is one of the last regions of the brain to myelinate (Romanes, 1947). The present work has shown that active myelination continues in this region during early post-natal life. The only distinguishing feature of the corpus callosum of normal and swayback lambs was the presence in the latter of randomly distributed focal axonal swellings packed with mitochondria and other organelles. Similar structures have been found in the stumps of crushed sciatic nerves, (Webster, 1962; Holtzman and Novikoff, 1965) in the proximal segment of severed axons in the dorsal columns and infrequently also in the florid lesions of experimental allergic encephalomyelitis (Lampert, 1967). They are indicative of injury to the axon and have been called reactive changes (Lampert, 1967). The presence of these structures in normally myelinating tissue is further evidence for the concept that swayback is a disease of the neuron, and not a primary demyelinating disorder (Innes and Shearer, 1940; Innes and Saunders, 1962).

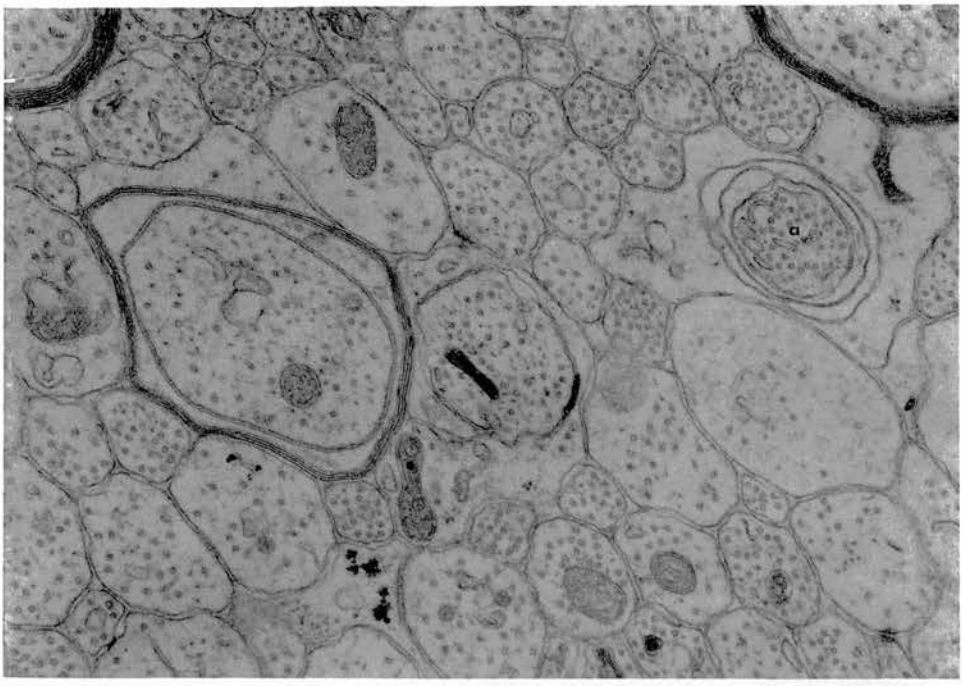

Fig. 1

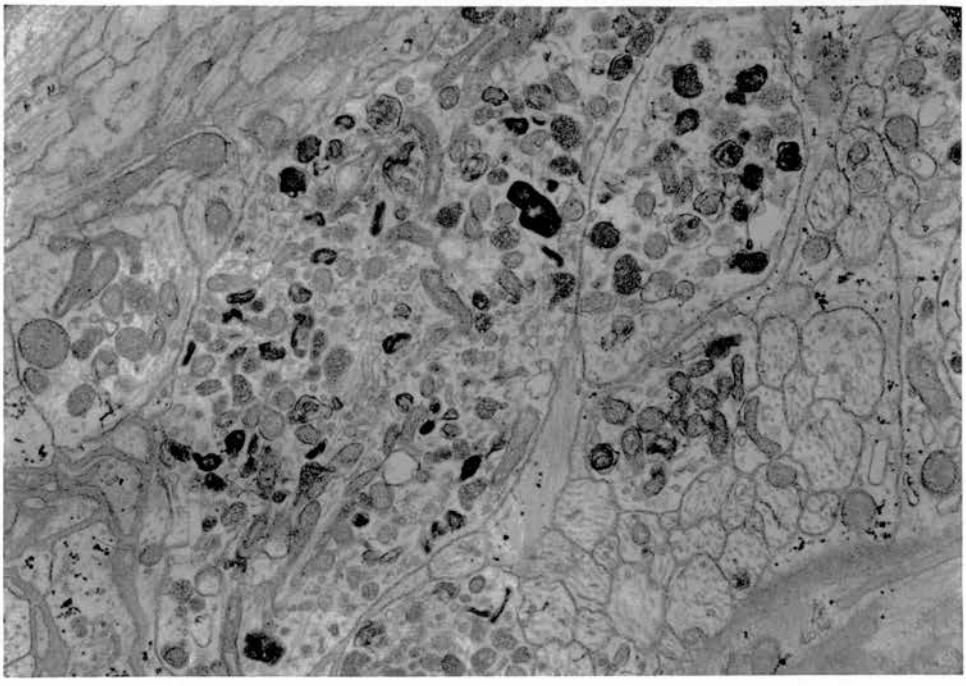

Fig. 2

Fig. 1. The corpus callosum of a swayback lamb. There are many unmyelinated axons. The axon in the centre of the field is covered by a single revolution of a cell process. The axon indicated by (a) is wrapped by several revolutions of a single cell process. Thin, but well-formed myelin sheaths are evident. $\times 30{,}000$

Fig. 2. There are several enlarged axons packed with mitochondria, homogenous and laminated dense bodies, tubules and multivesicular structures. $\times 16{,}000$

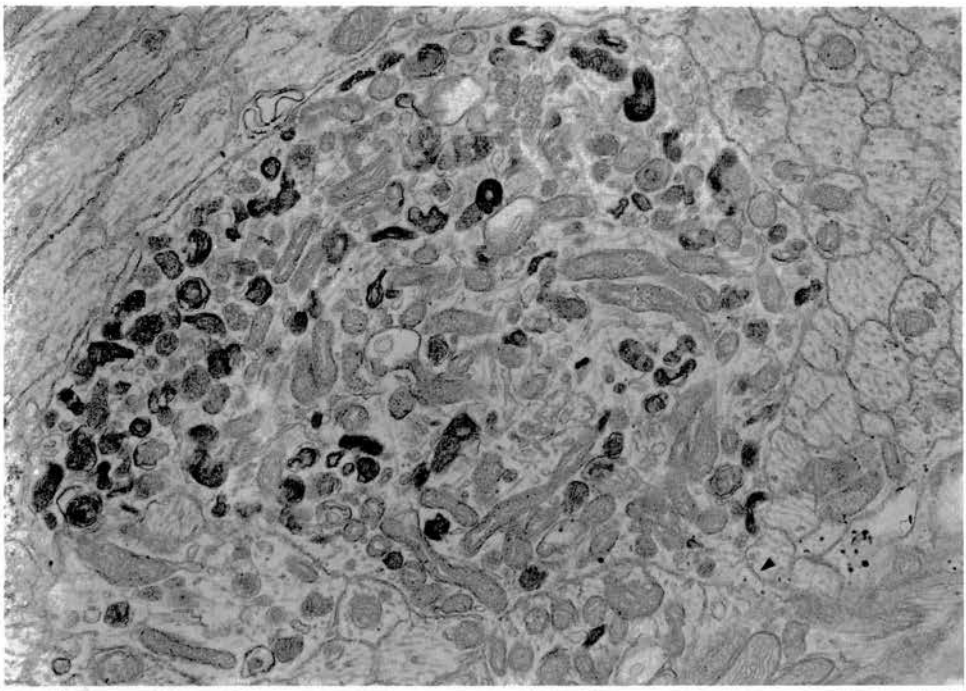

Fig. 3

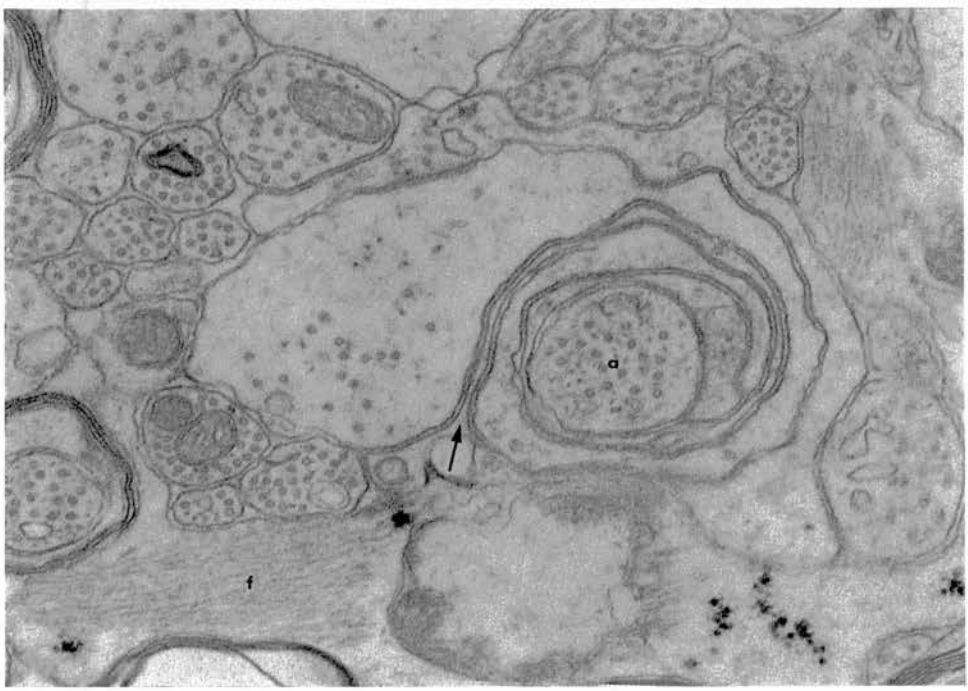

Fig. 4

Fig. 3. A dilated segment of this axon is filled with organelles. The arrow indicates a constriction where this portion is in continuity with another segment of the axon. ×16,000

Fig. 4. The axon (a) is surrounded by the processes of two cells. The larger process has several cytoplasmic tubules and terminates before reaching the axon. The process indicated by the arrow reaches the axon and forms an inner loop. It arises from a cell with fibrils (f) and glycogen. ×45,000

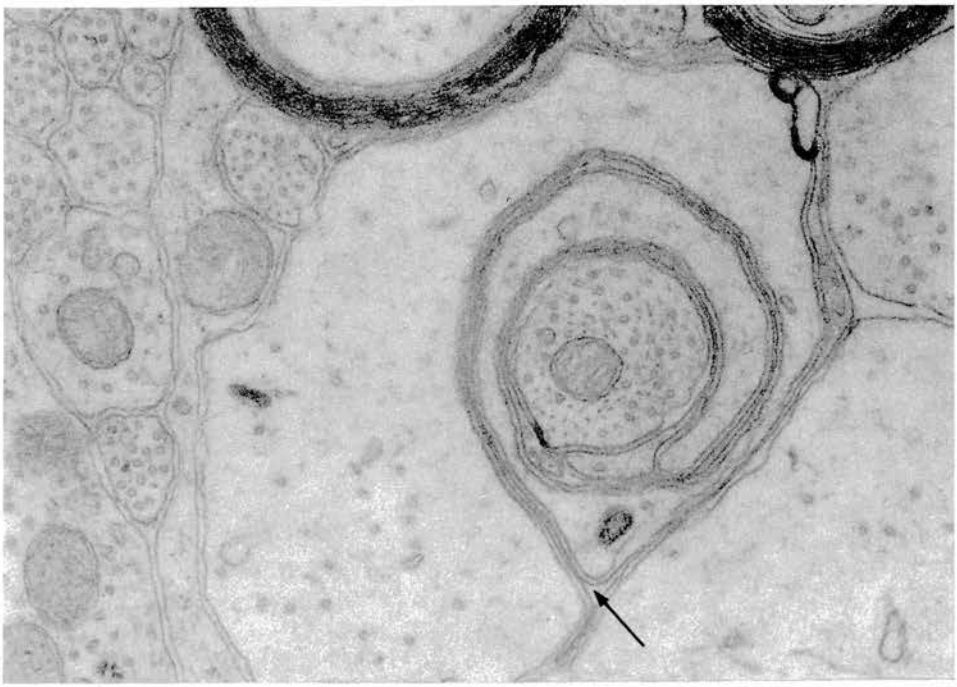

Fig. 5

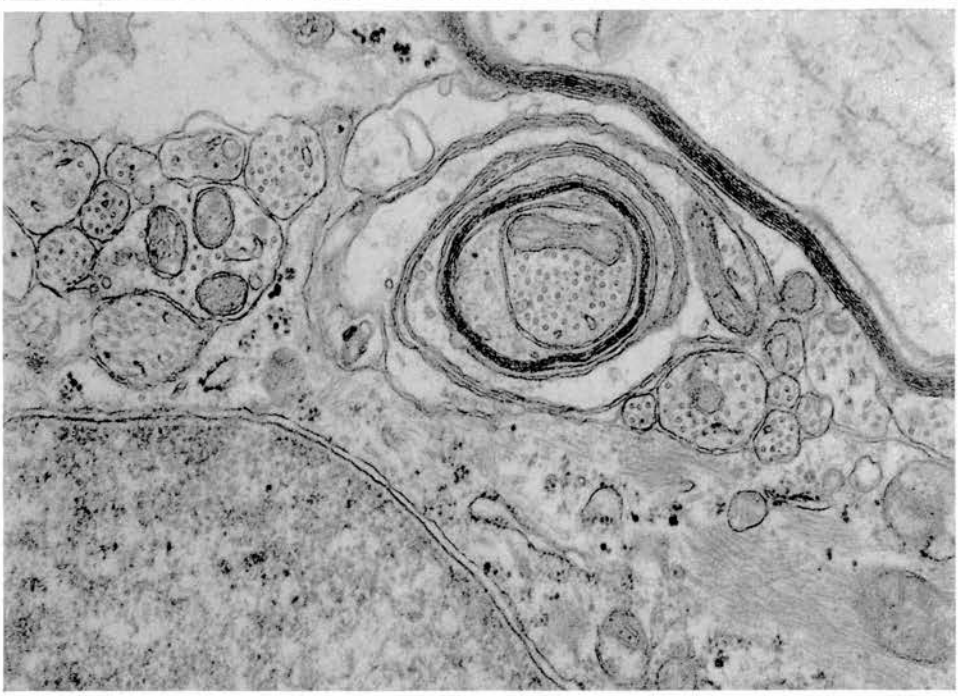

Fig. 6

Fig. 5. The central axon contains a mitochondrion, fibrils and tubules and is surrounded by two cell processes. The thin process (arrow) reaches the axon; the larger cell process ends after two revolutions. Portions of two myelinated axons and several unmyelinated axons are present. ×45,000

Fig. 6. Several lamellae of structural myelin surround the axon before the terminal loop of another cell process is evident between the lamellae. ×35,000

The appearance of the corpus callosum of these young lambs irrespective of whether or not they were affected with swayback is of interest. They contained numerous thin myelin sheaths similar to those observed in the corpus callosum of mice and rats (Schultz *et al.*, 1957) and are consistent with the Type A model of Hirano and Dembitzer (1967). However, myelin profiles which cannot be reconciled with any of the schematics proposed by Hirano and Dembitzer for wrapping by a single cell process were frequently observed in both swayback and normal lambs. They can only be interpreted as the result of two independent processes wrapping the axon. Similar profiles have been seen in the optic nerve of Anurans (Maturana, 1960), in tissue cultures of cerebellum (Ross *et al.*, 1962) and in experimental demyelination and remyelination. It has been suggested that they represent the unwinding of the sheath by another cell process (Maturana, 1960), a competition between processes in the formation of the myelin sheath (Bunge *et al.*, 1961), a trapping of an extraneous cell process (Bunge *et al.*, 1961) or an error in myelin formation (Lampert, 1965). If the final product is to be a normal myelin sheath then one process must be withdrawn before the final configuration is obtained. It seems unlikely that competition for axons would be the causative factor in our lambs, since so many unmyelinated axons are present in the immediate vicinity. That the interdigitating process is a manifestation of demyelination as described by Lampert and Carpenter (1965) is equally unlikely in these lambs in view of its frequent occurrence in normal material. Other than to indicate its appearance and to describe it as a normal phenomenon in this study we have no explanation for its occurrence.

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Cardiovascular System in Naturally Occurring Copper Deficiency and Swayback in Sheep

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SUMMARY

Experimental copper deficiency is associated with lesions of the cardiovascular system in several species of animals. A study was made on the vascular system of swayback lambs obtained in Scotland. Although swayback is a spontaneous neurodysgenesis dependent upon severe copper deficiency, vascular lesions were not observed. The present investigation included light and electron microscopic examinations, tests of mechanical properties, and biochemical analyses (particularly of mucopolysaccharides) of aorta. The absence of lesions may be explained by (*a*) insufficiently low levels of tissue copper in swayback lambs; (*b*) selection of lambs and sheep before the onset of vascular lesions (since lesions in experimentally copper-deficient swine are found only during the rapid growth phase at 2 to 3 months of age); or (*c*) factors other than copper deficiency operative in the pathogenesis of the naturally occurring sheep disease.

Lesions of the cardiovascular system are a common sequel to experimental copper deficiency in swine[6] and domestic fowl.[5] In association with disruption of elastic laminae and altered mechanical properties,[2] there is a decreased elastin[8] and increased mucopolysaccharide content of affected aortas.[3,8] Simple experimental copper deficiency has not been produced in ruminants, but naturally occurring copper deficiency in sheep is widespread and often associated with swayback in lambs.[1,7] Vascular abnormalities have not been reported in these animals and it therefore seemed appropriate to study blood vessels in lambs affected with swayback and in the dams.

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Materials and Methods

Two age-balanced groups of sheep were obtained in Scotland. The group of sheep that was copper deficient consisted of 14 swayback lambs, 12 hours to 60 days old, and 4 dams of swayback lambs. Four normal lambs and 2 postparturient ewes were

used as controls. All sheep were killed; samples of liver were collected and assayed to determine the copper status of each sheep, and the aorta, certain peripheral arteries, and blocks of all organs were fixed in formalin. In a few sheep, a segment of the abdominal aorta was isolated between clamps and perfused *in situ* with buffered 3% glutaraldehyde to obtain blocks for electron microscopy. Other unfixed samples of aorta were packed on ice (in insulated containers) and dispatched to Utah by air express for study of biochemical and mechanical properties. Subsequently, 7 normal lambs were obtained in Utah for additional estimations of aortic mucopolysaccharide content.

Each thoracic aortic specimen was divided into 2 or 3 parts. The first was extracted with saline buffer as previously described for aortas of pigs.[8] The buffer extracts were set aside for measurement of viscosity, and the tissues were treated with acetone, alcohol, and ether, and dried. Mechanical properties of rings cut from the 2nd segments of the aortic specimens were investigated according to methods previously described.[2] Six rings from each aorta were each stretched 3 times, the 1st and 2nd stretches on each ring being reversed after loading to approximately 100 Gm., and the 3rd progressing to breaking point. The 3rd aortic segments, taken only from some of the swayback lambs and adult sheep, were dehydrated in acetone and combined into groups for isolation and identification of mucopolysaccharide.[3] The normal lambs obtained in Utah were used as controls to the swayback sheep.

Results and Discussion

Histologic differences were not found between the blood vessels from lambs and sheep that were copper deficient or controls by either light or electron microscopic examination. The relative viscosity was the same for all 4 groups (normal lambs, normal ewes, swayback lambs, and dams of swayback lambs) and contrasts sharply with the finding of viscous extracts from aortas of pigs that were copper deficient compared with control pigs. The fat-free residues of aorta in all 4 groups of sheep were not significantly different when expressed as percentage of wet weight of aorta. The percentage residue weight of aorta for all groups of sheep is markedly lower than that found in the pig (20 to 25%).[8]

No significant differences in total mucopolysaccharide content in aorta were

TABLE 1—Certain Biochemical and Physical Properties of Cardiovascular Tissues in Lambs with Swayback (Copper Deficiency)

Identification of lamb		Aorta				
No.	Age (days)	Fat-free residue (% wet weight)	Relative viscosity of buffer extract	Total mucopolysaccharide* (% dry weight)	Tensile strength (kg./sq.cm.)	Copper content of liver (p.p.m. dry weight)
CONTROL						
A18	1	16.4	1.17	...	3.5	41.4
A1	3½	12.3	1.24	...	7.7	144.6
A2	6½	15.1	1.13	...	10.8	225.1
A13	14	15.0	1.19	...	5.6	45.7
SWAYBACK						
A8	½	13.5	1.15	...	4.1	4.6
A9	½	13.2	1.15	...	6.6	10.1
A3	2	15.5	1.11	...	6.6	10.9
A4	2	14.6	1.17	...	5.1	11.7
A5	2	17.1	1.22	...	7.9	11.5
A6	2	16.9	1.10	...	8.4	14.6
A11	3	14.8	1.17	...	4.6	8.6
A29	21	13.3	1.12	0.54	10.6	8.2
A30	14	...	1.12	0.54	...	224.7**
A31	14	...	1.12	0.54	...	84.3**
NORMAL†						
A+B	15	...	...	0.70	...	...
C+D	80	...	...	0.80	...	...
E+F+G	35	...	...	0.81	...	...

*Based on acetone-dried aorta powder. **Swayback lambs which had been given copper supplementation prior to death. †Obtained from farms in Utah.

TABLE 2—Certain Biochemical and Physical Properties of Cardiovascular Tissues in Adult Sheep with Copper Deficiency

Identification of sheep		Aorta				
No.	Age (years)	Fat-free residue (% wet weight)	Relative viscosity of buffer extract	Total mucopolysaccharide* (% dry weight)	Tensile strength (kg./sq.cm.)	Copper content of liver (p.p.m. dry weight)
CONTROL						
A26	7	12.9	1.12	...	6.6	281.4
A27	8	12.8	1.12	0.81	9.3	119.4
COPPER DEFICIENT						
A12	7	...	...	0.70	...	4.7
A20	8	16.8	...	0.70	7.0	17.3
A21	6	19.1	...	0.70	8.9	8.0
A22	7	18.5	1.15	...	9.4	4.7
A23	6	13.7	1.14	...	12.4	6.6

*Based on acetone-dried aorta powder.

found between normal sheep and sheep that were copper deficient (Tables 1, 2), although the mucopolysaccharide yield from the swayback lambs seemed slightly smaller. The mucopolysaccharide composition of aortas of lambs seems similar to that of other species.[3] The major component is chondroitin sulfate C, although chondroitin sulfate B and heparitin sulfate are found in relatively small amounts. There may also be a small amount of chondroitin sulfate A. Differences in the relative proportions of mucopolysaccharides could not be detected between normal and experimental sheep.

Estimations of tensile strength were calculated as breaking load in kilograms per square centimeter of cross-sectional area of the 2 limbs of the stretched rings, and are given as the means of 6 values for each aorta. The form of the load-strain curves is identical with that seen with pig aorta.[2] No differences, either in the form of extension curves or in values of ultimate tensile strength, were found between swayback and control lambs. The aortas of the ewes behaved similarly and only differed from those in the lambs in the generally higher values of tensile strength.

It would seem from this study that cardiovascular lesions did not occur either in lambs with congenital swayback or in the dams and may not be a feature of copper deficiency in the sheep. The report of a dissecting aneurysm in a ram from an area in New Zealand[4] that has swayback in sheep is of interest, but may represent an isolated incident similar to idiopathic cystic medial necrosis of the aorta in man. It must be stated, however, that the mean level of copper in the livers of the swayback lambs and their mothers was 9 p.p.m. dry weight, as compared with the level of 3 p.p.m. dry weight in experimental pigs with elastin defects.* The difference is small but may be significant, especially since factors other than copper deficiency may play a role in the pathogenesis of the naturally occurring sheep disease. Furthermore, elastin defects occur in young, rapidly growing pigs of comparable age to lambs with the delayed form of swayback, and this series does not contain examples of the latter. The duration of deficiency, its intensity, complicating factors, and species and age differences may all be relevant to the understanding of the biological properties of copper.

*Weissman, N., and Coulson, W. F., Department of Pathology, University of Utah College of Medicine, Salt Lake City, Utah: Unpublished data, 1965.

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Early Morphological and Histochemical Consequences of Peripheral Nerve Section in Lambs of Normal and Low Copper Status

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EARLY MORPHOLOGICAL AND HISTOCHEMICAL CONSEQUENCES OF PERIPHERAL NERVE SECTION IN LAMBS OF NORMAL AND LOW COPPER STATUS

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PLATES LXIV–LXVIII

FOR many years nerve section has been used intensively in anatomical, physiological and pathological studies of the nervous system. The morphological changes and the concepts arising from them have been comprehensively reviewed (Ramón y Cajal, 1929; Guth, 1956; Wohlfart, 1961) and termed axonal reaction.

An observation that cells whose axons had been cut were refractory to poliomyelitis virus (Howe and Bodian, 1941) focused attention upon the metabolism of cells undergoing axonal reaction. It was later shown that the inhibition of the response to poliovirus in cells undergoing axonal reaction in the monkey was associated with lowered cytochrome oxidase and raised acid phosphatase activity (Howe and Mellors, 1945). An increase in lysosomes reflecting raised acid phosphatase activity has since been confirmed in axonal reaction in several species including the rat, cat, rabbit and monkey (Bodian and Mellors, 1945; Barron and Sklar, 1961; Novikoff and Essner, 1962; Holtzman, Novikoff and Villaverde, 1967). Changes in the conformation of the Golgi apparatus also occur in axonal reaction (Penfield, 1920; Novikoff and Essner; Barron and Tuncbay, 1964), and Holtzman *et al.* (1967) have suggested that the lysosomes develop from a Golgi-related smooth endoplasmic reticulum and accumulate in the centre of the cell together with numerous mitochondria.

Neuronal changes resembling those of axonal reaction occur in swayback, a naturally occurring disease of newborn and young lambs. These include chromatolysis (Barlow *et al.*, 1960), lowered cytochrome oxidase activity (Barlow, 1963), retispersion and fragmentation of Golgi. On the other hand, loss of lysosomes and condensations of the Golgi network were also prominent features in swayback (Barlow and Cancilla, 1966).

Swayback was originally described as a demyelinating encephalopathy with affinities to Schilder's disease (Innes and Shearer, 1940) and with possible relationships to multiple sclerosis (Campbell *et al.*, 1947; Campbell, 1963, 1967), but it has since been shown that the myelin changes in swayback are secondary to axonal degeneration and under certain circumstances are Wallerian in type (Cancilla and Barlow, 1966, 1968). This finding suggested that some spontaneous form of axon rupture might be implicated in the pathogenesis of the neuronal changes. Since the natural disease depends upon a low foetal and puerperal copper status (Innes and Shearer; Howell and Davison, 1959; Barlow *et al.*; Mills and Williams, 1962) and copper deficiency leads to reduced

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cytochrome oxidase activity and decreased phospholipid synthesis (Gallagher, Judah and Rees, 1956; Howell and Davison; Fell, Mills and Boyne, 1965), it appeared pertinent to examine the effect of copper status on the response to nerve section. This paper describes the findings in young lambs during the 3 wk after section of the sciatic nerve.

Materials and methods

The lambs used were obtained mainly from one batch of Blackface ewes. Immediately before mating the ewes were divided into two groups and housed. One group was fed on a semisynthetic diet containing 1·2 p.p.m. copper (Suttle and Field, 1968), the other group was given this diet supplemented with copper to a level of 11·2 p.p.m. Ten lambs were obtained from the deficient group and 4 from the control group. The latter was therefore supplemented to a total of 9 lambs with the offspring of Greyfaced ewes that had been bred to a Blackface ram and maintained on a normal commercial ration.

The copper status of each lamb was determined by atomic absorption spectrophotometry of blood samples taken at 24 hr, and blood, brain and liver samples taken at the time of death. During the first fortnight of life none of the subjects showed any locomotor disability or other evidence of disease.

Sciatic nerve section was performed with aseptic surgical procedures on 14-day-old lambs under deep pentobarbitone anaesthesia. The site chosen was as close as possible to the point of emergence of the left sciatic nerve from the greater sciatic foramen. The nerve was approached through a small incision extending back and slightly downwards from a point about $\frac{1}{2}$ in. (12 mm) behind the crest of the ilium and midway between the sacral spinous processes and the greater trochanter of the femur. The nerve was exposed by blunt dissection of the overlying gluteal muscle and a length of about 1 cm was removed with a sharp scalpel to minimise the chance of reunion. The lambs were killed for examination 1, 5, 10, 15 or 21 days post-operatively.

Histological methods. The spinal cord was removed immediately after death and serial blocks 5 mm thick were taken from the lumbosacral intumescence between spinal segments L3 and S3. The right side of each block was marked by a fine track of India ink injected into the dorsal horn with a tuberculin syringe. Blocks no. 4 and 6 in the series were quenched in a solid CO_2-isopentane mixture for cryostat sections and oxidase histochemistry, and the remainder of the series transferred immediately to ice-cold formol-calcium (4 per cent. formaldehyde, 1 per cent. $CaCl_2$) for 36 hr before frozen sections were cut for phosphatase and lipid histochemistry. Portions of these blocks were also retained in formol-calcium together with other representative regions of the central nervous system (CNS) for general morphological study. Transverse and longitudinal blocks of both the proximal and distal stumps of the left sciatic nerve were also fixed in formol-calcium for both frozen and paraffin sectioning.

The peroneus tertius muscle from each hind limb was removed, attached to a library card and fixed in 4 per cent. formaldehyde in saline for 6 hr. Blocks were then taken from the junction of the proximal and middle thirds for cholinesterase histochemistry and for transfer to formol-calcium for paraffin and frozen sections.

Histochemical methods included cytochrome oxidase (Burstone's method, with α-naphthoic acid as a coupler, 60 minutes' incubation, Pearse, 1961, p. 901); succinic dehydrogenase with Nitro-BT, 60 minutes' incubation (Nachlas *et al.*, 1957); acid phosphatase, 30 minutes' incubation (Holt, 1961); thiamine pyrophosphatase, 25 minutes' incubation (Novikoff and Goldfischer, 1961); Gomori's choline-esterase after Coupland and Holmes, 30 minutes' incubation; OTAN for hydrophobic and hydrophilic lipids (Adams, 1959); sudan black for terminal nerve bundles (Cavanagh, Passingham and Vogt, 1964); and sudan IV for neutral fat. Routine histological methods included haematoxylin and eosin, luxol fast blue and Smith-Quigley for myelin, Holmes and Gros-Schultz for neurofibrils and gallocyanin-chrome-alum for Nissl granules.

Quantitative methods. The variation in severity and extent in chromatolytic changes following axon section is well known (Marinesco, 1898; de Neef, 1901; Romanes, 1951), and shows that chromatolysis *per se* is an unsatisfactory basis for the quantification of nerve cell damage. The capriciousness and difficulty of standardising the preparations of several of the other methods in use rendered these methods also unsuitable. During the qualitative examination it became apparent that the acid phosphatase method of demonstrating lysosomes was likely to be of value, since with it changes were observed most regularly. The sampling site can be standardised and section thickness can be fairly well controlled since the method utilises fixed tissue, and provided care is taken with incubation conditions reasonably reproducible preparations of a permanent nature may be expected.

Accordingly a section from about the junction of the last lumbar–first sacral segment (block 8 or 9 according to the size of individual and whether 6 or 7 lumbar vertebrae were present) was selected. Comparable sections from 9 lambs in each group, balanced for age, were randomised, the India ink marker dot obscured and each half of the section labelled A or B in random fashion. Thereafter all cells were examined at $\times 160$ magnification with the aid of a square-ruled graticule in a square eyepiece field. A decision was made as to whether the lysosome content of each nerve cell was normal or reduced. Total cells and cells depleted of lysosomes were counted. When the entire series had been counted the sections were re-randomised and the procedure repeated. For statistical analysis of the results it was assumed that had the nerve not been cut the total number of cells on each side would have been the same and that any variations in technique or physiological status of the cells would affect both sides of the section equally.

RESULTS

Liver and brain copper levels are more appropriate indices of copper status than blood. The mean values in parts per million dry weight were for the deficient group, liver 8·3 (range 0·2–37·4) and brain $6{\cdot}5 \pm 2{\cdot}26$ (3·2–9·8), and for the control group, liver 100·2 (30·8–262) and brain $13{\cdot}32 \pm 2{\cdot}1$ (10·5–14·9). No evidence of incipient swayback or other intercurrent neurological disease was found during the general neuropathological examination.

The spinal cord

Qualitative results. Changes in the CNS are referable to nerve section and are located in the neurones on the left side of the caudal intumescence within spinal segments L5–S2 (blocks 6–11). The changes are confined to those nerve cells in the ventral horn whose distribution is shown in fig. 1. At L5 affected cells lie in the corridor AL-A′L′. The distribution changes systematically from block to block so that in S2 altered neurones lie within the corridor AS-A′S′.

At 24 hr after nerve section, changes are confined to a few cells in the sectioned side of block 8 (S1) of the one copper-deficient lamb examined at this time. The changes consist of dilatation of the vesicles of the Golgi apparatus and a slight movement of the network to the margins of the cells. There appears to be a slight depletion in the lysosome content of the same cells. The over-all density of the cytochrome oxidase reaction product in the nerve cells and neuropil is appreciably less than that in the control lamb, but no selective cellular differences are present.

At 5 days post-section, chromatolysis is present in all the blocks from L5 to S2, though the number of affected cells is surprisingly low. Swollen cells whose cytoplasm is packed with abnormally fine Nissl substance are also frequently

encountered on the sectioned side and contrast sharply with the chunky Nissl substance of normal ventral horn cells (fig. 2). In both lambs from the deficient group, these reactive neurones show a granular disintegration of neurofibrils (fig. 3), which is not seen in either of the control lambs. Neurones of the sectioned side in both groups, however, show depletion of cytochrome oxidase activity, which in some cells of the copper-deficient animals appears to be total. The copper-deficient animals, however, can be clearly distinguished from the controls by lowered cytochrome oxidase activity throughout the section. Cells of the unsectioned side frequently show a density of reaction product similar to that of cells on the sectioned side in the control lambs (figs. 4–6). Succinic

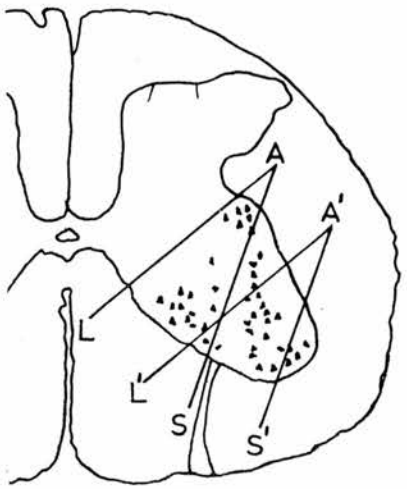

FIG. 1.—Diagrammatic TS spinal cord at lumbosacral junction. The corridor AL–A′L′ represent the distribution of neurones showing axonal reaction at L5, AS–A′S′ those so affected at S2.

dehydrogenase activity is also reduced in affected cells; the decrease in reaction product is most severe in the central cytoplasm. With respect to the activity of this enzyme there is no difference between the copper-deficient and control groups. Similarly, in both groups phosphatase histochemistry reveals changes of equal severity in affected neurones of both copper-deficient and control groups. Lysosomes are reduced and the Golgi apparatus shows a proliferation of tiny vesicles throughout the cytoplasm (fig. 7).

By the 10th day after section frankly chromatolytic cells are more frequently encountered in all the 6 (3 deficient and 3 control) lambs examined at this age (fig. 8). Oxidase histochemistry shows the same pattern as at 5 days, but phosphatase histochemistry reveals more advanced changes. Retispersion of the Golgi is frequent (fig. 9) and in some cells fragmentation and lysis of substantial portions of the network have occurred (figs. 10 and 11). Depletion of lysosomes is widespread and often very severe amongst affected cells (figs. 12 and 13). No clear differences between the copper-deficient and control groups were recognised.

AXONAL REACTION IN LAMBS

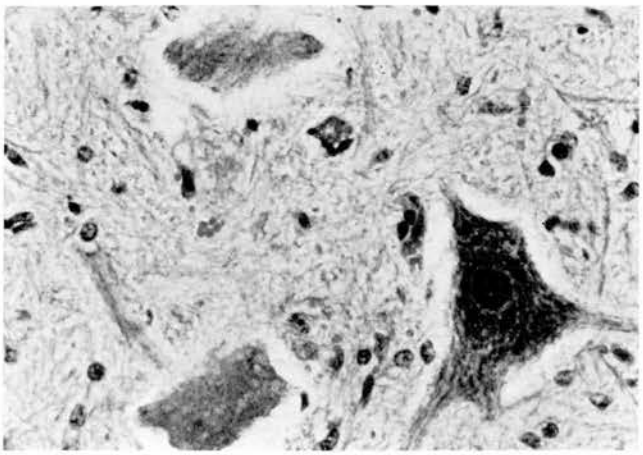

FIG. 2.—Axonal reaction in a low-copper lamb 5 days post-operatively. The two cells at the left of the photograph show abnormally fine Nissl granules compared with that at the right. Gallocyanin. ×400.

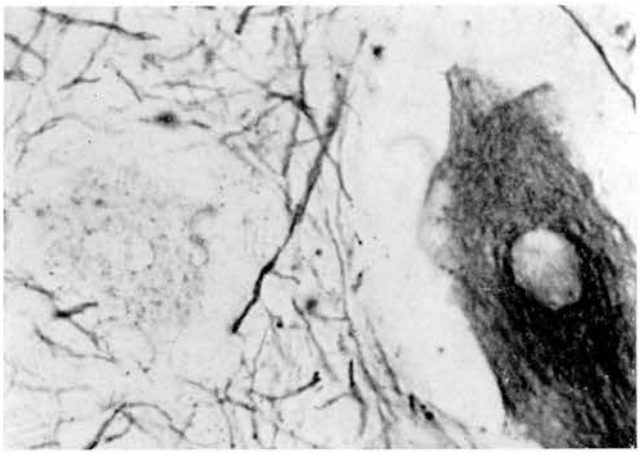

FIG. 3.—Copper-deficient lamb 5 days after sciatic nerve section. Granular disintegration of neurofibrils is particularly severe in the left-hand cell. Holmes. ×675.

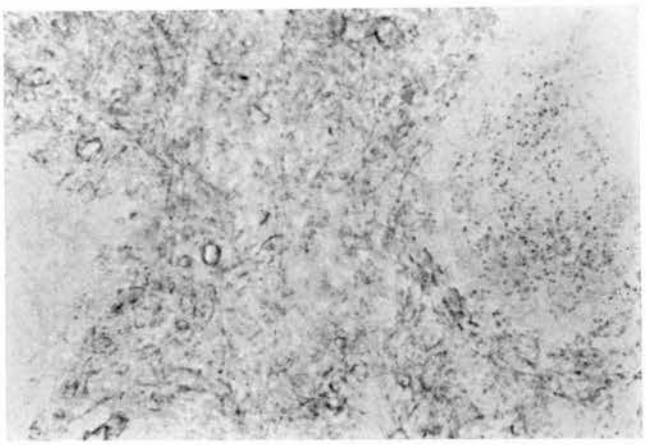

FIG. 4.—Axonal reaction in copper-deficient lambs 5 days after sciatic nerve section. Cytochrome oxidase activity is reduced in both cells (cf. fig. 6) and is total in the left cell. Cytochrome oxidase. ×430.

AXONAL REACTION IN LAMBS

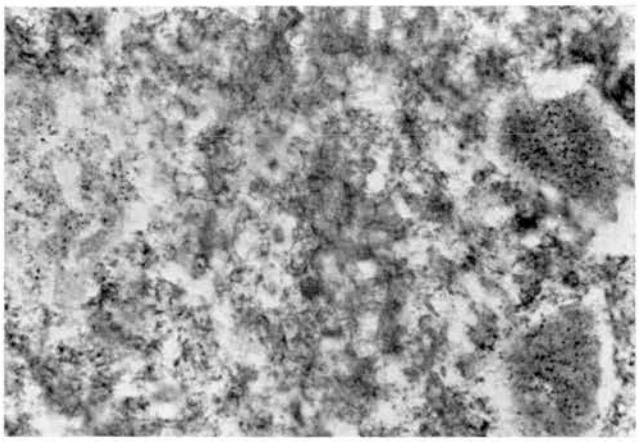

FIG. 5.—Axonal reaction in control lamb 5 days after sciatic nerve section. Cytochrome oxidase activity is reduced, but not so severely as in fig. 4. Cytochrome oxidase. ×430.

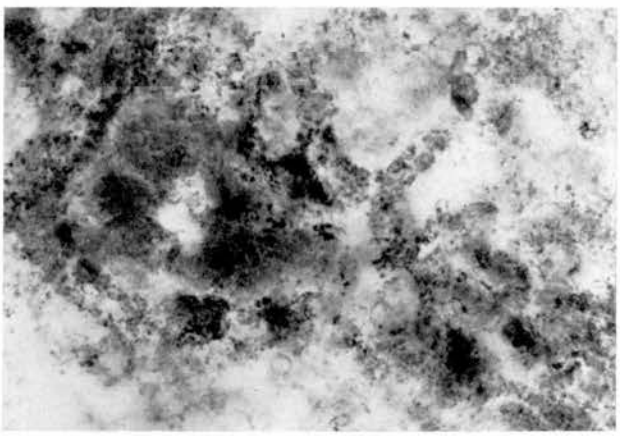

FIG. 6.—Control lamb unsectioned side, 5 days after section of sciatic nerve. Strong cytochrome oxidase activity of cell body and neurophil (cf. figs. 4 and 5). Cytochrome oxidase. ×450.

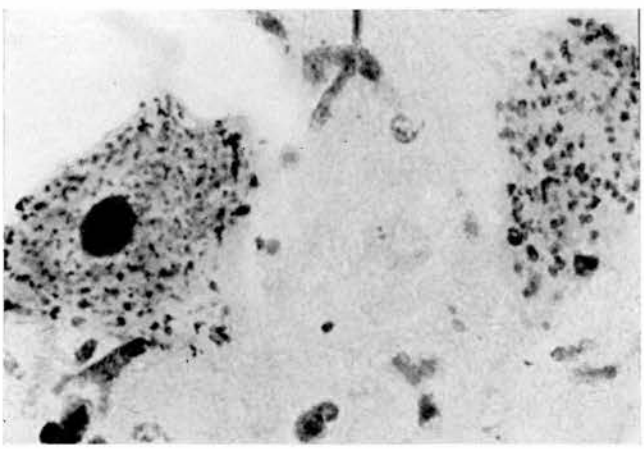

FIG. 7.—Axonal reaction in copper-deficient lamb 5 days after sciatic nerve section. Proliferation of fine vesicles of the Golgi apparatus. Thiamine pyrophosphatase (TPP). ×400.

AXONAL REACTION IN LAMBS

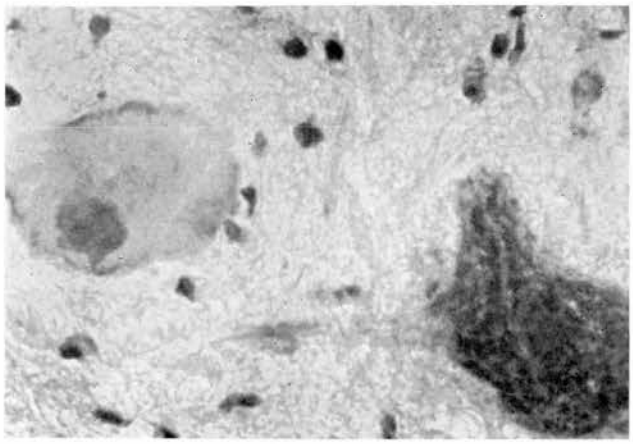

FIG. 8.—Axonal reaction in control lamb 10 days after sciatic nerve section. Chromatolysis. Haematoxylin and eosin. ×430.

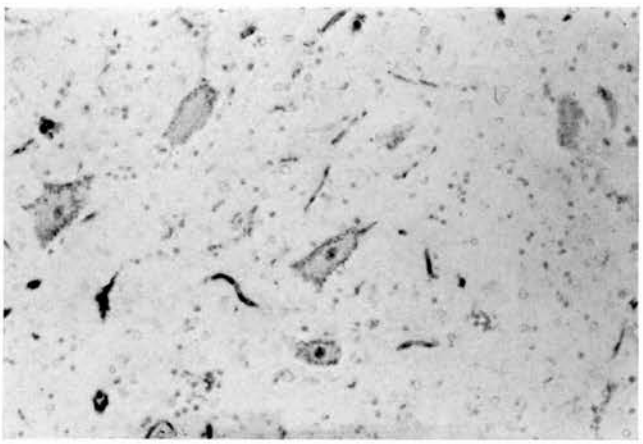

FIG. 9.—Axonal reaction in copper-deficient lamb 10 days after sciatic nerve section. Retispersion of Golgi apparatus. TPP. ×110.

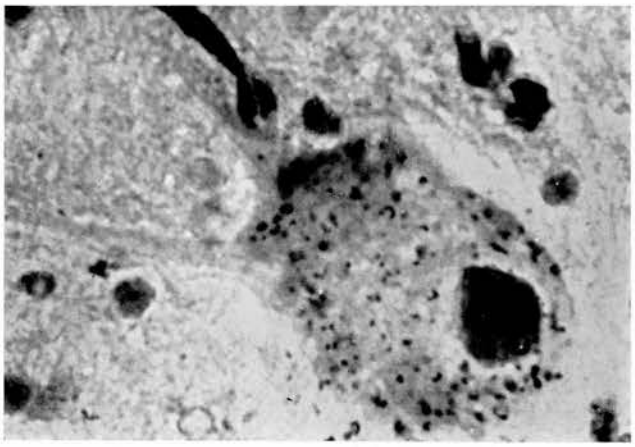

FIG. 10.—Axonal reaction in copper-deficient lamb 10 days after sciatic nerve section. Early fragmentation of the Golgi apparatus. TPP. ×675.

AXONAL REACTION IN LAMBS

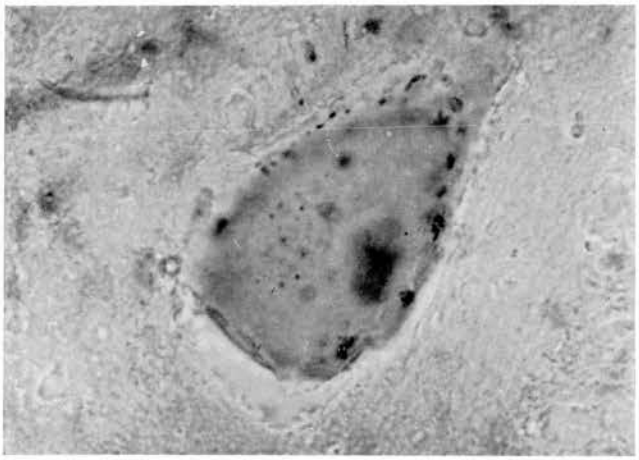

FIG. 11.—Axonal reaction in control lamb 15 days after sciatic nerve section. Dissolution of the Golgi apparatus. TPP. ×675.

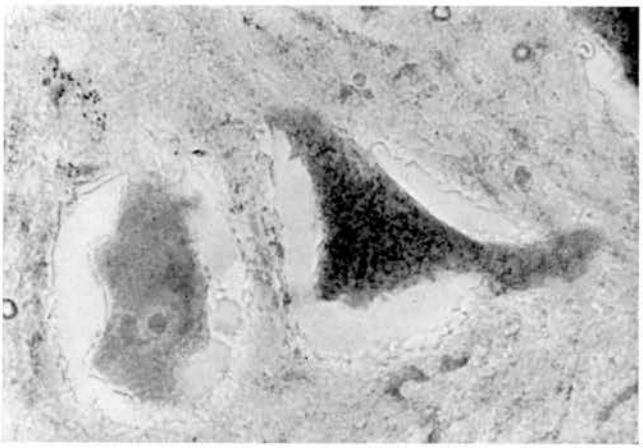

FIG. 12.—Axonal reaction in copper-deficient lamb 10 days after sciatic nerve section. Depletion of lysosomes in the cells on left is almost total. Acid phosphatase (AcPase). ×430.

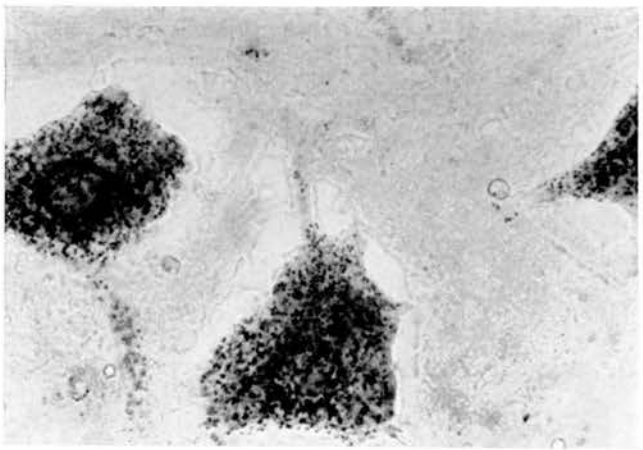

FIG. 13.—Same lamb as in fig. 12, but unoperated side. Dense population of lysosomes. AcPase. ×430.

AXONAL REACTION IN LAMBS

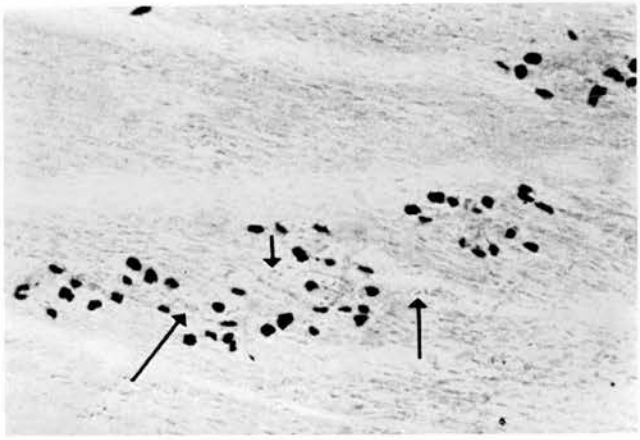

FIG. 14.—Peroneus tertius muscle sectioned side, copper-deficient lamb 15 days after sciatic nerve section. Granular ghost-like outlines of some terminal nerve fibres are indicated by the arrows. The muscle fibres are atrophic. Cholinesterase-Gros-Schultz (CGS). ×110.

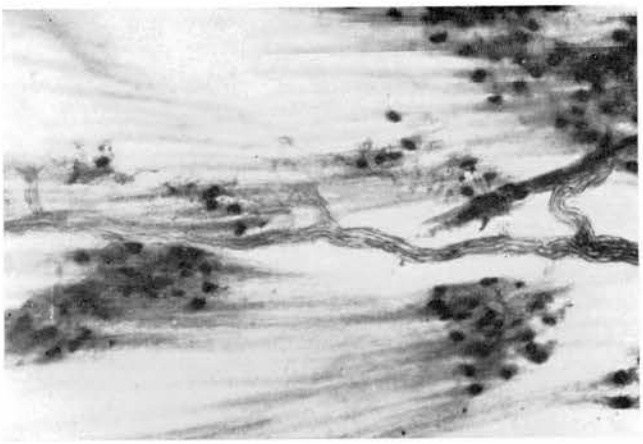

FIG. 15.—Same lamb as in fig. 14. M. peroneus tertius of unaffected side. The terminal nerve bundles stain strongly and there is apparent diffusion of the enzyme reaction product from the motor end-plates. CGS. ×110.

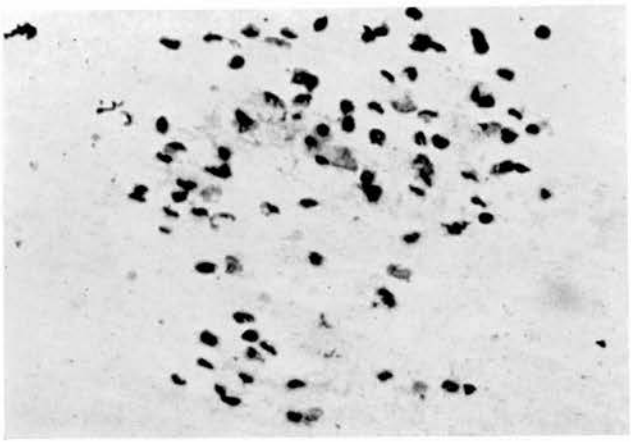

FIG. 16.—M. peroneus tertius of copper-deficient lamb 21 days after sciatic nerve section. A few motor end-plates show fragmentation and dissolution. CGS. ×430.

The pattern of neuronal reaction to nerve section remained unchanged during the remainder of the experiment except for cytochrome oxidase activity in control lambs. In one out of two 15-day-survival lambs and in the sole 21-day survival lamb of the control group no cells with depleted enzyme activity are present, though in all the comparable copper-deficient animals such cells are common.

Quantitative results. The foregoing results have indicated that the lysosome content may be a sensitive marker of the reaction of the neurone to axonal damage. The randomisation of sections and sides of each section eliminated any observer bias due to previous experience and balanced the effects of random variation in the observer's criterion of depletion during counting. The re-randomisation of the material and a second count showed random variation between counts (table) that indicated that no observer bias developed during the counting process.

Student's " t " test showed that significantly more depleted cells were present on the sectioned side than the unsectioned side of control lambs ($P<0{\cdot}05$) and this difference was highly significant in the copper-deficient lambs ($P<0{\cdot}0005$). For both sound and sectioned sides the differences between the copper-deficient and control groups were, however, not significant. No trends attributable to duration of survival after nerve section were found in either group.

The proximal stump of the sciatic nerve

At 24 hr post-section there is haemorrhage and oedema of the perineurium and activation of histiocytes. Small numbers of lipid droplets with the staining characteristics of myelin are present close to the cut ends of the nerves. The myelin sheaths are foamy for a distance of 0·4–0·6 mm back from the incision, and the axons are swollen and palely basophilic.

At 5 days post-section the proximal stump is embedded in a loose granulation tissue of proliferating epineurial cells and fibroblasts. Mitosis is frequently seen. This granulation tissue contains polymorphonuclear leucocytes and numerous macrophages. Some macrophages contain undigested droplets of myelin lipid. Retrograde swelling and foamy degeneration of the myelin sheaths extend over about 1 mm, but retrograde axonal swelling is much more extensive. A proportion of axons show spherical or torpedo-shaped reaction bulbs.

By the 10th post-sectional day the stump is enclosed in a scar from proliferating epineurial fibroblasts. Nerve fibres extend well into the scar and are myelinated almost to their tips. Many reaction bulbs are present in the scar, but there are also small numbers of very slender new axons some of which also show fine myelin sheaths. Occasionally these fine axons have flexed upon themselves to form Perroncito spirals. An increased density of Schwann cell nuclei is associated with the terminal portions of the nerve fibres. Macrophages remaining within the scar tissue contain lipid, with predominantly the staining qualities of hydrophobic neutral fats.

TABLE

Cell counts in spinal cord

Status of animals	Survival after section (days)	Sound side						Sectioned side					
		Total cells			Lysosome-depleted cells			Total cells			Lysosome-depleted cells		
		Count I	Count II	Mean	Count I	Count II	Mean	Count I	Count II	Mean	Count I	Count II	Mean
Controls	1	107	94	100·5	52	36	44	82	90	86	36	37	36·5
	5	93 92	102 73	97·5 82·5	30 66	23 40	26·5 53	78 84	78 113	78 98·5	61 33	58 77	59·5 55
	10	120 88 103	85 82 99	102·5 85 101	30 62 37	25 59 32	27·5 60·5 34·5	105 86 116	81 90 124	93 88 120	67 56 61	40 37 56	53·5 46·5 58·5
	15	98 75	80 75	89 75	25 26	22 15	23·5 20·5	78 89	73 71	75·5 80	32 59	24 42	28 50·5
	21	65	62	63·5	25	17	21	88	86	87	49	51	50
				Mean	88·5		Mean	34·6		Mean	89·6		Mean	48·7
				S.D.	±12·6		S.D.	±13·8		S.D.	±12·7		S.D.	±9·8
Copper-deficient	1	91	99	95	26	21	23·5	91	98	94·5	71	67	69
	5	73 123	65 119	69 121	10 33	12 25	11 29	93 151	92 144	92·5 147·5	46 69	47 65	46·5 67
	10	88 82 106	79 77 95	83·5 79·5 100·5	37 40 41	25 43 37	31 41·5 39	97 85 97	90 67 105	93·5 76 101	62 37 54	49 23 53	55·5 30 53·5
	15	88 81	84 81	86 81	19 25	26 31	22·5 28	77 63	71 87	74 75	46 34	52 50	49 42
	21	57	68	62·5	35	22	28·5	84	115	99·5	49	78	63·5
				Mean	86·4		Mean	28·2		Mean	94·8		Mean	52·9
				S.D.	±16·4		S.D.	±8·5		S.D.	±21·1		S.D.	±11·9

During the remaining period of the study the scar tissue surrounding the stump becomes progressively more compact and firmly integrated with the peri- and epi-neurium. Vigorous growth of new fine axons is evident, but in 1 copper-deficient and 2 control 15-day-survival lambs and in both lambs of the 21-day-survival group foamy myelin spherules extend proximally along the nerve for 2·6–4·0 mm, and are associated with the reappearance of macrophages containing hydrophilic myelin lipid. These appearances suggest that myelin degeneration due to the presence of scar tissue may recur during repair.

During the period under examination there were no material differences between copper-deficient and control lambs.

The peripheral stump and terminal axons

At 24 hr after section, haemorrhage and oedema of the peri- and epi-neurial sheaths are evident over several centimetres of the distal stump together with histiocytic infiltrations and the formation of foamy myelin spherules. By 5 days marked shrinkage of the nerve has occurred, associated with an apparent increase in Schwann cell nuclei, many of which are pyknotic. Axons are fragmented and 50–70 per cent. have disappeared. The myelin tubes retain their hydrophilic staining properties, but are collapsed or vesiculated and infiltrated by macrophages. The intramuscular terminal axons appear as ghosts. By the 10th post-operative day the peripheral segment is a densely cellular straplike band of tissue containing islands of hydrophobic lipids. Axons have completely disappeared at all levels. This appearance remains unchanged throughout the remainder of the experiment and no differences between the deficient and control groups are observed.

The motor end-plates and muscles

With the chosen incubation period for cholinesterase, normal muscle shows a granular precipitate of the reaction product extending beyond the motor end-plates. At 24 hr after section the amount of this excess precipitate is reduced, and by 5 days has disappeared from the peroneal muscle on the sectioned side (figs. 14 and 15). The end-plates themselves, however, show no morphological changes until 15 days after section of the nerve, when shrinkage of the terminal expansion and fragmentation of the end-plates occur irregularly (fig. 16). Concomitantly atrophy of muscle fibres with loss of clarity of the striations and proliferation of sarcolemmal nuclei become evident. No difference in the rate of progress or the nature of the changes was observed between the copper-deficient and control groups.

Discussion

The results presented here indicate that the changes occurring in the peripheral nerve, motor end-plate and muscle after sciatic nerve section in the young lamb are closely comparable to those described for other mammalian species. The cells whose axons had been cut, however, showed changes distinctly different from those found in rodents, carnivores and primates.

After unilateral sciatic nerve section affected cells were found entirely within the ventral horn of the sectioned side between L5 and S2 spinal segments. The position of such cells varied systematically between segments, but throughout they conformed closely to lamina IX in the cat (Rexed, 1954). The earliest histological changes resembled those seen in the rabbit and cat (Romanes, 1941–42, 1951) and consisted of abnormally fine Nissl substance in a slightly swollen cytoplasm. As Geist (1933) and Romanes (1941–42, 1951) found, the frequency of actual chromatolysis was surprisingly low and may be attributable to both the site and method of sectioning the nerve (Marinesco, 1898; de Neef, 1901).

Histochemically, affected cells showed a reduction of cytochrome oxidase activity at 5 days post-section, which persisted throughout the remainder of the experimental period in the copper-deficient lambs, but appeared to be of a transitory nature in the controls; in these some recovery was evident by the 15th post-sectional day. In the deficient lambs, however, this reduction in enzyme activity was more severe than in the controls, as it was superimposed on the general effect of low copper status (Fell *et al.*, 1965). The situation in young lambs differed from that in cat and monkey (Howe and Mellors, 1945), in which reduction in enzyme activity developed more slowly, but persisted for 100 days after section.

Reduction of succinic dehydrogenase activity was also evident in the lambs from the 5th day after section and persisted throughout the experimental period. The depletion of this enzyme was most marked in the perinuclear region of the cell and appears to differ from the findings of Holtzman *et al.* (1967) in the ganglion nodosum of the rat, in which accumulations of mitochondria occurred in the centre of the cell.

The sequential changes in thiamine pyrophosphatase in the Golgi apparatus after nerve section in the lamb closely resembled those detected in other species by the use of either silver staining or enzymatic techniques (Penfield, 1920; Novikoff and Essner, 1962; Barron and Tuncbay, 1964). However, with respect to acid phosphatase the lambs behaved in a strikingly different manner: a rapid and persistent *depletion* of lysosomes occurred after section. This is the reverse of the situation seen in several other species (Barron and Sklar, 1961; Novikoff and Essner; Holtzman *et al.*), when the same technique was used (Holt, 1961).

Most other students of axonal reaction have utilised young adult animals, and Brodal (1939) has shown that the response to axon section may vary with age. This may be the explanation of some of the enzymatic differences described here, but not of the unusual lysosome response. The depletion in lysosomes that occurs after sciatic nerve section in sheep takes place irrespective of the age of the subject (Barlow, unpublished results), and in young lambs it is highly significant (table: $P<0{\cdot}0005$).

This effect would appear to be another example of a species-specific response, to some of which attention has already been drawn by Geist and by Cammermeyer (1968). The depletion of lysosomes may be relevant in considerations of susceptibility to a variety of pathological processes, e.g., copper deficiency in the neonatal lamb.

In this experiment, 2 groups of lambs of differing copper status were used, but the deficient group was deliberately not so depleted of copper as to show any evidence of swayback. The mean brain copper level (6·5±2·26 p.p.m. dry weight) was considerably above that which Mills and Williams (1962) regarded as the critical value below which the natural disease develops. This could be the reason why effects attributable to copper deficiency in this experiment were minimal. The low copper status, however, contributed to the reduction in cytochrome oxidase activity, and it is tentatively suggested that a more significant effect might be found in an experiment of longer duration.

This experiment has shown that the retrograde neuronal changes following axon section are similar in type though milder in degree than those in natural swayback. The findings support the view that disruption of the axon may be fundamental in the pathogenesis of this disease.

SUMMARY

The changes occurring in the cells of origin, the peripheral nerve and muscle after sciatic nerve section have been studied in young lambs of normal and low copper status. Apart from the retrograde neuronal changes the results are similar to those found in other species. The neuronal lesions were histologically similar to those occurring in rat, cat, rabbit and monkey, but histochemically several differences were found, notably with respect to acid phosphatase activity. Lysosomes were depleted after nerve section in lambs, in contrast to a generally reported increase in other species. The difference was not attributable to age. The possible significance is briefly discussed. No significant effect of copper status was evident.

I am grateful to the staff of the Biochemistry Department for providing the animals used in this experiment and for carrying out the copper assays, to Mr J. C. Rennie for help with the surgery, and to Mrs C. Whitehead for skilled technical assistance.

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EXPERIMENTAL COPPER DEFICIENCY IN SHEEP

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INTRODUCTION

A large number of clinical abnormalities of grazing sheep and cattle have been attributed to Cu deficiency because they are associated with hypocupraemia and low liver Cu concentrations: they respond to Cu therapy (Underwood, 1962). Marked hypocupraemia can, however, be found in clinically normal animals (Allcroft and Lewis, 1957; Barlow, Purves, Butler and MacIntyre, 1960; Todd, Milne and How, 1967). Furthermore, the symptoms associated with Cu deficiency in sheep vary from location to location: swayback (enzootic ataxia) is the only clinical abnormality regularly associated with 'Cu deficiency' in the United Kingdom, whereas in Australasia, anemia, loss of wool crimp and achromotrichia have also been observed (Allcroft and Lewis, 1957).

It is, therefore, desirable to determine under experimental conditions the true symptoms of Cu deficiency and the order in which they appear. A preliminary account of our attempts to do this has already been published (Suttle and Field, 1967).

MATERIALS AND METHODS

Animals. In Exp. 1, ten castrate male and eleven female Cheviot × Border Leicester lambs, aged 10 to 12 weeks and weighing approximately 18 kg. were used. In Exp. 2, six castrate Merino lambs weighing approximately 20 kg. were used.

Treatments. In Exp. 1, lambs were weaned gradually on to a semi-purified diet containing 1·2 μg. Cu/g. DM with 10 μg. Cu/g. added as Cu SO_4 $5H_2O$. After 2 weeks, the Cu supplement was withdrawn from the diet of 5 male and 6 female lambs. Exp. 2 was started several weeks after weaning, four of the lambs being given the unsupplemented diet.

Basal diet. In Exp. 1 the basal pelleted diet was that described by Suttle and Field (1968a), the principal ingredients being oat hulls, dried skim milk, starch, sugar and urea. Vitamin A was increased from 1000 to 4000 I.U./kg. after the first lambing and sodium chloride from 0·5 to 1·5 per cent. after 5 months following an outbreak of urolithiasis in which one wether lamb died. The diet contained on average 0·2 mg. Mo and 0·73 g. S/kg. DM. The daily food allowance was increased from 0·75 to 1·75 kg./day during the growth period. Mature animals generally received 1 kg./day, but allowances were increased to 1.3 kg. during late pregnancy and to 2 kg./day during lactation. In Exp. 2, the food intake was limited to a maximum of 1 kg./day and the experiment lasted 14 months. For a further three months the unsupplemented wethers were given a diet similar to that described by Howell (1968) and containing 0·6 μg. Cu/g.

Management. Animals were individually penned in a low Cu environment (Suttle and Field, 1968a) and given deionised drinking water. In Exp. 1, the wether lambs were slaughtered after 17/18 months. Ewe lambs were tupped by Scottish Blackface

rams at approximately 21 and 33 months of age in the same environment (Suttle and Field, 1969). Viable offspring were left to suckle their mothers and were examined frequently for signs of ataxia. Survivors from the first and second lamb crop were slaughtered at 6 and 8 to 10 weeks of age, respectively, and the ewes after 40 months on the dietary treatments.

Blood samples were taken from the jugular vein at frequent intervals from wethers, ewes and lambs (Suttle and Field, 1968a) and milk samples were obtained from two supplemented and four unsupplemented ewes at intervals during the second lactation.

Methods. Cu in blood, tissues and diets was estimated by the method of Brown and Hemingway (1962) until the first lambing and by atomic absorption spectrophotometry thereafter (Suttle and Field, 1968a). Benzylamine oxidase activity in plasma was assayed by a manometric method modified from that of Blaschko and Bonney (1962) by using 0·2 M Tris-buffer : other biochemical methods have been described in earlier papers (Suttle and Field, 1968a, b). The histological methods were those of Barlow *et al.* (1960) with the addition of OTAN for distinguishing normal and degenerating myelin. Brain, liver, heart and kidney were generally examined from wethers, ewes and lambs; in addition, the psoas muscle of wethers was sampled for Cu estimation. Histological examinations on the second lamb crop were confined to nervous tissue, and the coronary and splenic arteries.

Statistics. Logarithmic transformations were performed on groups of data whose variances differed widely. Twin lambs are given a single mean value and overall treatment means are given with their standard errors.

RESULTS

Experiment 1

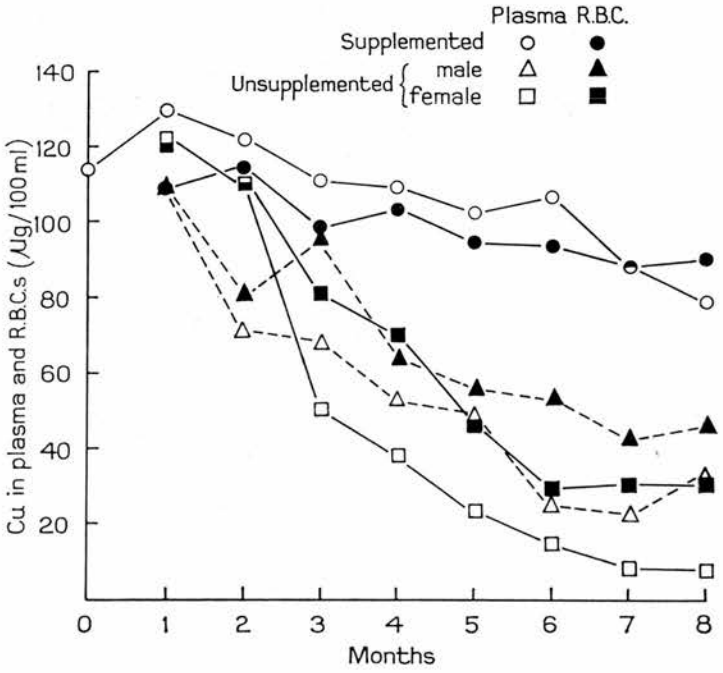
Growth Rate and Feed Conversion Efficienecy

There were no significant differences in growth, appetite or feed conversion efficiency between the supplemented and unsupplemented groups, the mean growth rates being 132 ± 4 and 128 ± 3 g./day and feed conversion efficiencies 11·7 ± 0·9 and 12·5 ± 0·7 g. feed/g. liveweight gain, respectively, for the first 12 months of the experiment. There was little further growth and in each group a mature liveweight of about 60 kg. was attained. All the ewes remained in good condition until the first lambing and feed was rarely refused.

Cu in Plasma and Erythrocytes

A fall in both plasma and R.B.C. Cu concentrations was observed in unsupplemented lambs after 2 to 3 months (Fig. 1). Thereafter, plasma Cu values fell most rapidly in unsupplemented females and the minimum value reached after 7 months was 8 μg./100 ml. as compared with 23 μg./100 ml. for male lambs. A similar sex effect was evident in the R.B.C.s which generally contained more Cu than the plasma. In supplemented lambs the values in both blood fractions remained within the normal range, but decreased slightly during the course of the experiment, R.B.C.s generally containing less Cu than plasma. From the results given in Table 1 it can be seen that in unsupplemented ewes plasma Cu concentrations remained relatively low throughout both pregnancies, but increased between pregnancies. Values for supplemented ewes remained within the normal range throughout.

Fig. 1.



Mean Cu concentrations in plasma and R.B.C.s in Cu-supplemented, unsupplemented male and unsupplemented female lambs

TABLE 1

MEAN PLASMA COPPER CONCENTRATIONS

Supplemented and unsupplemented ewes during pregnancy and in their lambs at birth

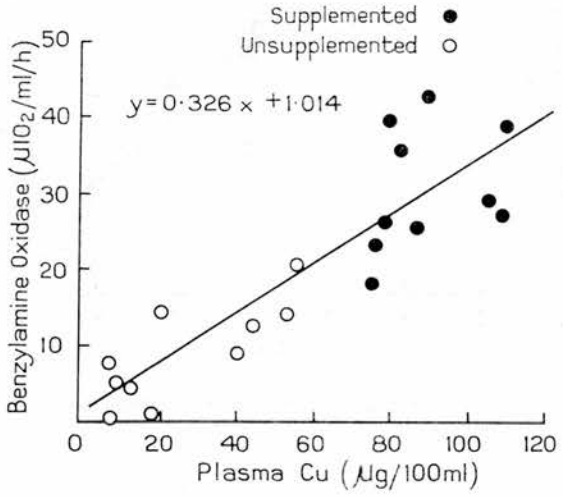
	Ewes					*Newborn lambs*	
	Pregnancy (months)						
Group	*1st*	*2nd*	*3rd*	*4th*	*Parturition (µg.Cu/100 ml. plasma)*		*No. of * values*
1st pregnancy Supplemented	87·0	79·1	90·6	—	89·3 ± 10·9	28·4 ± 4·1	5
2nd pregnancy	174·0	138·6	122·0	120·7	110·7 ± 8·4	35·9 ± 6·4	4
1st pregnancy Unsupplemented	17·4	14·6	9·3	—	11·1 ± 2·2	9·0 ± 1·7	6
2nd pregnancy	47·0	42·2	23·1	14·3	14·1 ± 2·5	20·0 ± 3·0	5

* Twin lambs given single mean value

Plasma Monoamine Oxidase Activity

Mean values of 9 and 21 $\mu 10_2$/h/ml. plasma were recorded for monoamine oxidase activity in the unsupplemented and supplemented groups, respecively, after 16 to 24 weeks and the difference was significant ($P < 0{\cdot}001$). Enzyme activity was found to increase linearly with increases in plasma Cu concentration and the relationship between the two variables is given in Fig. 2; the correlation co-efficient was 0·88 ($P < 0.001$). There was, however, no difference between unsupplemented males and females.

Fig. 2.



Relationship between benzylamine oxidase activity and Cu concentration in plasma of unsupplemented and supplemented lambs.

Wool Crimp

Loss of wool crimp was suspected in three unsupplemented lambs after 6 months treatment and a distinct band of poorly crimped wool was visible after 11 months (Fig. 3); these lambs, one male and two female, were not those with the lowest plasma Cu levels. There was subsequently a restoration of crimp to the staple of the affected lambs towards the end of the growth period. Supplemented lambs showed no loss of crimp.

Haemogloblin Values

Haemoglobin concentrations and haematocrit values were not affected by removing the Cu supplement from the diet. Mean haemoglobin concentrations in the unsupplemented and supplemented ewes were 15·0 ± 0·8 and 15·7 ± 0·7 g/100 ml. after 8 months on experiment, 12·1 ± 0·4 and 12·4 ± 0·2 g/100 ml. after 15 months and 9·7 ± 0·75 and 11·6 ± 0·66 at first parturition, respectively.

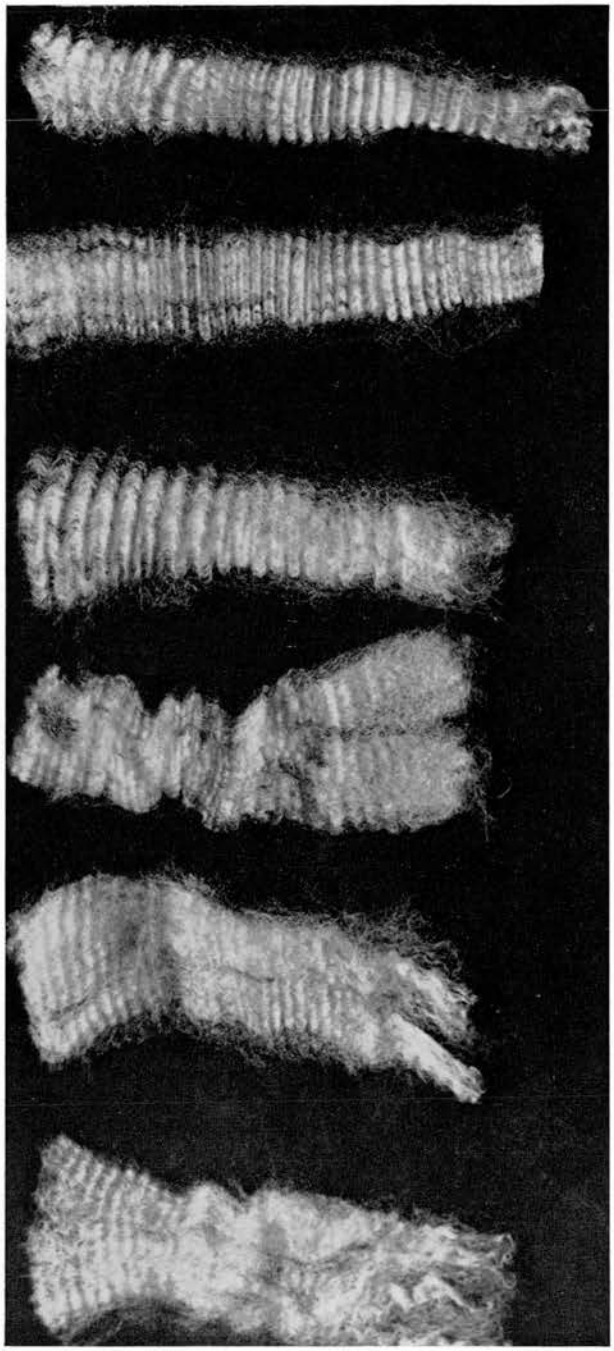


Fig. 3.

Temporary loss of wool crimp in three unsupplemented Half-Bred lambs (left) after 11 months' treatment compared with wool of three supplemented lambs.

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Values for ewes after the second pregnancy and for the newborn lambs from both crops were all within the normal range, as were haematocrit values at all samplings.

Clinical Condition of Ewes and Lambs

All ewes held to their first service within 16 days of introducing the ram and all produced lambs, a high proportion of twins being obtained in both crops from the two groups. The lambs were generally well developed physically at birth. However, a number of lambs in the first crops from both groups showed severe respiratory difficulty and inco-ordination and they either died or were slaughtered within hours of birth. The vitamin A supplement was increased prior to the second lambing and there were no signs of this abnormality in lambs of the second crop from either group. The detailed results for both crops are given in Table 2.

Of the six viable lambs in the first crop, the four from the supplemented group remained healthy until slaughter at six weeks: one of the unsupplemented lambs showed signs of ataxia and died at 13 days with an umbilical abscess, while the other remained healthy. Of the six viable lambs from the second unsupplemented lamb crop, three showed clinical signs of delayed swayback, one at 3 weeks and two at 10 weeks of age: the five viable lambs from the second supplemented crop remained healthy.

Three unsupplemented ewes prolapsed at their first lambing and developed gross oedematous swellings around the vulva; supplemented ewes did not prolapse and the abnormality did not recur at the second lambing. One supplemented ewe was lost after a caesarean delivery and an unsupplemented ewe died after retaining an immature twin foetus at the first lambing.

Lamb Pathology.

The pathological findings have also been summarised in Table 2. All lambs from the first crop had an intracranial abnormality: it was most severe in lambs dead or dying at birth and was a major contributory cause of death. The lesion consisted of a partial herniation of the cerebellum anteriorly so that the fissura prima lay beneath the tentorium cerebelli, and posteriorly formed a 'tail' in the foramen magnum. Local softenings were noticeable in the constricted area, but the cytoarchitecture was undisturbed and the lesions appeared to be the result of deformity of the posterior cranial vault.

In seven out of the ten first-crop lambs examined in the unsupplemented group, chromatolysis in the red nucleus and/or the ventral horn of the spinal cord together with thinning and/or degeneration of the myelin in the long spinal tracts were observed. In addition to these lesions, one lamb showed cerebral oedema and another showed gelatinous cavitation of the cerebrum. Since these lesions were accompanied by low brain Cu and cytochrome oxidase activities, they would normally be considered to be consistent with a diagnosis of swayback (Barlow *et al.*, 1960). However, chromatolysis in either the red nucleus or ventral horns of the spinal cord was also evident in three of the ten first-crop lambs from supplemented ewes with normal brain cytochrome oxidase activities and Cu concentrations: in two cases there was also myelin thinning in the long tracts. In the second lamb crop there were no cases of the intra-cranial abnormality. The three

TABLE 2

SUMMARY OF THE MAIN CLINICAL, BIOCHEMICAL AND PATHOLOGICAL RESULTS
Both lamb crops from supplemented and unsupplemented ewes

		1st lamb crop									*2nd lamb crop*								
Group	*Ewe No.*	*Lamb birth wt. (kg.)*	*Brain Cu (μg./g. DM)*	*Liver Cu DM)*	*Brain cytochrome oxidase (μl O_2/ mg. DM/h)*	*Pathological findings* 1	2	3	4	*Conditions at birth*	*Lamb wt.*	*Brain Cu (μg./g. DM)*	*Liver Cu DM)*	*Brain cytochrome oxidase (μl 0·2/mg. mg. DM/h)*	*Pathological findings* 1	2	3	4	*Condition at birth*
Cu-Supplemented	1	—	12·0	89	N.D.	−	−	−	+	Died	5·47	16·5	159	195	−	−	−	−	Normal
		—	12·8	113	N.D.	−	−	−	+	Killed†	3·65	16·9	152	108	−	−	−	−	Normal
	2	2·86	12·8	710	235	−	−	−	+	Died after	—	—	—	—	0	0	0	0	
		2·89	12·9	572	167	−	−	−	+	Caesarean delivery									
	3	3·06	14·0	477	283	−	+	+	+	Killed†	5·33	16·4	149	105	−	−	−	−	Normal
		3·06	11·2	365	168	−	+	−	+	Normal									
	4	3·76	11·0	196	142	−	−	−	+	Normal	4·08	10·9	336	N.D.	−	−	−	−	Died at
		3·29	10·0	274	205	−	+	+	+	Killed†	4·67	9·6	269	N.D.	−	−	−	−	birth
	5	3·81	12·5	—	258	−	−	−	+	Normal	4·05	12·7	—	195	−	−	−	−	Normal
		3·36	10·8	120	228	−	−	−	+	Normal	3·68	10·1	66	113	−	−	−	−	Normal
	6	—	5·1	4·0	N.D.	−	+	+	+	Died	4·95	4·3	2·6	—	−	−	−	−	Died at birth
		—	1·1	8·5	67	−	+	+	+	Killed†	4·56	3·2	4·9	51	−	+	+	−	Normal
	7	4·95	9·0	5·5	N.D.	−	+	+	+	Ataxia—hand fed	5·55	5·0	3·3	33	−	+	+	−	Normal
Un-supplemented	8	3·10	4·4	6·5	151	−	−	−	+	Killed†	3·22	8·5	10·6	146	−	−	−	−	Normal
		3·65	4·2	7·0	81	−	−	−	+		4·33	6·1	7·6	85	−	−	−	−	Normal
	9	4·12	2·5	4·0	0	+	+	+	+	Died	5·35	3·4	5·2	147	−	−	−	−	Normal
		3·68	2·3	0·5	25	+	+	+	+										
	10	3·43	3·2	4·8	44	−	+	+	+	Killed†									
		—	—	—	—	?	?	?	?	Foetus retained: ewe died	—	—	—	—	0	0	0	0	
	11	3·62	2·5	6·5	35	−	+	−	+	Normal	4·91	1·4	2·4	37	−	+	+	−	Normal
		3·25	2·5	5·0	53	−	−	−	+	Killed†	2·94	—	—	—	?	?	?	?	Died

† Lambs showed rapid deterioration in condition, respiratory difficulty and inco-ordination.
N.D. Not determined.

Key to pathological findings:

1. Cerebral cavitation or gelatinous white matter.
2. Chromatolysis of red nucleus and/or vestibular nucleus and/or ventral horns and/or reticular formation.
3. Tract degeneration in dorso-lateral and nucleomarginal funiculi.
4. Intra-cranial abnormality (see text).

cases of delayed clinical swayback showed neuropathological changes consistent with this diagnosis and there was no evidence of such changes in the C.N.S. of lambs from supplemented ewes.

No histological abnormalities were found in the coronary or splenic arteries or other tissues.

Tissue Cu and Brain Cytochrome Oxidase in Lambs

The individual results for brain and liver Cu, and brain cytochrome oxidase activity are given in Table 2. In the first lamb crop there were no consistent differences between viable and non-viable lambs, and the mean liver ($P < 0.001$) and brain ($P < 0.01$) Cu concentrations and brain cytochrome oxidase activities ($P < 0.01$) were lower in the lambs from unsupplemented ewes. The mean Cu concentrations in kidney were 16·8 ± 3·3 and 17·9 ± 7·05, respectively for unsupplemented and supplemented lambs and in the heart 8·9 ± 0·75 and 13·2 ± 0·78 μg./g. DM, respectively; the decrease in heart Cu was significant ($P < 0.05$).

In the second lamb crop the degree of tissue Cu depletion in unsupplemented lambs was generally similar to that in the first lamb crop. Only one unsupplemented ewe (8) failed to produce offspring with swayback lesions in either crop and her lambs were all of relatively high Cu status. Mean Cu concentrations in the kidney were 11·8 ± 0·62 and 20·3 ± 2·42, respectively, for unsupplemented and supplemented lambs and in the heart 10·9 ± 1·53 and 17·0 ± 0·65 μg./g. DM respectively; the difference for both tissues was significant ($P < 0.01$).

Plasma Cu in Lambs

The mean values for newborn lambs are given in Table 1; those for unsupplemented lambs are similar to those of their mothers and lower than those for supplemented lambs. Values in supplemented lambs increased rapidly after birth to reach normal values after 1 week, but those for unsupplemented lambs remained at about 20 μg./100 ml. until slaughter.

Cu in Milk

Milk from unsupplemented ewes contained on the average 26 ± 2·2 μg. Cu/100 ml. and there were no marked changes as lactation progressed. By contrast, values for supplemented ewes fell progressively from 116 to 52 μg./100 ml. between the 1st and 52nd day of lactation.

Copper Concentrations in Tissues from Wethers and Ewes

The mean tissue Cu concentrations for wethers killed after 18 months and ewes killed after 40 months treatment are given in Table 3. The Cu concentrations in liver and brain were lower in the unsupplemented ewes than in the wethers and in both sex groups the values were significantly less than those for supplemented animals after logarithmic transformation of the data. Of the other tissues only the value for skeletal muscle ($P < 0.05$) in the unsupplemented ewes was significantly lower ($P < 0.01$) than that for the corresponding supplemented animals. Pathological changes were not detected in any of the ewe or wether tissues examined.

L

TABLE 3

Tissue copper concentrations (μg/g DM) in wethers and ewes given a diet low in copper with or without a Cu supplement for 18 months and 40 months, respectively

Group	*No. of sheep*	*Liver*	*Brain*	*Skeletal muscle*	*Cardiac muscle*	*Kidney*
Wethers						
Unsupplemented	4	9·1 ± 1·3	8·0 ± 0·31	2·1 ± 0·28	10·4 ± 1·07	10·4 ± 1·51
Supplemented	5	663·8 ± 132·7	15·5 ± 1·98	2·2 ± 0·35	9·7 ± 1·55	14·9 ± 3·77
Ewes						
Unsupplemented	5	6·2 ± 0·66	5·2 ± 0·22	2·5 ± 0·59	11·5 ± 1·05	—
Supplemented	4	504·7 ± 109·8	27·5 ± 1·51	4·7 ± 0·07	13·2 ± 0·68	—

Experiment 2

The results of feeding Merino lambs on the unsupplemented diet were generally similar to those obtained with the half-bred lambs. Overall growth rates were slower at 0·088 kg./day, but there was no difference between supplemented and unsupplemented animals. Plasma Cu concentrations in unsupplemented animals did not begin to fall until the sixth month of the experiment when the values were 54, 53, 58 and 80 μg./100 ml. compared with 80 and 97 in supplemented lambs. The subsequent rate of fall was slower than that shown by the unsupplemented male half-breds and after 14 months the unsupplemented values were 20, 11, 12 and 26 μg./100 ml. After transfer to the very low Cu diet, values fell to 8 to 11 μg./100 ml. Haemoglobin levels remained within the normal range throughout, but loss of wool crimp became evident in one unsupplemented lamb after 12 months and in the remainder after 14 to 15 months.

DISCUSSION

A complicating feature of the first experiment was the appearance of pathological criteria for swayback in lambs of normal Cu status in the first lamb crop. All lambs in that crop had an intracranial abnormality affecting the cerebellum and there is strong circumstancial evidence that this was caused by a deficiency of vitamin A. The initial dietary vitamin A supplement was insufficient for late pregnancy (A.R.C. 1965) and the deficiency may have been aggravated by losses of the vitamin during the pelleting process (Pickford, 1968); after the supplement was increased the defect did not appear. Furthermore, similar lesions have been observed in vitamin A deficient rats (Wohlbach and Bassey, 1941; Wohlbach, 1946). We suggest that the chromatolysis and myelin thinning found in three lambs from the first supplemented crop developed as secondary consequences of the intracranial defect. The severe distortion of the cerebellum could quite conceivably have caused pressure anoxia, mechanical trauma or failure to make synaptic connections leading to chromatolytic degeneration of the neurones. These lesions cannot, therefore, be regarded as indicative of swayback when another neurological defect is present and the pathology of unsupplemented lambs in the

first crop will not be discussed further since it cannot be attributed simply to Cu deficiency. Clinical signs of vitamin A deficiency were not observed at other stages of the experiment and it is considered that the differences in clinical abnormalities and biochemical parameters observed during those stages can be attributed to the experimental treatments.

By comparing the results of attempts which have been made to produce a simple deficiency of Cu under experimental conditions, it is possible to draw some tentative conclusions concerning the true symptoms of Cu deficiency and the order in which they appear. It is also possible to contrast the requirements, in terms of Cu intake, for various functions under experimental and field conditions. In the studies of Howell (1968) and of our own the first sign of deficiency, a fall in blood and plasma Cu concentrations, occurred after 2 to 3 months; the time taken for hypocupraemia to develop will obviously depend on the initial liver Cu stores as well as Cu uptake. The correlation between Cu concentrations and monoamine oxidase activity in plasma has also been demonstrated by Mills, Dalgarno and Williams, (1966).

Loss of wool crimp was the first clinical abnormality to be recorded and this is in accordance with the observations of Marston (1950) among grazing sheep in Australia. At Cu intakes of 0·04 mg./kg. liveweight (2·1 mg./day) the requirement for normal crimp formation was, however, met when the half-bred lambs stopped growing. Requirements for crimp formation in the Merino were of a similar order.

The first measurement of tissue Cu concentrations, in wethers after 18 months treatment, showed that liver Cu concentrations had been reduced to levels associated with swayback (Barlow *et al.*, 1960) and in both unsupplemented lamb crops many lambs had Cu concentrations and/or cytochrome oxidase activities in brain tissue of a similar order to those found in natural delayed cases of swayback (Mills and Williams, 1962). The incidence of delayed swayback in the second lamb crops from Cu-depleted ewes in this and other experiments (Suttle and Field, 1969) and the absence of lesions in contemporary controls shows that swayback can be produced simply by depleting the ewe of Cu. Although the incidence of swayback in both experiments was low (40 and 20 per cent.), intakes of 0·02 to 0·03 mg./kg. liveweight (1·2 to 1·8 mg./day) during pregnancy and 0·04 mg./kg. liveweight during lactation must be regarded as insufficient for normal nervous tissue development under our conditions. The delayed swayback which developed can be grouped with impaired crimp formation as an early consequence of simple Cu deficiency. Lewis, Terlecki and Allcroft (1967) have recorded a 41 per cent incidence of swayback in the offspring of ewes given a low Cu diet, but the precise role of dietary Cu is difficult to ascertain in their experiments since the ewes were generally of low initial Cu status, some coming from farms where swayback was endemic, and no controls were used.

In two pairs of lambs from the second crop where only one died at birth, lesions consistent with swayback were found only in the lamb which survived. Since the biochemical and histological findings for unsupplemented twins of equal longevity (four pairs) have always been similar in these studies it appears that those lesions in delayed swayback which are histologically evident can develop completely in the postnatal period. The marked reduction in Cu concentrations in

milk from deficient ewes in this and other experiments (Suttle and Field, 1969), which is in accordance with observations on cattle and sheep from Cu-deficient areas (Beck, 1941), also suggests that the Cu-depletion begun in utero will be continued during lactation. Assuming a milk intake of 1 to 2 l./day, the Cu intake of our unsupplemented lambs would have been reduced by 50 to 75 per cent. to 0·25 to 0·5 mg. Cu/day without, however, affecting their growth rates or haemoglobin levels.

The absence in our studies of the anaemia, stunted growth and reproductive failure found by Howell (1968) may indicate that these abnormalities result from a relatively severe deficiency of Cu since Howell used a diet of lower Cu content than ours (0·3 μg. Cu/g. DM, Howell, personal communication). This suggestion is supported by our finding that the addition of Cu antagonists, in the form of molybdenum and sulphate to a low Cu diet induced anaemia and reproductive failure (Suttle and Field, 1968a, b; 1969). Both anaemia (Lahey, Gubler, Chase, Cartwright and Wintrobe, 1952; Mills and Murray, 1960; Hunt and Carlton, 1965) and reproductive failure (Dutt and Mills, 1960; Howell and Hall, 1969) have, of course, been produced in monogastric species given Cu-deficient diets and anaemia has been associated with Cu-deficiency in grazing sheep (Bennets and Chapman, 1937; Marston, 1950).

Other factors may, however, have contributed to the different responses. In Howell's study the ewe lambs were mated at 8 months of age and the demands for growth and pregnancy were superimposed. Furthermore, those unsupplemented animals which survived pregnancy weighed 50 per cent. less than supplemented animals indicating a marked difference in food intake and/or food conversion efficiency. If food intakes differed widely, paired feeding experiments would be needed to establish whether the anaemia and reproductive failure were primary consequences of Cu deficiency or secondary consequences resulting from undernutrition. It appears that under our conditions Cu intakes of less than 0·03 mg./kg. liveweight are required to affect adversely these particular body functions.

Recent studies have shown that breeds of sheep differ in their susceptibility to swayback, the Cheviot being less susceptible than the Scottish Blackface, but more susceptible than the Welsh Mountain breed (Weiner, 1966; Weiner and Field, 1966). Similar differences may affect susceptibility to anaemia since haemoglobin levels which were unchanged in our Cheviot × Border Leicester sheep were reduced in Scottish Blackface ewes and their lambs given the unsupplemented diet (Suttle and Field, 1969). It is interesting to note that Howell (1968) produced severe Cu deficiency in a relatively insusceptible breed, the Welsh Mountain. The Merino is not particularly susceptible to Cu deficiency, and differences between the Cu deficiency syndromes observed in the field in Australia and in the U.K. (see Allcroft and Lewis, 1957) are, therefore, unlikely to be attributable to differences in breed: it seems probable that the syndromes reported in Australia result from a more severe Cu depletion.

In conclusion it must be stressed that the Cu intakes required to produce Cu deficiency under our experimental conditions are much lower than those found in 'Cu-deficient' pastures. Thus it is unusual to find Cu concentrations in herbage of less than 3 μg./g. DM and in swayback areas in the United Kingdom considerably higher values have been reported (Allcroft and Lewis, 1957). This suggests

that in swayback areas there are factors which considerably reduce the retention and/or utilisation of ingested Cu and the aetiology of swayback will probably remain obscure until the nature of these factors is ascertained. Although the minimum requirement for Cu under our conditions is of the order of 2 mg./day and equivalent to only 40 per cent. of that suggested by an Agricultural Research Council working party (A.R.C. 1965) for mature sheep, there are clearly field situations in which 2 mg. Cu/day would constitute a grossly inadequate intake.

SUMMARY

Two groups of weanling half-bred lambs were given a diet low in Cu, with or without a Cu supplement; castrate males were killed after 18 months, but females were kept on the diets for 40 months and produced two lamb crops. Unsupplemented animals developed low plasma and tissue Cu concentrations and there was a temporary loss of wool crimp between 6 and 12 months; growth, appetite and feed conversion efficiency were not, however, impaired. Lambs from the first crop from unsupplemented ewes had extremely low tissue Cu concentrations and brain cytochrome oxidase activities: lesions associated with swayback were observed in 7 out of 10, but they could not be attributed to Cu deficiency, since some of the pathological criteria for swayback were found in three lambs of normal Cu status from supplemented ewes. The latter lesions were thought to be secondary consequences of an intracranial deformity present in all first crop lambs at birth and attributable to vitamin A deficiency during late pregnancy. In the second lamb crop the intracranial abnormality did not recur: three out of seven lambs of low Cu status from unsupplemented ewes developed delayed swayback, while there were no comparable lesions in control lambs. Anaemia was not observed in ewes or lambs and the reproductive capacity of the two groups was equally good. These findings are discussed in relation to the specificity of the pathological criteria for swayback and the contrasting Cu requirements for particular body functions under experimental and certain field conditions.

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A Condition in the Goat Resembling Swayback in Lambs

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SUMMARY.—A single female kid was found to have lesions of nerve cells and fibres in the brain stem and spinal cord which were identical with those of swayback. The animal showed a quadriplegia from birth and its mother had a low blood copper value. These findings are considered good evidence for a diagnosis of swayback.

SWAYBACK is a neuropathy of young animals having a world-wide distribution. The relevant literature up until 1956 has been reviewed by Innes and Saunders (1957) since which time contributions have been published by Barlow (1958), Gracey and Todd (1958), Behrens and Schulz (1959), Howell and Davison (1959), Barlow *et al.* (1960), Schulz and Behrens (1960) and Spais, Palsson and van Bogaert (1961).

The condition occurs as an ataxia of new-born and young animals characterised pathologically by a deficiency of myelin in the cerebral hemispheres and/or chromatolysis and necrosis of large multipolar nerve cells of the brain stem and ventral horns of the cord, together with degeneration of nerve fibres especially in the dorsal part of the lateral funiculi and the sulcomarginal funiculi. The affected animal and its dam also invariably have a low copper status.

Such have been the findings of several authors (Bennetts & Beck, 1942; McDonald, 1942; Tabusso, 1942; Schulz *et al.*, 1951; and Dandemaev & Abramova, 1956). They were adopted as diagnostic criteria by Barlow *et al.* (1960) and found satisfactory in 79 cases from 20 different sources, collected over a 4-year period.

Recently, however, Spais *et al.* (1961) have reported that they did not find lesions in either the spinal tracts or ventral horn cells in clinically similar material from Greece and Iceland, and Innes and Saunders (1962) state definitely that the characteristic chromatolytic changes described by Innes 25 years ago were localised in the red nucleus.

Though swayback is usually regarded as a disease of sheep similar clinical signs have been observed in other species. Tabusso (1942) suggested that the alpaca, llama, vicuna and guanaco were also susceptible and Cunningham (1950) reported ataxia in a copper-deficient calf. "Lamkruis" in South Africa which closely resembles swayback is also said to occur in goat kids (Schulz *et al.*, 1951). The same authors mention a paresis in the bontebok, duiker and steenbok which also has similar clinical manifestations. Unfortunately, in none of these instances has the result of any histological or biochemical examination been reported. It, therefore, seems important to record in some detail this case resembling swayback in a new-born kid.

History

The dam which gave birth to this affected kid was one of a small herd maintained at the Hannah Dairy Research Institute for experimental work. They were kept on restricted grazing from April to October and were housed during the winter (each in a separate pen). During the winter a ration containing beanmeal, bruised oats, flake maize and decorticated groundnut cake (6:2:1:1) was fed with hay to appetite. The dam in question was 3 years old and had the previous spring given birth to a pair of healthy male twins, after which she produced 825 lb. milk in a 196-day lactation. During the following autumn she escaped from her pen and mated with her half-brother. Towards the end of the resulting pregnancy her appetite was poor and she lost condition. After 156 days

gestation she bore a singleton—the affected kid. At birth the kid was incapable of movement and adopted the posture of a lying dog with the neck curled round and the head against the flank. When carried to the dam and supported the kid was able to suck, and as a result appeared to have gained some strength by the second day. It was able to raise its head for short periods, but when it did this, the head swayed from side to side. It was also capable of slight movements of the forequarters but was incapable of locomotion.

Having made no marked improvement by the 3rd day the kid was taken to the West of Scotland Agricultural College's Veterinary Investigation Department where it was sacrificed. No gross abnormalities were observed *post mortem*, but since the clinical picture had been so like that of swayback the brain and cord were removed, fixed in 15 per cent. formol-saline and sent to the Moredun Institute for histological examination.

Investigation

Pathology

The naked eye appearance of the central nervous system was normal. Histological examination showed a mild oedema of the pia-arachnoid, and slight distension of the perivascular spaces. Neither gelatinous lesions nor cavitations were observed in the *centrum semiovale*, and no abnormalities were recognised in the cerebellum. A few cells of the red nucleus showed swelling, severe central chromatolysis, or hyaline necrosis. Cells degenerating in a similar way were also noted in the medial vestibular nucleus, the reticular formation, and the ventral horns of the spinal cord, particularly at the level of the cervical and lumbar expansions (Fig. 1). Appropriately treated frozen sections of spinal cord revealed a diffuse distribution of Marchi-positive and sudanophilic fibres throughout the white matter, with focal intensification in those parts of the lateral funiculi adjacent to the dorsal nerve roots (Fig. 2.).

Biochemistry

When a presumptive diagnosis had been reached blood samples were taken from the dam of the affected kid and from 3 other dams in the herd. The results of copper estimation are shown in the table.

	Dam of affected kid	Other dams 1	2	3
Blood Cu μgm. per 100 ml.	21.1	38.4	68.2	58.9

Data on blood copper levels in normal goats are not available but the lower limit of normality in sheep has been regarded as 60 μg per 100 ml. blood (Barlow *et al.*, 1960). Judged by these standards it can be seen that the mother and one other of the dams sampled had abnormally low blood copper values.

Discussion

The changes observed in the nerve cells and fibres of the brain stem and cord of this kid are identical with those constantly found in a previous series of

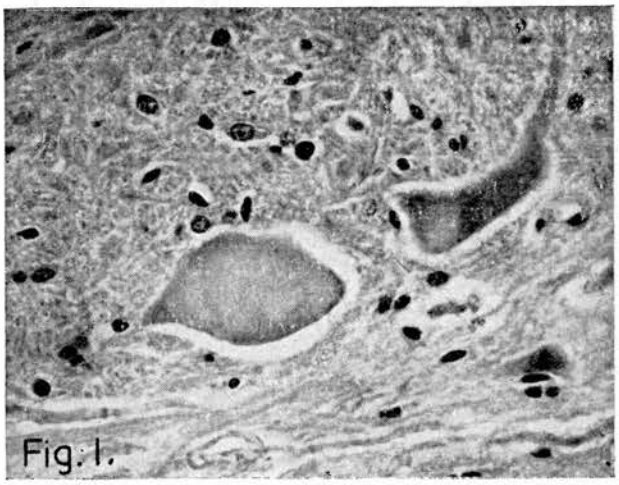

FIG. 1.—Severe chromatolysis, ventral horn cell of spinal cord (H. & E. ×396).

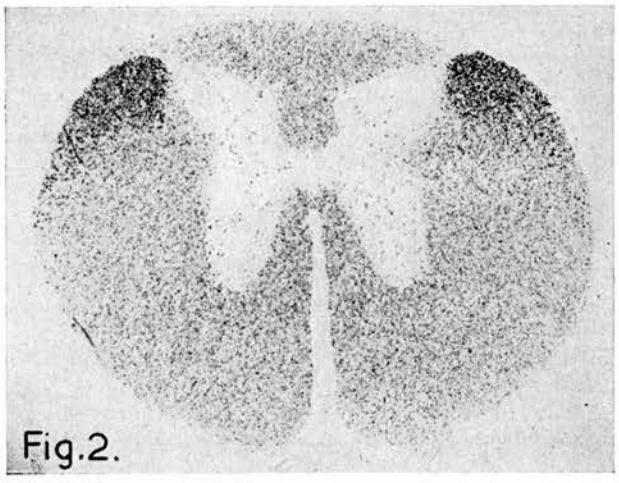

FIG. 2.—Transverse section of spinal cord showing myelin degeneration. Note the aggregations of Marchi-positive fibres in the dorsal part of the lateral funiculus (Glees ×15).

swayback cases in lambs. The presence of quadriplegia and a low maternal blood copper in addition, make a strong case for regarding this as an example of swayback in the goat. If it is such, it is of interest in that for the whole of pregnancy the dam was confined indoors and fed on concentrates and hay. This type of diet, whilst containing average amounts of copper has been shown to result in elevated liver copper values in housed sheep (Allcroft & Lewis, 1957).

Swayback appears to be a metabolic disorder associated with copper deficiency and hitherto cases confirmed by histological and biochemical examination have only been described in sheep. When it is considered that clinically similar ataxias have been observed in other ruminant species sharing "affected" grazings with sheep, and like them showing rapid intra-uterine development of the nervous system, the absence of such reports may be significant. Barlow (1958) suggested that a factor additional to copper deficiency is involved in the aetiology of swayback and the foregoing considerations suggest

that such a factor might be very host specific. The present case in a goat does not lessen the weight of such an argument in view of recent work (Dalimier, 1959; Bratanov & Dikov, 1960), showing that sheep × goat hybrids ("ovids" and "caprids") are possible.

Acknowledgment.—Thanks are due to Dr. E. J. Butler, Moredun Institute, for blood copper estimations.

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17.

PATHOLOGICAL AND BIOCHEMICAL STUDIES OF AN OUTBREAK OF SWAYBACK IN GOATS

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INTRODUCTION

In a preliminary report, Barlow, Robertson, Owen and Proudfoot (1962) described a condition resembling swayback in a female kid born to a goat of a small experimental herd kept at the Hannah Institute. The kid was unable to stand for three days after it was born but survived through being artificially fed on its dam's milk. After a clinical diagnosis of swayback the animal was killed and histological examination of its central nervous system (CNS) revealed neurone degeneration in the red nucleus and defects of myelin, particularly in the lateral funiculi adjacent to the dorsal nerve roots. All the Hannah Institute breeding goats were on a diet designed to be poor in greenstuff and analysis showed low concentrations of copper in the blood of the adult animals. The parallel with swayback in sheep (Innes and Shearer, 1940; Barlow, Purves, Butler and Macintyre, 1960 a, b) and the lack of any report of histological or biochemical studies of paresis in the goat led us to keep the herd on the same type of diet for another two breeding seasons. The present paper is an account of this investigation in which in 1962 two more cases of paresis were encountered thus confirming that this condition was swayback as defined by Barlow *et al.* (1960 a, b). In 1963 although no overt signs of swayback were seen, histological evidence of the same morbid process was obtained. Since copper and vitamin A deficiencies can both interfere with myelination of foetal nerves (Owen, 1965) studies of the distribution of both copper and vitamin A in the body were made. So far as we know there is no relevant information in the literature on the levels of copper, vitamin A or cytochrome oxidase in normal goats and kids and consequently it is not possible to make a complete assessment of our results at the present time.

METHODS

Animals. A herd of eight female British Saanen goats maintained by the Biochemistry Department was used. The goats were two to five years old and in their first to fourth pregnancies. One five-year-old male castrate was also on the diet. At birth all kids were allowed to suckle their own dams and were thereafter bottle-fed

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from the combined milk of the dams on the diet. During pregnancy and for a period afterwards all dams were kept in individual pens.

Diet. When the dams were confined to their pens their diet consisted of hay and a concentrate mixture of natural foods. No mineral or vitamin supplements were given. In 1961 and 1962, from the beginning of April to the beginning of October, the goats were grazing a sward grown from a seed mixture of 8 lb. S 22 Italian rye-grass, 1 lb. Dorset Marl, broad-leaf red clover and 1 lb. Canadian Altaswede long-flowering red clover, this mixture being sown at 20 lb. per acre in alternate years on each of two adjacent but separately fenced three-quarter acre fields, one of which was ploughed every second year. This system of management minimizes the worm burdens carried by goats. Regular worm-egg counts on the faeces, by the McMaster flotation method, showed negligible infestation throughout.

In 1963 none of the goats was allowed to graze till the end of June. At all times other than those mentioned above the goats were confined to stalls and allowed to eat only the experimental diet. The concentrate mixture consisted of the following parts by weight : bruised oats, 6; bean meal (*Vicia faba*), 2; flaked maize, 1; decorticated groundnut meal (*Arachis hypogea*), 1. From its composition it can be assumed that the concentrate mixture was very poor in carotene and in other precursors of vitamin A. The hay was part of a large consignment used for another experiment (Hart, 1964). It was low in molybdenum and copper and was free from clover. Only one source of hay was used each year. In 1961 and 1962 the hay was made from timothy grass in the Carse of Stirling and in 1963 frome a rye-grass, cocksfoot, fescue mixture in Yorkshire. Hay is known to be an inadequate source of carotene (Owen, 1965) and becomes poorer in carotene as winter progress (Chanda, Clapham, McNaught and Owen, 1951). The analysis of the diet is given in Table 1.

Management. Outbreeding was practised. In 1963 each goat was weighed weekly throughout pregnancy and on the day after parturition. In 1962 blood samples were taken monthly during pregnancy and less frequently thereafter for estimation of copper and vitamin A by the method of Butler (1962).

In 1962 thirteen kids were born alive and one died in utero while being delivered surgically from a goat which had just died at the onset of parturition. In 1963 there were fifteen kids. The young were weighed and examined clinically each day till slaughter at 5 to 23 days of age. At the end of the 1963 season two adults which had given birth to affected kids were also killed and examined. At slaughter samples of tissues were taken for chemical analysis and pathological examination.

Histological methods. The tissues of the CNS were examined by the methods used by Barlow *et al.* (1960b) and Barlow (1963a). For histochemical demonstration of cytochrome oxidase Burstone's method as used previously by Barlow (1963b) was applied to standard blocks of the parietal region of the cerebrum, midbrain, cerebellum, medulla and spinal cord. A sample of cortex from the corresponding region of the opposite cerebral hemisphere was manometrically assayed for succinic dehydrogenase and for cytochrome oxidase (Schneider and Potter, 1943). Portions of the midbrain were taken for copper and iron analysis.

Chemical methods. Copper in the diet, blood and tissues was estimated spectrophotometrically with dithizone as described by Butler and Newman (1956, 1964), iron by the method of Trinder (1956) and molybdenum spectrophotometrically by the dithiol method of Bingley (1959), modified by Hart (1964). Sulphate was estimated spectrophotometrically by means of benzidine in a 0·01N hydrochloric extract of the diet (Dick and Bingley, personal communication). Total sulphur in the diet was measured by refluxing 10 to 20 grammes of food with fuming nitric acid followed by double oxidation of an aliquot in an apparatus similar to that of Revol and Ferrand (1935), but modified by Owen (unpublished) to include ball-joints above both condensers. Phosphate was removed (Owen, 1936) prior to the micro-estimation of the total sulphur as sulphate, using the benzidine method of Dodgson and Spencer (1953), but with washed celite (Johns-Manville & Co. Ltd., London) to bind the precipitate. Magnesium was determined spectrophotometrically as its Titan Yellow complex

using a modification of the procedure of Mason (1952). Calcium was isolated as the oxalate and determined in the flame photometer of Evans Electroselenium Ltd., which was also used for estimating potassium. Calcium was also independently determined by permanganate oxidation of its oxalate as described by Owen (1939) and Owen, Irving and Lyall (1939). Phosphorus was determined in ashed diet by two independent methods, Fiske and Subbarow (1925) and Martin and Doty (1949).

Vitamin A was estimated by the antimony trichloride reaction as described by Owen (1963).

RESULTS

Diet

The chemical analysis of the diet (Table 1) shows a low level of copper in the hay and considerably greater amounts in the concentrates. The ratio of calcium to phosphorus is between a third and a quarter for the concentrates, but is reversed in the hay so that the overall ratio falls within normal limits. The average intake of concentrates was 3 lb. per day: assuming an intake of 3 lb. of hay per day the total intake of calcium and phosphorus would be less than is usually supplied to milking animals.

Growth Rates

Comparison of the rate of increase in weight in 1963 with gains made in 1958 indicated that the diet was suboptimal in 1963. In both years the herd consisted of eight animals of average age three years and of comparable parity. The average weight per dam in 1958 was 100 lb. at the beginning and 144 lb. at term, when 14 kids of average weight 6·2 lb. were born. In 1963 the dams increased from 98 lb. at conception to only 123 lb. at term when 15 kids of average weight 6·1 lb. were born. The weights of the dams in 1963 were consistently lower during pregnancy than in 1958.

Clinical Results

In 1962 two kids (IIIa, IIIb) born to dam III were very slow to stand and suffered from neonatal headshaking. After a fortnight, during which they were hand-fed from bottles of their own dam's milk, they had recovered enough to be able to run awkwardly. Three of the goats (I, V and IX) died rather suddenly while receiving the diet. No. I died at parturition, and V about four weeks after parturition. No. IX was the adult male castrate.

Histochemistry and Pathology

In 1962 the cytochrome oxidase activity in five regions of the CNS of the live-born kids was assayed histochemically, the intensity of the reaction in cells and fibres being graded from trace amounts (+) to very intense activity (++++). Each set of five results so obtained was averaged to give the index which is recorded in Table 2. Cytochrome oxidase was also measured manometrically with results which are also in Table 2, which shows that, by either method, only the pair of ataxic twins were significantly different from the others. The reduction in activity of cyctochrome oxidase which occurred within cells and along the course of unmyelinated fibres in grey matter was undistinguishable from that occurring in swayback lambs (Figs. Ia and Ib).

TABLE 1

CHEMICAL COMPOSITION OF THE DIET

	Concentrate mixture				*Hay*				
*Sample date and No.**	*1962*		*1963*		*1962*	*1963*			
Food constituent in dry matter	*1*	*2*	*1*	*2*	*1*	*1*	*2*	*3*	*4*
Dry matter (%)	72·8	82·4	87·2	—	89·7	88·9	88·6	84·6	—
Copper (parts/million)	8·0	10·0	9·1		2·9	4·9	3·4	3·5	—
Molybdenum („)	—	—	—	0·4 – 0·6					0·2 – 0·3
Total sulphate (as % S)	—	0·05	0·08	—	0·21	0·07	0·18	0·22	—
Total sulphur (as % S)	—	—	—	0·13 – 0·16					0·50 – 0·58
Calcium (%)	0·14	0·13	0·19	0·13	—	0·49	0·73	0·54	0·45
Total phosphorus (%)	0·50	0·49	0·37	0·38	0·16	0·15	0·19	0·17	0·18
Magnesium (%)	0·27	0·18	0·14	—	0·07	0·13	0·13	0·12	—
Potassium (%)	1·09	0·84	0·64	—	1·99	1·54	1·96	1·43	—

Note. Analyses of the concentrate mixture 1963, 2, and the hay 1963, 4 were done at the Hannah Institute. All the other analyses were done at the Moredun Institute. At the former Institute calcium was estimated by permanganate, at the latter by flame photometer. At the former phosphorus was by Martin and Doty's (1949) method and at the latter by that of Fiske and Subbarow (1925).

*The numbers indicate samples taken at different times of the year.

TABLE 2

HISTOCHEMISTRY OF BRAIN, AND COPPER, IRON AND VITAMIN A IN THE TISSUES

Kids from dams on a diet poor in copper and carotene, and copper in the blood of the dam at parturition

					Brain of kids						*Blood of kids*		*Liver of kids*			*Kidney of kids*
1962	*No. of kid*	*Sex*	*Birth weight (lb.)*	*Age when killed (days)*	*Succinic* dehydrogenase*	*Cytochrome oxidase Manometric**	*Cytochrome oxidase Histochemical*	*Cu µg/g in dry wt.*	*Fe µg/g in dry wt.*	*Blood Cu of dam at term µg/100ml*	*Cu µg/100ml*	*Vitamin A i.u/100ml*	*Cu µg/g in dry wt.*	*Fe µg/g in dry wt.*	*Vitamin A i.u/g fresh*	*Vitamin A i.u/g fresh*
	Ia	M	7·5	0	—	—	—			14·0	—	—	4·3	2799	6	
	IIa	F	9·0	18	22	116	++++			34·0	37·1	376	8·5	63	28	
	IIIa	F	6·5	17	37	37	+			16·2	33·1	308	5·5	446	18	
	IIIb	F	6·5	17	26	42	+				49·8	246	8·5	1014	30	
	IVa	F	7·25	16	15	89	++			33·3	38·7	113	6·6	206	22	
	IVb	F	5·5	16	2	289	++++				44·4	97	8·2	139	24	
	Va	M	8·25	21	21	100	++++			51·5	64·4	71	9·4	222	10	
	Vb	M	8·00	21	19	99	++++				56·4	97	9·1	86	12	
	VIa	F	7·75	16	17	96	++++			47·1	54·7	153	7·8	506	16	
	VIb	M	9·0	16	10	70	++				39·1	105	6·9	492	12	
	VIIa	F	7·75	14	16	108	++++			66·7	78·9	107	8·9	102	21	
	VIIb	MF†	8·25	15	13	82	+++				71·5	53	6·8	353	26	
	VIIIa	F	7·25	13	14	68	+++			49·8	63·1	57	7·6	871	13	
	VIIIb	M	8·25	13	8	145	++++				68·4	71	10·4	114	16	
Average for 1962					16·7	103				31·9	53·8	143	7·8	528	18	
1963	IIα	M	7·75	8	16	131		12·4	65	45	31·6	—	6·3	91	19	7
	IIβ	M	7·75	8	15	163		12·2	56		33·6	—	5·5	88	15	7
	IIIα	F	6·75	6	21	209		4·4	—	28	25·8	—	4·8	676	20	7
	IIIβ	F	6·25	6	11	107		4·8	42		29·1	—	4·6	201	17	3
	IVα	F	5·75	23	22	162		10·9	—	46	51·6	312	6·8	—	17	4
	IVβ	F	5·25	23	26	193		—	73		58·4	220	7·7	81	18	4
	VIα	M	9·00	8	31	151		12·4	67	50	52·4	—	6·2	275	58	6
	VIβ	F	6·50	8	23	101		10·7	53		44·0	—	9·5	307	77	7
	VIIα	MF†	8·00	23	20	143		13·7	60	65	83·5	264	18·5	69	19	5
	VIIIα	F	4·75	22	44	210		6·9	55	—	13·3	311	4·3	1405	10	5
	VIIIβ	F	5·00	22	28	186		8·0	57		16·9	295	5·2	174	13	4
	Xα	M	6·75	16	14	123		10·4	87	58	50·2	—	8·1	255	14	8
	Xβ	F	6·25	16	13	154		11·8	65		58·9	—	10·5	338	8	7
	XIα	M	7·50	5	17	140		6·7	37	50	35·6	266	5·2	2446	14	4
	XIβ	F	6·50	5	21	143		11·4	57		46·2	352	8·6	164	9	5
Average for 1963					21	154		9·8	60	49	42·1	289	7·4	469	21·9	5·5
Overall average					19	130				39·9	44·0	194	7·6	499	20·0	

* Microlitres oxygen taken up per hour per mg dry weight.
† Hermaphrodite

No macroscopic lesions were found. Histological changes in the nervous system were present in 4 kids, the ataxic twins IIIa and IIIb of 1962 and two clinically unaffected animals (IVa 1962 series, and XIα in 1963).

Though the lesions observed in the kids were directly comparable with those in swayback lambs (Barlow, 1963a) there were no lesions of the forebrain and the lesions found elsewhere were relatively mild. Chromatolysis was seen amongst the cells of the red and vestibular nuclei and the ventral horns of the spinal cord of all four kids (Fig. 2), but neurone necrosis was infrequent and was seen only in kid IIIb which was the most severe clinical case (Fig. 3).

In ataxic twins, IIIa and IIIb, there was also thinning of the granular layer of some folia of the cerebellum, and occasional Purkinje cells were either necrotic and situated far out in the molecular layer, or were buried in the granular layer (Fig. 4). Frank demyelination of the long tracts of the spinal cord was observed only in kid IIIb (Fig. 5), and two of the remaining three animals showed sudanophilic droplets in and between the fibres. The lesions occurred in the dorsal part of the lateral funiculus and the ventral angle of the sulcomarginal funiculus. No fibre changes were seen in kid XIα.

No lesions of the CNS were found in adult goats, but both dam I, which died during parturition and its moribund surgically-delivered kid Ia (Tables 2 and 3) had skeletal lesions. Histological examination of the tibiae and costochondral junctions revealed osteomalacia in the mother and rickets in the foetus.

Thus on clinical and pathological grounds it is evident that only the two kids, IIIa and IIIb, fully satisfied the criteria of swayback, for the histological changes which were observed in kids IVa and XIα were not accompanied by manifestations of paresis.

Biochemical Results

Copper in tissues of adults. The biochemical data relating to the adult animals are set out in Table 3. Dam III which gave birth to the clinically affected twins had the lowest average blood copper level during pregnancy. The concentration of copper in the blood of dam IV, which produced a kid showing lesions in the CNS, was not significantly different from the concentrations in the blood of the other adults. There was a significant variation ($P<0{\cdot}001$) of blood copper from goat to goat. The concentrations of copper and iron in the liver of dam III were not markedly different from those found in goats which produced unaffected kids.

Vitamin A in colostrum and liver of adults. The values for vitamin A in colostrum (Table 3) may be compared with values obtained in 1964 from British Saanen goats on a complete diet with access to fresh grazing. These latter ranged from 900 to 3,000 i.u. vitamin A per 100 ml. (Owen, 1964, unpublished observations), whereas none of the colostra in Table 3 reached even the lower end of this range. The liver vitamin A values of adults were also low as compared with normal values obtained previously (Chanda and Owen, 1952).

Copper in tissues of kids. Table 2 shows the data obtained for the kids. The blood and liver copper values for the affected kids (IIIa and IIIb) were not significantly different from those for the unaffected kids. As might be expected there were positive correlations ($0{\cdot}01>P>0{\cdot}001$) between the copper content of

TABLE 3

BIOCHEMICAL STUDY OF ADULT GOATS

(Pregnant or lactating females and one male castrate)

	Copper (μg/100 ml) in blood of dam on date shown							*Vitamin A in mammary secretion (i.u. per 100 ml.)*				*Analysis of tissues post mortem*			
												Liver			*Brain*
No. of dam	*June 1961*	*Dec. 1961*	*Jan. 1962*	*Feb. 1962*	*Mar. 1962*	*At parturition 1962*	*Average for each goat*	*Pre-colostrum*	*Colostrum*	*Post-colostrum*	*Milk*	*Vitamin A i.u/g fresh wt.*	*Copper μg/g dry wt.*	*Iron μg/g dry wt.*	*Iron μg/g dry wt.*
I*	38	16	18	11	14	14	19	278	413			172	3·1	491	—
II	—	35	—	18	25	34	28	94	475	45	68				
III†§	21	—	14	10	15	16	15	216	625	57		77	4·6	374	99
IV	59	—	26	30	36	33	37	—	686	90					
V‡	—	—	71	81	74	52	70		440			170	—	—	
VI§	—	72	59	60	40	47	56	227	—			58	8·4	316	102
VII	—	102	69	47	61	67	69	237	—		78	97	—	—	—
VIII	68	—	72	49	45	50	57								
IX‡ (Male Control castrate)	—	17	—	11	7	14	12					100	1·7	728	—
Averages:	47	48	47	35	35	36		210	528	64	73	112	4·5	477	101

* Died at parturition. Kid stillborn (male). Both dam and kid had skeletal lesions.
† Gave birth to swayback twin female kids.
‡ Died while on diet.
§ Killed for histological study after 1963 kidding.

the blood of the kid and that of its mother at term ($r = +0{\cdot}702$) and also between the copper content of the blood of the kids and the concentrations of copper in their liver and brain ($r = +0{\cdot}726$ and $+0{\cdot}690$ respectively with $P<0{\cdot}01$ in each case).

The cytochrome oxidase activities for the two clinically affected cases were lower than in any of the other kids, but were not related to the copper status for the two correlation coefficients, liver Cu × brain cytochrome oxidase $= -0{\cdot}076$ and brain Cu × brain cytochrome oxidase $= +0{\cdot}332$, were not statistically significant.

Vitamin A in tissues of kids. High values of vitamin A were found in the blood of many of the kids and these were associated with the lower values of blood copper. Conversely the larger values of blood copper were associated with the smaller values of blood vitamin A so that there was both in 1962 and 1963 an inverse relationship between vitamin A and copper in the blood of the kids. For the whole series, omitting the aberrant values of kid VIIa, the coefficient of correlation was $r = -0{\cdot}622$ with $P<0{\cdot}01$.

DISCUSSION

There is no doubt that the histological lesions found in the central nervous system of at least two of the kids were characteristic of swayback in lambs, and so confirmed our earlier report of the occurrence of this disease in the goat. The cause of the disease was not established, but in view of current theories of its aetiology in lambs it is relevant to consider the possible importance of dietary and nutritional factors. Other factors should not, however, be excluded and the fact that all the cases of frank swayback which occurred in 1961 and 1962 came from the same dam and different sires indicates the possible involvement of a maternal factor. The field evidence of Barlow *et al.* (1960 a, b) and the lack of success of attempts to produce swayback by feeding sheep on a defined diet (Underwood, 1962) have led to the general belief that unidentified factors in addition to copper are aetiologically important. For example, Barlow *et al.* (1960 a, b) found an apparent association between the incidence of swayback and the aspect of the hill slopes on which the sheep over-wintered.

In lambs the only nutritional factor identified so far is a low copper status but its rôle in the aetiology of the disease is still obscure. Many attempts have been made to reproduce swayback by feeding ewes on experimental diets during pregnancy and with one exception they have been unsuccessful. Mills and Fell (1960) and Fell, Williams and Mills (1961) reported the occurrence of the disease in lambs whose mothers had received large amounts of sulphate or molybdate or both. Molybdate and sulphate can each inhibit the retention of copper (Dick, 1956). The significance of these experiments in relation to the aetiology of the disease is not clear and an attempted confirmation elsewhere was not successful (Butler and Barlow, 1963). The levels of molybdate and sulphate in the diet of the goats were not excessive and provided average intakes of about 1 mg. and 10 g. per day, respectively.

The average daily intake of copper was about 15 mg. and, assuming that the levels of copper normally present in the blood and tissues of sheep and goats are similar, was not sufficient to maintain the copper status of the dams at normal

levels. An intake of about 5 mg. per day appears to be adequate for this purpose in sheep. In the absence of any other data for goats, however, this interpretation must be regarded as merely tentative. It is important to note that there was no significant difference between the copper status of affected and healthy kids and that the values are not as low as those associated with swayback in lambs. For example, a mean liver value of 5·54 parts Cu per million parts of dry matter (SE = ±0·32) has been obtained for 25 swayback lambs belonging to the same age group as the kids (0 to 3 wks.) and this is significantly lower than the mean value of 7·59 p.p.m. (SE = ±0·51) obtained for the kids ($0{\cdot}01>P>0{\cdot}001$). The blood copper levels of ewes bearing affected lambs (Barlow *et al.*, 1960b) are also considerably lower than those of the adult goats, the mean values and standard errors being 12·8 ± 1·3 and 43·0 ± 3·9 μg/100 ml. respectively ($P<0{\cdot}001$).

Howell and Davison (1959) reported a reduction of cytochrome oxidase activity in the brains of swayback lambs and this finding has since been confirmed by Mills and Williams (1962) and Barlow (1963b). Any discussion of the significance of the activities of this enzyme found in the brains of the kids is restricted by the lack of normal data. The two kids which fully satisfy all the diagnostic criteria for swayback (IIIa and IIIb) showed lower activities than any of the other kids and these are within the range of 33 to 139 qO_2 which we have found so far in swayback lambs of the same age. The fact that sheep and goats are interfertile (Barlow *et al.*, 1962) provides further justification for this comparison. It would be unwise, however, to attach much importance to the comparison since the values for half the unaffected kids also fall within this range. There was no correlation between these values and the copper content of the brain or liver.

A new biochemical feature of the present work is the finding of an inverse relationship between the levels of copper in whole blood and vitamin A in the serum. The swayback kids of 1962 were among the group which showed abnormally high concentrations of vitamin A in the blood serum. It is a reasonable hypothesis that vitamin A, having found its way into the blood of the kid from its dam's milk, was inhibited from being stored in the liver when the blood contained insufficient copper, for the kids with the lowest copper levels in their blood had the highest vitamin A values and conversely. Supporting this hypothesis are the low levels of vitamin A in adult livers and colostrum and the experiments of Simek, Mandl, Travnicek and Syrinek (1961) and Moore, Constable, Day, Impey and Symands (1964). Simek *et al.* (1961) found that copper deficiency limited the power of the pig to store vitamin A in its liver and Moore *et al.* (1964) noted that one effect of feeding copper to rats previously deficient in it was to cause the appearance of fat droplets in the cytoplasm of the liver cells. The latter observation is significant because vitamin A is normally stored in the liver fat. There was no relationship between blood or liver copper values and liver vitamin A in the kids. This is not surprising since in the rat vitamin A is maintained at normal levels in the blood till the liver is nearly empty (Owen, 1965) so that there is no correlation at any stage of depletion between vitamin A in the blood and in the liver. Vitamin A is carried to the liver by blood lipoproteins and vitamin A in the blood is maintained by an equilibrium between it and liver vitamin A alcohol which is only a minor part of liver stores. Liver stores in the sheep are chiefly in the less labile form of vitamin A palmitate (Ganguly and Mahadevan, 1964).

Though copper deficiency and vitamin A deficiency have been studied separately in relation to myelin defects in different species (Mellanby, 1934; Irving and Richards, 1938; Owen, 1951, 1965) the present experiments appear to be the first in which both copper and vitamin A have been studied at the same time and in the same species of animal, in relation to the integrity of the CNS. The results may have a bearing on the conclusion of Allcroft and Lewis (1956, 1957) that factors additional to copper deficiency are necessary for the production of swayback as it occurs in lambs in the field. In this context the large amount of non-sulphate sulphur which was found in the hay deserves further study.

CONCLUSIONS

A small herd of stall-fed goats, from which a case of suspected swayback was reported in 1962, was maintained during two further successive pregnancies on the same type of diet which consisted of meal and hay and had a low content of carotene.

The diet failed to produce as great an increase in weight during pregnancy as is usually observed, and in 1962 two kids were born with symptoms of swayback. At necropsy characteristic lesions were found in the cells and tracts of the central nervous system (CNS) together with low activity of cytochrome oxidase. Two other kids showed lesions in the CNS but were clinically normal. Three adult goats died while on the diet.

The blood copper values of all the dams were low in comparison with normal sheep, but the former values and those for the copper content of the blood and liver of the kids were all higher than those associated with swayback in lambs. The levels of copper and vitamin A in the blood of the kids were inversely related, and vitamin A levels in the livers of four dams and one male castrate were low. The possibility is discussed that vitamin A metabolism may be deranged in animals throwing swayback young.

The copper content of the brain and liver of the kids was proportional to the content of copper in their blood. Copper in the blood of the kid was also proportional to the copper in the dam's blood at term.

The results confirm the author's earlier report that "swayback" occurs in the young of the goat.

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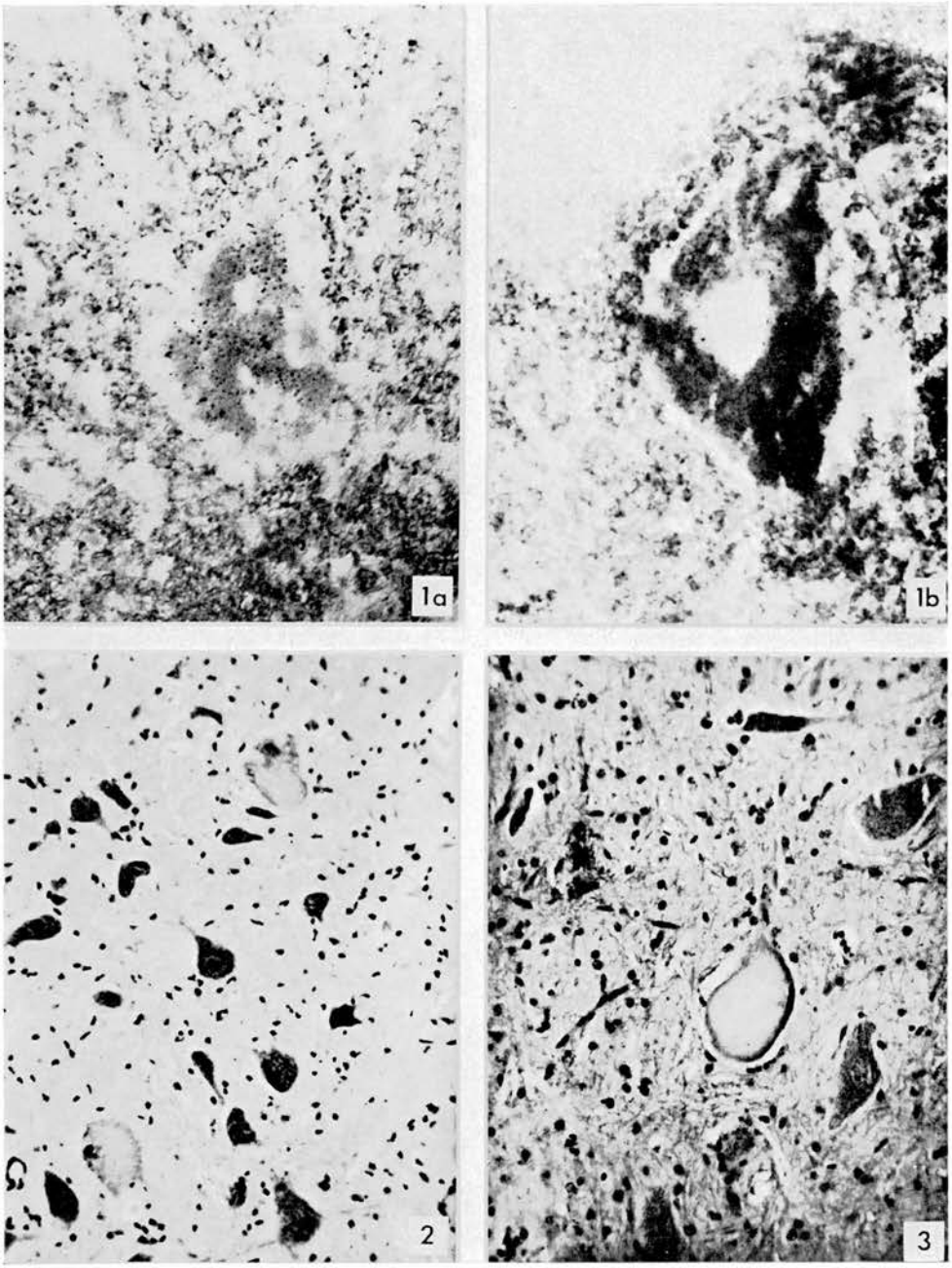

Fig. 1a. Cytochrome oxidase activity in the red nucleus of ataxic kid. (Burstone x 500).
Fig. 1b. Cytochrome oxidase activity in the red nucleus of unaffected kid. (Burstone x 500).
Fig. 2. Chromatolysis in the red nucleus of the brain of ataxic kid. (Neutral red, Luxol fast blue x 150).
Fig. 3. Necrosis of a cell of the ventral horn of spinal cord of axatic kid IIIb. (Hæmatoxylin and Eosin x 150).

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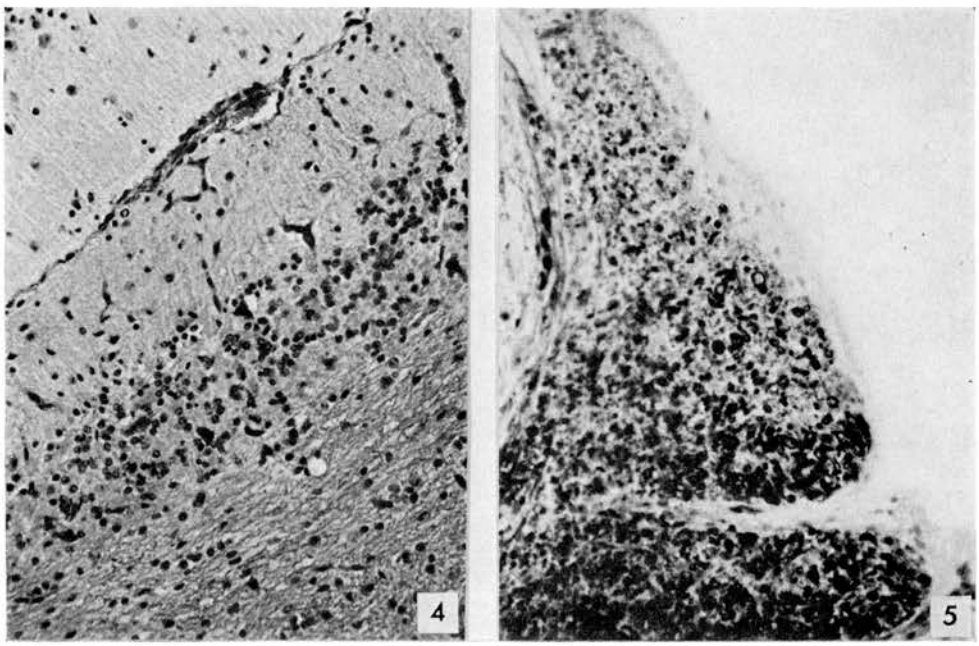

Fig. 4. Cerebellum of attaxic kid IIIa (or b) showing thinning of the granular layer and heterotopia of Purkinje cells. (Hæmatoxylin and Eosin x 100).
Fig. 5. Thinning of myelin in the dorsal part of lateral funiculus of the spinal cord of ataxic kid. IIIb. (Smith Quigley x 150).

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Dietary Production of Congenital Copper Deficiency in Swine [1]

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ABSTRACT A method for the production of copper deficiency in the adult sow and newborn pig is described. Attempts to produce neonatal deficiency in the pig by the depletion of normal maternal copper reserves through repeated breeding with a copper-deficient ration were unrewarding. It was necessary to raise gilts from birth to maturity in a copper-depleted state before copper deficiency was observed in their offspring. Brain and liver copper levels as low as 2.3 ± 1.5 and 2.4 ± 0.9 μg/g dry weight, respectively, and serum copper levels of 9 ± 3 μg/100 ml were achieved in the newborn deficient animals. These animals were asymptomatic and lacked gross lesions but manifested a slight reduction in tensile strength of the aorta and a rapid development of anemia in early postnatal life.

Naturally occurring copper deficiency associated with lesions in the nervous or cardiovascular systems has been reported in lambs and kids with swayback (1, 2), in cattle with falling disease (3), in pigs with paresis (4–6) and in the red deer with ataxia (7). The effects of deficiency are manifested in utero in the lamb and goat, while in cattle, the pig and red deer they first appear in maturing or mature animals. In addition, extensive and detailed studies have been made of experimentally induced copper deficiency in several species. From these experiments there have resulted descriptions of anemia in rats (8) and swine (8–11), skeletal changes in dogs (12) and swine (13) and cardiovascular lesions in swine (14), chicks (15–17) and rabbits (18). All of these studies were made on animals rendered deficient after birth; relatively few studies have been made of the production of copper deficiency in the fetus. Copper deficiency has been produced in the newborn rat and has resulted in decreased viability, anemia, edema, hemorrhages and abdominal hernias (19). Attempts to produce swayback experimentally have been indirect and have met with equivocal results (20, 21). Recent studies with guinea pigs have indicated that congenital copper deficiency may be associated with alterations in the nervous system.[2,3] Our studies of the effect of copper deficiency on the nervous system of the lamb (1, 22–24) and on the cardiovascular system of swine (14) have led us to a closer examination of the role of this metal in the fetus. The present study records our observations on the dietary production of maternal and fetal copper deficiency in swine.

MATERIALS AND METHODS

The animals selected for this study were a breed of miniature pig (25). Sows were of the Pitman-Moore strain [4] and the boars were of the Hanford strain.[5]

The first experiment was designed to induce copper deficiency in the adult sow by a combination of low copper diet and drain on copper stores from repeated gestations. Two sows were started with the experimental diet in the last half of pregnancy. Alterations were made in the diet at various periods until an acceptable, low copper-containing mixture was achieved, which would sustain pregnancy. These al-

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[2] Tsai, M. D., G. J. Everson and R. Shrader 1964 Copper deficiency in the guinea pig. Federation Proc., 23: 133 (abstract).
[3] Everson, G. J., and Tong-In Wang 1967 Copper deficiency in the guinea pig and related brain abnormalities. Federation Proc., 26: 633 (abstract).
[4] Vita-Vet Labs, Marion, Indiana.
[5] We thank Dr. L. Bustad and Dr. H. Ragan of Richland, Washington, for these animals.

TABLE 1

Manipulation of daily diet and duration of feeding during sequential pregnancies of sows (exp. 1)

	Days fed diet [1]						
	58	12	44	58	108	39	53
Milk undiluted, ml [2]	1620	2430	1620	1600	1600	1600	1600
Total solids, g	421	632	421	416	416	416	416
Glucose monohydrate, g [3]	—	—	—	200	200	200	200
Sucrose, g	300	125	150	—	—	—	—
Sulphide water, ml [4]	ad lib.	ad lib.	ad lib.	ad lib.	ad lib.	1600	2400
Cellulose, g [5]	400	600	400	—	—	—	—
Nonnutritive fiber, g [6]	—	—	—	250	200	100	100
Vitamin supplement, g [7]	18.6	7.8	9.3	2	2	1	1
NaCl, g	6	10	6	6	6	2	4
Calcium glycerophosphate, g [8]	—	30	—	20	—	—	—
Magnesium sulphate, g [9]	2	8	2	2	3	—	—
Iron supplement, ml [10]	1	1	1	1	1	5	5
Mineral supplement, ml [11]	4	4	4	4	4	5	5
Copper supplement, ml [12,13]	10	10	10	10	10	5	5

[1] Time starts in middle of first pregnancy.
[2] Carnation Evaporated Milk, Carnation Company, Los Angeles; 100 ml contained: (in grams) protein, 7.4; fat, 8.4; carbohydrates, 10.5; minerals, 1.6; and moisture, 78.1.
[3] Cerelose, Corn Products Company, New York.
[4] Ten milliliters of a 0.36% solution of sodium sulphide in 39 liters of tap water; allowed to stand for 24 hours.
[5] Alphacel, Nutritional Biochemicals Corporation, Cleveland.
[6] General Biochemicals Inc., Chagrin Falls, Ohio.
[7] Vitamin diet fortified, vitamin D omitted; 1 kg supplied (in grams): vitamin A conc, 4.5; α-tocopherol, 5; ascorbic acid, 45; inositol, 5; choline chloride, 75; menadione, 2.25; p-aminobenzoic acid, 5; niacin, 4.5; riboflavin, 1; pyridoxine·HCl, 1; thiamine·HCl, 1; Ca pantothenate, 3; (in mg) biotin, 20; folic acid, 90; and vitamin B_{12}, 1.35; obtained from Nutritional Biochemicals Corporation.
[8] Nutritional Biochemicals Corporation.
[9] Mallinckrodt Chemical Works, St. Louis.
[10] Carbonyl iron powder, Antara Products, General Aniline and Film Corporation, New York; 72 mg/ml, 72 g powder added slowly to 330 ml reagent grade concentrated HCl. Deionized water added to dissolve the salt and make volume up to 1 liter.
[11] Reagent grade salts in deionized distilled water: (in grams/liter) manganese chloride, 1.8; aluminum sulfate, 0.6; sodium fluoride, 3.1; zinc sulfate, 1.8; cobalt nitrate, 1.8; and nickel acetate, 2.8.
[12] Two grams of copper sulphate/liter of deionized distilled water.
[13] Control sow only.

terations are summarized in table 1. The animals were housed in specially constructed stainless steel pens with slotted oak floors coated with an epoxy resin. Stainless steel equipment was used to prepare and feed the diet. The sows were rebred after each gestation. Two litters were obtained from the control sow and 3 litters from the experimental sow on this regimen. The newborn animals were bled for serum copper determinations and then slaughtered for liver and brain copper analyses.

The second experiment was designed to assure a copper-poor state in the sow by rearing the animals from birth with a copper-deficient diet and maintaining them with the diet through each gestation. The animals were weaned to a standard milk diet at 4 days of age and placed in individual pens constructed of galvanized iron fitted with stainless steel feed trays. The diet consisted of equal parts of canned evaporated milk and sulphide water fed at the rate of 230 ml/kg per pig a day supplemented from day 7 with iron and minerals, prepared as in table 1, in the amount of 0.5 ml and 0.2 ml/kg per pig a day, respectively, to a maximum of 5 ml/day. Control animals received the copper supplement at a rate of 0.5 mg/kg per pig a day to a maximum of 2.5 mg. Two control sows, two deficient sows and a control sow transferred to a deficient diet at 30 days of age formed the experimental group. The animals were weighed weekly, at which time blood samples were taken for serum copper analyses and determination of the volume of packed red cells (VPRC). The deficient animals received a 2.5 mg copper supplement when the VPRC approached 30% or the serum copper level was below 40 μg/100 ml. After each animal reached 10 kg in weight, additions to the basic milk-sulphide water mixture fed daily were in increments of 120 ml/kg per pig. The

TABLE 2

Daily diet for sows (exp. 2)

	6 months to 1 year of age	Last 6 weeks of first gestation	Maintenance diet [1]
Milk undiluted, ml [2]	2400	2400	1600
(Milk, total solids, g)	(624)	(624)	(416)
Sulphide water, ml [2]	2400	3000	1600
Glucose monohydrate, g [2]	400	450	400
Nonnutritive fiber, g [2]	50	50	25
NaCl, g	2	2	3
Vitamin supplement, g [2]	2	2	4
Iron supplement, ml [2]	5	5	5
Mineral supplement, ml [2]	5	5	5
Copper supplement, ml [2,3]	5	5	5

[1] Restricted diet supplies 1 mg copper/day; supplemented diet supplies 3.5 mg copper/day.
[2] Prepared or as described in table 1.
[3] Control sows only.

TABLE 3

Copper content of blood and tissues of newborn pigs from successive litters of sows fed the control or deficient diet (exp. 1)

	Litter no.	Serum copper	Brain copper	Liver copper
		μg/100 ml	*μg/g dry wt*	*μg/g dry wt*
Control	1	164 [1] ± 13 [2] (7) [3]	18 ±0 (2)	146 ± 14(2)
	2	55 ±15 (6)	14.8 ± 1.3(4)	160 ± 39(4)
Deficient	1	132 [1] ± 7 (5)	19 ±1 (2)	196 ± 19(2)
	2	83 ±2 (5)	17 ±0 (2)	168 ± 22(2)
	3	40 ±8 (9)	17.2 ± 2.1(7)	100 ± 21(7)

[1] Whole blood copper.
[2] Mean deviation (arithmetic average of all the differences between the observations and their mean).
[3] Numbers in parentheses indicate number of animals examined.

diet was judged adequate by a steady weight gain and any leveling off of weight was balanced by a dietary increase. Vitamin supplements, nonnutritive fiber, sodium chloride and glucose monohydrate [6] were added from 4 months of age in successive months until the diet in table 2 was achieved at 6 months of age. When the animals were near maturity they were transferred to the larger, specially constructed pens described in the first experiment. By this method the animals were raised to sexual maturity in a copper-depleted state and subsequently bred.

Two litters were obtained from each of the copper-deficient animals and one litter was obtained from each of the control sows and the control sow transferred to a deficient diet at 30 days of age. Newborn animals were bled for serum copper and VPRC determinations and then killed for liver and brain copper analyses. Three piglets from the second litter of a copper-deficient sow were weaned onto the standard milk-sulphide water diet at 4 days of age and killed at intervals up to 25 days of age for tissue copper determinations. Complete autopsies were performed. Segments of descending thoracic aorta and loops of skin from the hind limb just proximal to the foot were obtained for tensile strength measurements (26–28). The samples for copper analyses were examined with a Perkin-Elmer model 303 atomic absorption spectrometer.[7]

RESULTS

In the first experiment, both the control and the experimental animals were maintained in good condition. The newborn young were generally active and, except for an occasional runt, were of normal weight. There was a reduction in the liver copper content and serum copper of the third litter of the depleted sow but apart from this, the brain, serum and liver copper values were unchanged (table 3).

[6] Cerelose, Corn Products Company, New York.
[7] Unpublished data, N. Weissman and B. J. Lythgoe.

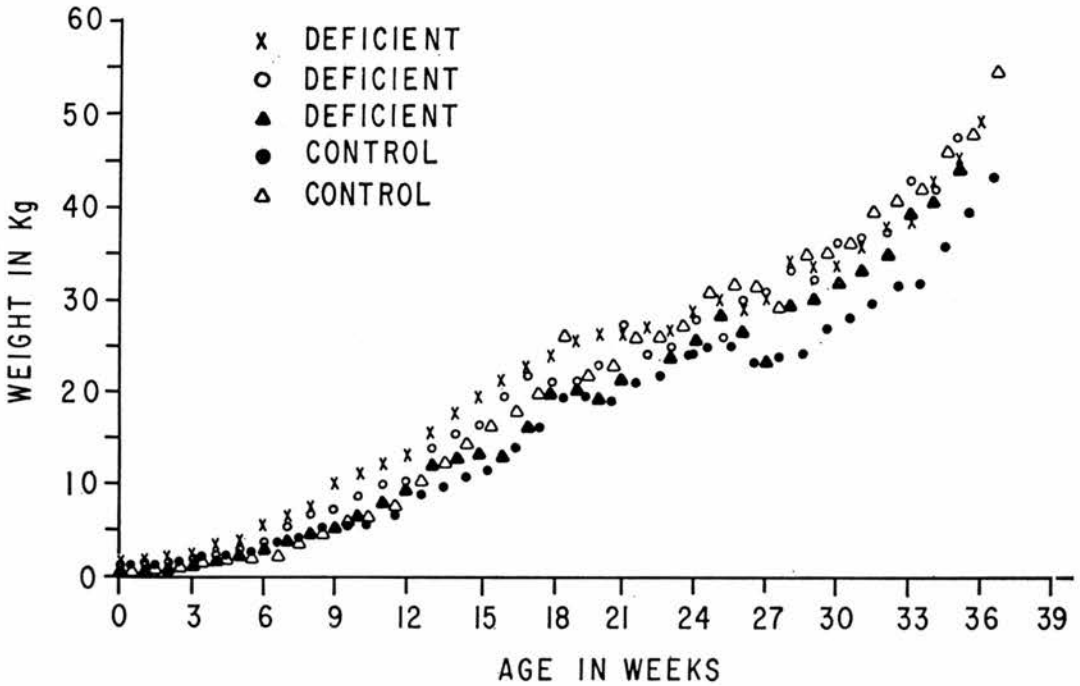

Fig. 1 Growth of animals in experiment 2. The animal indicated by X was a control transferred to a deficient diet at 30 days of age.

In the second experiment, the gilts of both the copper-supplemented and copper-restricted groups showed similar weight gains during the first 36 weeks of life (fig. 1). The details of the serum copper determinations of each of the animals from this same period are shown in figure 2. The decline in serum copper in the two deficient animals began at 4 weeks of age and reached the extremely low values of 6 and 9 μg/100 ml by 8 weeks of age. The copper-supplemented animal that was reversed to a deficient diet at one month of age reached similar low serum copper levels at almost the same time. Eight supplements of copper were administered to the animals fed the deficient diet from the ninth to thirty-fourth week of age. The animals have been followed for an additional year with the diet and no further copper supplementation has been required to maintain a serum copper level in the range of 10 to 30 μg/100 ml in the deficient animals and 20 to 50 μg/100 ml in the control sow transferred to the deficient diet at 30 days of age. The VPRC has been between 35 and 45 in all the animals.

Each gilt farrowed its first litter between 12 and 13 months of age. The newborn animals were of normal weight, generally active, and did not appear abnormal. The copper content of the brain, liver and serum of the piglets derived from the copper-restricted sows was significantly reduced compared with that of the offspring of the supplemented animals (table 4) and all the newborn of experiment 1.

Newborn animals maintained with a deficient diet for up to 25 days developed severe anemia associated with severe copper deficiency (table 5). No consistent alteration was noted in the tensile strength of the aorta and skin although the lowest values were found in the deficient animals (table 6).

DISCUSSION

Milk or milk products are the base in many of the diets used in the production of experimental copper deficiency (8–11,

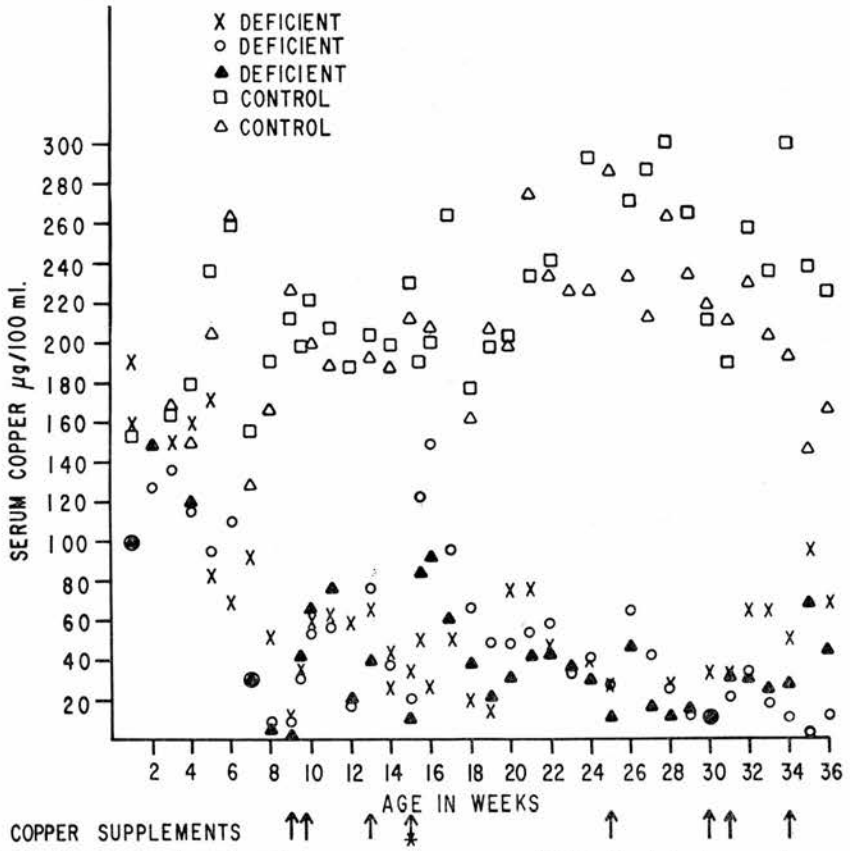

Fig. 2 Development and maintenance of a copper-deficient state in growing animals (exp. 2). The copper supplements were 2.5 mg except for one supplement of 7.5 mg. The animal indicated by X was a control transferred to a deficient diet at 30 days of age.

TABLE 4

Copper content of blood and tissues of newborn pigs from successive litters of sows fed the control or deficient diet from 4 days of age (exp. 2)

	Litter no.	Serum copper	Brain copper	Liver copper
		µg/100 ml	*µg/g dry wt*	*µg/g dry wt*
Control	1	43.3±5 [1] (6) [2]	15.3 ±1 (4)	117 ±18 (4)
Deficient A [3]	1	24.5±8.5 (4)	5.4 ±1.2(5)	4.4± 1.3(5)
Deficient B	1	—	7.5 ±0.4(5)	4.8± 0.6(5)
	2	11.3±2.4 (5)	7.5 ±0.6(6)	5.9± 1.8(5)
Deficient C	1	19 ±5.7 (6)	4.0 ±0.8(6)	3.3± 1.3(6)
	2	9 ±3 (2)	2.35±1.5(2)	2.4± 0.9(2)

[1] Mean deviation (arithmetic average of all the differences between observations and their mean).
[2] Numbers in parentheses indicate number of animals examined.
[3] Control sow transferred to a deficient diet at 30 days of age.

13–19). Although these diets are low in copper they are effective mainly in young animals when the demands of growth for copper together with the low dietary supply combine to deplete the available tissue reserves. This is well-demonstrated by our experiments in which significant neonatal copper deficiency was produced only in the litters of sows reared from birth with a copper-deficient diet. Despite the stress of 3 gestations it was not possible to deplete the copper stores of a commercially bred

TABLE 5

Terminal copper content and volume of packed red cells (VRPC) in piglets maintained beyond birth

Age at killing	VPRC	Serum copper	Brain copper	Liver copper
days [1]	%	*μg/100 ml*	*μg/g dry wt*	*μg/g dry wt*
14	20	3.2	2.8	2.9
19	10	19.4	2.9	1.75
25	10	19.8	1.8	1.4

[1] One animal at each age.

TABLE 6

Tensile strength of aorta and skin of newborn piglets

Group	No. of animals	Aorta	Skin
		kg/cm²	*kg/cm²*
Control	4	22.5 ± 7.6	50.2 ± 14.0
Deficient A1	7	13.9 ± 6.1	49.4 ± 12.0
B1	6	18.0 ± 3.9	35.9 ± 5.4
C1	6	12.2 ± 6.2	48.2 ± 14.8

adult sow fed the milk diet. In our second experiment the deprivation of the dietary copper from birth in the breeding stock has been shown to be effective in producing congenital copper deficiency. Such congenital deficiency has been asymptomatic and without gross cardiovascular or central nervous system lesions though deficient pigs reared for a short time developed severe anemia. This suggests that the fetal copper status was sufficient to sustain development but not the rapid growth of early extra-uterine life.

The anemia ascribed to copper deficiency is generally associated with a severe depletion of copper reserves and serum copper levels near 20 μg/100 ml (10, 11). The sows raised from birth in a copper-deprived state and their offspring were near this level. However, anemia was not observed in our animals. That such a condition was imminent is indicated by the rapid development of progressive anemia in the newborn animals maintained beyond birth.

Studies on the mechanical properties of the aorta of copper-deficient and control swine before and after elastin isolation and digestion have demonstrated a reduction of the tensile strength of the aorta from deficient animals (26–28). This abnormality has been attributed to altered elastin. In our experiments the values for the tensile strength of the aorta in the newborn deficient animals were generally lower than those in the controls. Although these data suggested the presence of altered elastin the values obtained were not low enough to be statistically significant. It is possible that the degree of copper deficiency was insufficient to interfere with elastogenesis although similar serum copper levels are usually associated with severe cardiovascular lesions in older swine (14). The tensile strength of the skin of most of the copper-deficient animals was within the normal range although in one group of deficient animals the tensile strength was low. The significance of this last observation will have to await further investigation. In previous studies no differences have been noted in the tensile strength of skin from older copper-deficient and control animals (28).

In both experiments all females had equal breeding opportunities but the copper-deprived stock in each experiment produced more litters than the control stock over the same period. In such small groups this difference may be coincidental, but considered in the light of the deleterious reproductive effects of copper deficiency in the rat (29) this result was unexpected.

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19.

EXPERIMENTAL COPPER DEFICIENCY IN MINIATURE SWINE

Biochemistry, Histochemistry and Pathology of the Central Nervous System

By

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INTRODUCTION

Studies on swayback have established a relationship between copper deficiency and disease of the nervous system in sheep (Bennetts and Chapman, 1937; Innes and Shearer, 1940; Howell and Davison, 1959; Barlow, Purves, Butler and Macintyre, 1960; Mills and Fell, 1960). Experimental studies (Everson, Tsai and Wang, 1967; O'Dell, 1968; Carlton and Kelly, 1968; Cancilla, Barlow, Weissman, Coulson and Carnes, 1967) and field investigations (Joyce, 1955; McGavin, Ranby and Tammemagi, 1962; Wilkie, 1959; Barlow, Butler and Purves, 1964; Terlecki, Done and Clegg, 1964; Marston, 1952; Owen, Proudfoot, Robertson, Barlow, Butler and Smith, 1965) have indicated that copper may be important for the development and maintenance of the nervous system in a variety of species. Direct experiment has been hampered, especially in ruminants, by the difficulty of establishing controlled copper deficiency. By careful dietary management (Cancilla *et al.*, 1967), copper deficiency has been produced in the sow and newborn piglet and has been maintained for up to three years through periods of rapid growth and reproductive activity. The results of these studies are presented here.

MATERIALS AND METHODS

Minipigs (miniature swine, Bustad, 1966) were fed a low copper diet and housed as previously described in detail (Cancilla *et al.*, 1967). The diet consisted of canned evaporated milk and sulphide water with added carbohydrate, minerals and vitamins. Control animals received a daily copper supplement. The pigs were housed in galvanised iron cages or stainless steel pens with slatted oak floors covered with an epoxy resin.

The effect of copper restriction was examined in sows which had completed up to three pregnancies, in their newborn piglets and in rapidly growing pigs. Two sows were reared from birth and one sow was reared from 30 days of age on the low copper diet. Two additional sows were reared on the diet supplemented with copper to a total of 3·5 mg./day. All were bred when sexually mature and produced up to three litters. The ages of the sows at killing are indicated in Table 1. The newborn

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piglets were killed at birth or were placed on the experimental diet and killed at intervals up to 104 days after birth. Another group of 18 pigs were reared from birth on the copper restricted or supplemented diet and they were killed at intervals up to one year of age (Table 2). All animals were weighed and bled regularly. The volume of packed red cells and serum copper assays were done to monitor the copper status of the animals. Complete autopsies were carried out and terminal blood samples and portions of liver and brain were assayed for copper by atomic absorption spectrophotometry (Cancilla *et al.*, 1967).

Blocks of cerebrum, brain stem, cerebellum and spinal cord were placed in cold formol calcium for 24 hours, washed and frozen sections cut for localization of acid phosphatase and thiamine pyrophosphatase as previously described (Barlow and Cancilla, 1966). Adjacent blocks were rapidly frozen on dry ice and used for histochemical localization of cytochrome oxidase (Pearse, 1961) and succinic dehydrogenase (Nachlas, Tsou, de Sousa, Cheng and Seligman, 1957). The remainder of the central nervous system (C.N.S.) was fixed in neutral formalin and representative blocks of cerebrum, cerebellum, brain stem and spinal cord were taken for paraffin and frozen sections. For electron microscopy, segmental perfusions of the spinal cord with buffered glutaraldehyde were carried out as previously described (Cancilla and Barlow, 1966). One micron sections from Epon embedded material were stained with cresyl violet for orientation by light microscopy after which ultrathin sections from selected areas were cut and stained with lead citrate or uranyl acetate. The specimens were examined and photographed with an R.C.A. EMU 3H or Siemens Elmiskop 1A electron microscope.

RESULTS

The biochemical data of the adult and rapidly growing animals are given in Tables 1 and 2 respectively. In addition, a total of six litters were produced by the copper deprived sows from which 25 piglets were examined at birth. In all these newborn animals tissue copper levels were much lower than those of newborn piglets from copper supplemented sows. The values in parts per million dry weight (p.p.m.) for individual litters varied from $2{\cdot}35 \pm 1{\cdot}5$ to $7{\cdot}5 \pm 0{\cdot}6$ for brain (control $15{\cdot}3 \pm 1$) and from $2{\cdot}4 \pm 0{\cdot}9$ to $5{\cdot}9 \pm 0{\cdot}6$ for liver (control 117 ± 18). Piglets reared on the deficient diet showed a further depletion in tissue copper at 25 days of age when copper levels of 1·8 p.p.m. in brain and 1·4 p.p.m. in liver

TABLE 1

TERMINAL COPPER CONTENT AND VOLUME OF PACKED RED CELLS (VPRC) IN ADULT SOWS

Diet	*No. of litters produced*	*Age at killing days*	*vprc %*	*Serum copper µg./100 ml.*	*Brain copper µg./g. dry wt.*	*Liver copper µg./g. dry wt.*
control	1	916	38	262	——	19·5
control	1	1139	44	278	26·5	22
deficient	1	797	40	32·5	5·5	2·7
deficient	2	1136	54	20·3	5·8	7·1
deficient	3	1138	50	27·7	7·2	6·0

were achieved. The piglet maintained on the diet for 104 days without supplementation was the only animal of the entire series to show evidence of neurological disease and this was subclinical. Terminal copper values in this animal were 6·8 and 3·5 p.p.m. for brain and liver respectively.

TABLE 2

TERMINAL COPPER CONTENT AND VOLUME OF PACKED RED CELLS (VPRC)

Animals reared from birth to one year of age

Diet	*Age at killing days*	*vprc %*	*Serum copper µg./100 ml.*	*Brain copper µg./g. dry wt.*	*Liver copper µg./g. dry wt.*
control	7	29	172	15·9*	147·1*
control	15	28	131	16·7*	77·5*
control	71	46	148	18·1*	21·2*
control	359	—	317	—	19·5
control	359	48	301	14	16·4
control	360	51	143	19·9	13·9
deficient	7	34·5	167	16·8*	206·8*
deficient	17	45	160	17·5	23·8*
deficient	28	34	52	—	10·3*
deficient	56	40	7·5	9·6*	2·6*
deficient	60	17	5·8	8·3*	2·1*
deficient	71	26	2·3	8·5*	3·7*
deficient	71	17·5	4·1	7·1*	2·4*
deficient	100	29·5	19·2	6·1	3·8
deficient	128	27	5·7	2·2	5·0
deficient	239	43	12	2	5
deficient	269	34	9·4	1·1	5·6
deficient	358	47	19·2	4·4	6·4

* Wet weight data converted to dry weight data (brain 17·7% solids/g. w.w. mean of 82 samples and liver 27·3% solids/g. w.w. mean of 286 samples).

Histological examination of the C.N.S. revealed a normal distribution of Nissl granules in the red nucleus, brain stem nuclei and ventral horn cells and the distribution of acid phosphatase and thiamine pyrophosphatase was normal. Succinic dehydrogenase activity was similar in both control and deficient animals, but by 71 days of age a moderate diffuse reduction in cytochrome oxidase activity was evident in the copper deficient piglets. This reduction in activity was more apparent in the neurophil than in the nerve cell bodies.

Examination of the spinal cord of the congenitally deficient piglet slaughtered at 104 days revealed a symmetrical pallor of myelin in the ventral and lateral columns (Figs. 1 and 2). Electron microscopy of these areas showed many intact myelinated fibres as well as fibres with alterations in the axon or myelin sheath. The axonal changes consisted of an increase in mitochondria and dense bodies or loss of the normal axolemmal outline with contraction of the axon and granular dissolution of its contents (Fig. 3). Vesicular profiles or portions of altered mitochondria were discernible in some of this granular material. The myelin sheath became distorted as it collapsed about the shrunken axon (Fig. 3). There was phagocytosis of degenerated axons and myelin by macrophages.

DISCUSSION

Copper deficiency (3 to 12 p.p.m. dry weight liver) has been implicated in several reports of spontaneous ataxia in pigs aged two weeks to several months (Joyce, 1955; Wilkie, 1959; McGavin *et al.*, 1962), but experimental reproduction of the condition does not appear to have been described. The association

of copper deficiency and swayback in lambs is well documented and in this disorder brain copper levels of 3 p.p.m. are not unusual (Mills and Williams, 1962).

In the present study, brain and liver copper levels of a similar order to those seen in these naturally occurring conditions were achieved during intrauterine development, in periods of rapid growth and during reproductive life and yet in only one animal was a significant alteration observed in the nervous system. Although it is not a specific alteration, the presence of this lesion suggests that the pig, or more particularly the minipig, may be susceptible to a process resembling that seen in swayback (Cancilla and Barlow, 1968). The infrequency with which such a lesion was encountered would indicate either that the minipig is remarkably resistant to this disorder or that other unknown factors may also be necessary for its production. In sheep, factors such as climatic variation (Butler and Barlow, 1963), dietary levels of molybdenum and sulphate (Mills and Fell, 1960) and genetic factors (Wiener, 1966; Wiener and Field, 1966) affect the availability and utilization of copper, and hence the incidence of swayback, but it appears from this experiment that factors independent of copper may also have a conditioning role. Despite the occurrence of widespread spinal tract degeneration, neuronal changes in the cells of origin of these fibres were not recognized, indicating either a total loss of affected neurons or sustained viability in the presence of severe axonal degeneration, a situation akin to the "dying-back" phenomenon (Cavanagh, 1964).

SUMMARY

Congenital and postnatal copper deficiencies have been produced in miniature swine. A subclinical myelopathy was found in a single animal at the age of 104 days. The ultrastructure of the lesion, although non-specific, resembled that of swayback in lambs. The possible pathogenesis of the disorder in this species and the factors influencing the overall incidence of disease in these experiments are briefly discussed.

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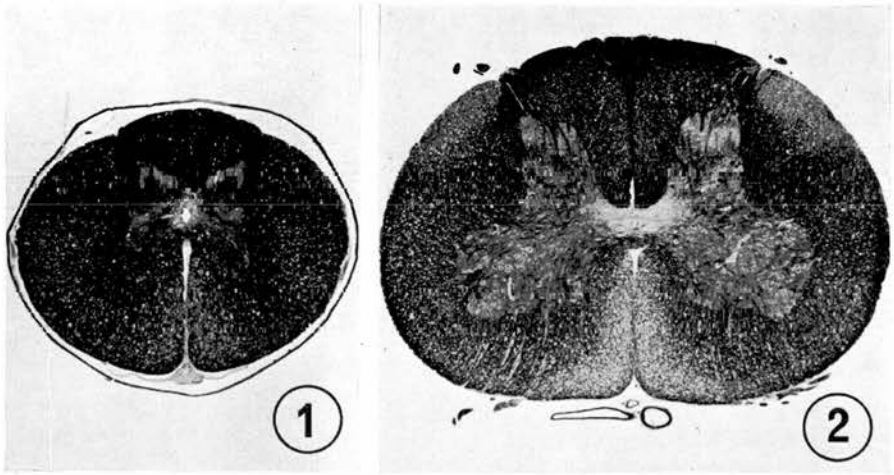

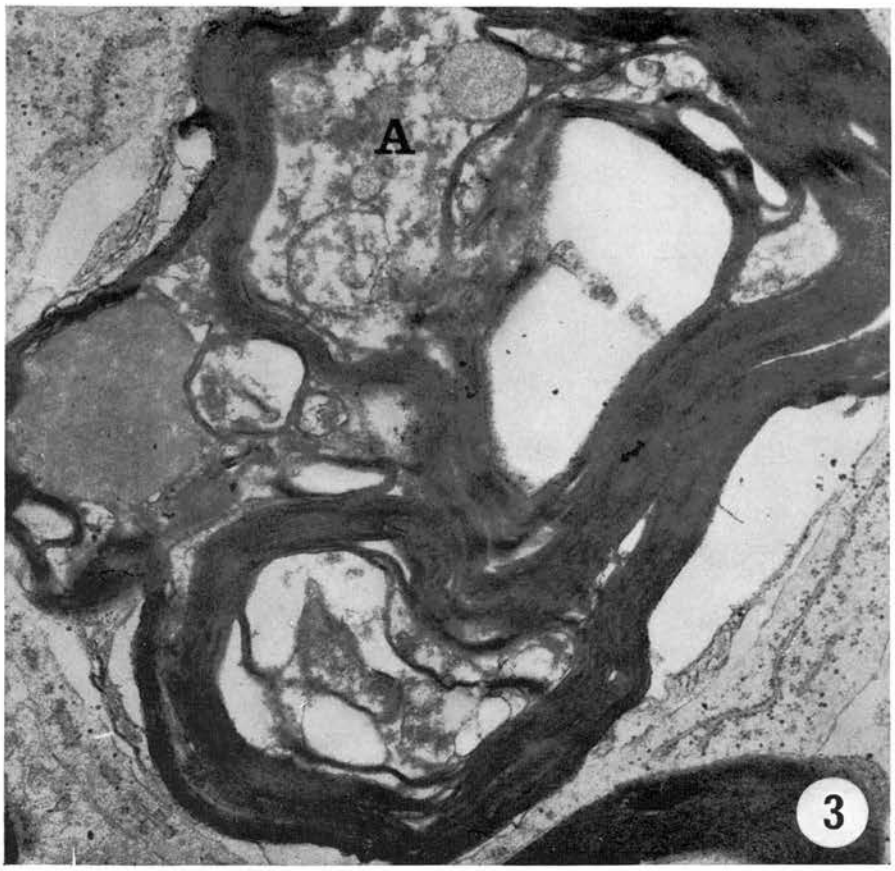

Figs. 1 and 2. Transverse sections of the spinal cord demonstrating demyelination of the ventral and lateral columns. Weigert's myelin stain × 5.

Fig. 3. Granular and vesicular degeneration of the axon (A) with loss of the axolemma. The myelin membranes are distorted as they have collapsed about the axon. Lead citrate × 22,500.

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20.

ON THE PATHOLOGY AND HISTOCHEMISTRY OF THE CENTRAL NERVOUS SYSTEM IN BORDER DISEASE OF SHEEP

BY

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On the Pathology and Histochemistry of the Central Nervous System in Border Disease of Sheep

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SUMMARY. A basis for the pathological confirmation of clinical Border disease in sheep up to 9 months of age has been shown. Three diagnostic criteria have been tentatively put forward:

(i) Abnormally pigmented, hairy birthcoats in normally smooth-coated breeds.
(ii) A flock history of poor growth and viability of lambs with no overt cause and/or a proportion with congenital rhythmic clonic spasm.
(iii) Hypomyelinogenesis and clusters of swollen interstitial glia in lambs under 6 months old.

The lesions are thought to resolve with time, so that diagnosis in older lambs becomes progressively more difficult. Swollen glial cells alone are non-specific but may have diagnostic value if other diseases can be excluded.

Some histochemical findings are discussed and the condition is compared with swayback.

THE EXISTENCE in lambs of abnormally pigmented, hairy birthcoats associated with poor growth and viability, and sometimes also with trembling and locomotor defects, has been described by Hughes, Kershaw and Shaw (1959). It was suggested that these abnormalities were due to a specific condition for which the name 'B' or Border disease was proposed. The condition occurred in the border counties of England and Wales, amongst Clun Forest and Kerry Hill sheep. The only pathological change found was hypomyelinogenesis and this was inconsistent, being recognized only in those animals with nervous symptoms, which are in a minority in most outbreaks. Similar neurological signs and pathology have been described in animals of several breeds and crosses under the title of 'Hypomyelinogenesis congenita' by Markson, Terlecki, Shand, Sellars and Woods (1959), but there is no mention of the fleece characteristics in these cases. More recently, Barr (1964) has reported a similar disease in various breeds and crosses. He mentions that, with the exception of a pair of Suffolk cross twins, no birthcoat changes were evident in the animals showing nervous signs, but his material was almost entirely from breeds which are normally not smooth coated.

The type of birthcoat, in sheep generally, has been shown to be under a considerable degree of genetic control (Schinckel, 1955) and there is wide variation in birthcoat between breeds. Therefore, the appearance of a hairy-coated lamb, if considered in isolation, is no criterion for the presence of Border disease. Likewise, poor viability and impaired growth can result from a number of causes. Thus, in the absence of nervous symptoms, clinical diagnosis of the disease is unsatisfactory, but if poor growth and viability are found in lambs which were hairy at birth in breeds normally having smooth birthcoats, Border disease may be strongly suspected.

The purpose of the present communication is to report the neuropathological findings in sheep which were suspected of Border disease on these grounds. They indicate that substantial improvement in diagnosis is possible.

MATERIALS AND METHODS

The work was based on 53 sheep, 23 of which showed the congenital, rhythmic, clonic spasms which are characteristic and have caused them to be known as 'shakers'. The other 30 were heterogeneous; some were suspected of Border disease on their fleece characteristics and inability to thrive, whilst others were included because of a family connection with clear-cut cases, or contact with them, though they themselves had never shown overt signs. In addition, control material for specific histochemical tests was obtained from 4 lambs with no known association with the disease. The sheep were from several sources and breeds and varied in age from 1 day to 3 years.

The birthcoats were classified for hairiness and abnormal pigmentation by several observers according to the source of the stock, with the result that somewhat different criteria were used, but the detailed differences between the various schemes for describing the extent of abnormal hairiness are unlikely to be of much significance in the present work. Dr. F. W. Dry or his associates classified a proportion of the lambs on a scale I (smooth coat) to VII (extreme hairiness) (Dry, 1955), whereas in the other cases an approximate equivalent to Dry's grades is used here for ease of presentation. The data for individual sheep are summarized in Table I. For the breeds concerned here hairiness equivalent to Grades V, VI and VII is attributable to Border disease, but for reasons which will emerge less hairy grades cannot necessarily be regarded as free of the condition in these breeds.

The animals were fully examined *post-mortem* and the entire CNS removed, being used for histology either in the fresh state immediately or following fixation in 10% formol-saline, 1% $CaCl_2$ in 10% formalin, or formol-ammonium bromide. H. & E. stained paraffin sections from coronal slices of the frontal and occipital lobes of the cerebrum; transverse slices of midbrain at the level of the red nucleus, medulla and cerebellum at the level of the middle cerebellar peduncle; and transverse and longitudinal slices of spinal cord at the level of the upper cervical and midthoracic segments and the cervical and lumbar enlargements were prepared, adjacent blocks being retained for frozen sections. Other methods included Luxol Fast Blue/Neutral Red, Smith-Quigley, osmium tetroxide-α-naphthylamine (OTAN) with and without alkaline hydrolysis (Adams, 1959; Adams and Bayliss, 1963), Sudan IV, Holmes' (1947) neurofibril method, Cajal's gold sublimate and Tsujiyama's (1961) method for oligodendroglia. Cytochrome oxidase was demonstrated immediately after death on fresh frozen sections from comparable regions using Burstone's method (Pearse, 1960a) as applied previously (Barlow, 1963b). In a limited number of cases Gomori-type lead nitrate reactions were used as cytological stains by the demonstration of sites of acid phosphatase activity (Pearse, 1960b) and also thiamine-pyrophosphatase activity, as a Golgi marker, was demonstrated by the method of Novikoff and Goldfischer (1961). Cholesterol and cholesterol esters were localized by a perchloric acid-naphthoquinone method (PAN) (Adams, 1961). In 8 lambs, succinic dehydrogenase and cytochrome oxidase were assayed manometrically (Schneider and Potter, 1943).

Initially all the regions described were examined by each method, though in later cases effort was concentrated on the occipital and cerebellar white matter and longitudinal sections of spinal cord. The multiplicity of methods for myelin and myelin lipids was necessary in view of the incompletely reliable nature of any single method in detecting minor changes in the myelin sheath.

RESULTS

No consistent abnormalities were found at the general *post-mortem* examination and microscopic neuropathological changes formed the most significant lesions. The changes were confined to the white matter and concerned the myelin sheaths and/or certain associated neuroglial cells, no damage to nerve cells or axons being demonstrated by the histological or cytological (phosphatase) methods.

From Table I it can be seen that no animals of fleece Grades I and II showed lesions despite breeding or contact relationships with the remainder. Forty-four sheep were classed as Grade III or hairier and 31 of these showed myelin and neuroglial changes: the 31 include

all the shakers except one. In the other 13 no myelin defects were detected, but 10 of them had neuroglial changes. The remaining shaker was the only one of these 10 under 6 months of age. Thus lesions of the nervous system were found in 41/44 sheep.

TABLE I
CLINICAL AND PATHOLOGICAL DATA OF 53 ANIMALS

Age in months	No. of animal	Age (months)	Breed	Sex	Fleece*	Shaker	Myelin lesions	Neuroglia
	1	0·5	Kerry ×	F	VII	+	+	+
	2	0·5	,, ×	F	,,	+	+	+
	3	0·75	,, ×	F	,,	+	+	+
	4	0·5	Jacob's	M	(VI–VII)	+	+	+
	5	0·5	Romney ×	F	,,	+	+	+
	6	<0·25	Suffolk	F	,,	+	+	+
Below 1	7	0·25	Clun × Suffolk	M	,,	−	+	+
	8	<0·25	Suffolk	M	,,	−	+	+
	9	<0·25	,,	F	,,	+	+	+
	10	<0·25	Clun	F	,,	+	+	+
	11	<0·25	Suffolk	F	,,	+	+	+
	12	0·25	Clun	F	(II–III)	−	−	−
	13	still-born	Suffolk	M	,,	?	−	−
	14	2	Kerry ×	F	(VI–VII)	+	+	+
	15	2	Suffolk ×	F	,,	+	+	+
	16	2·5	,, ×	F	,,	+	+	+
1–3	17	2·5	,,	F	,,	−	+	+
	18	2·5	Kerry ×	F	VII	+	+	+
	19	2·5	Clun	F	(VI–VII)	−	+	+
	20	2·5	Oxford	M	,,	+	−	+
	21	1·5	Kerry × Clun	M	,,	+	+	+
	22	3·0	Kerry ×	M	VII	+	+	+
	23	4·0	,,	F	,,	−	+	+
	24	3·75	Clun × Suffolk	M	(VI–VII)	−	+	+
	25	3·0	Kerry ×	M	VII	+	+	+
	26	4·0	Clun	M	(VI–VII)	−	+	+
3–6	27	5·0	Kerry	F	,,	+	+	+
	28	5·0	,,	F	,,	+	+	+
	29	4·5	Clun	M	,,	−	+	+
	30	4·5	Suffolk ×	M	,,	+	+	+
	31	5·0	Kerry	F	VII	−	+	+
	32	8·0	Kerry	F	III	−	−	+
	33	6·0	Clun	M	(VI–VII)	+	+	+
	34	6·0	,,	M	,,	+	+	+
	35	6·0	Kerry	F	I	−	−	−
6–9	36	6·0	,,	F	VII	−	−	+
	37	7·0	,,	F	I	−	−	−
	38	8·0	,,	F	VII	−	−	+
	39	8·0	,,	F	II	−	−	−
	40	8·0	,,	F	VII	−	−	+

TABLE I—*continued.*

Age in months	No. of animal	Age (months)	Breed	Sex	Fleece*	Shaker	Myelin lesions	Neuroglia
	41	9·0	Kerry	F	VII	—	—	+
	42	9·5	,,	F	,,	—	—	—
	43	9·5	,,	F	V	—	—	—
	44	10·0	,,	F	,,	—	—	+
9–12	45	11·0	,,	F	VII	+	+	+
	46	10·0	,,	M	III	—	—	+
	47	10·0	,,	M	,,	—	—	+
	48	9·0	,,	M	II	—	—	—
	49	9·0	,,	M	,,	—	—	—
	50	15	Kerry	M	IV	—	—	—
Over 12	51	15	,,	F	II	—	—	—
	52	36	,,	F	I	—	—	—
	53	36	,,	F	VII	—	—	+

* Fleece grade range I–VII; gradings according to the method of Dry (1955). Grades shown in brackets are for animals not originally scored by Dry's method but given here, for convenience, as approximations to Dry's classes.

The variation of the pathological findings with age for the 44 sheep is given in Table II. It can be seen that up to 6 months of age all showed neuroglial changes and 28/29 also showed myelin defects. In animals more than 6 months of age myelin changes were only found in 3/15, whilst in 3/9 animals over 9 months of age no neuro-pathological changes were detected.

TABLE II

VARIATON OF MYELIN AND CELL CHANGES WITH AGE IN 44 LAMBS WITH FLEECE GRADE III OR HAIRIER

Age (Months)	Number of sheep	Myelin changes	Neuroglial changes	No changes
0–1	11	11	11	0
1–3	8	7	8	0
3–6	10	10	10	0
6–9	6	2	6	0
9–12	7	1	5	2
over 12	2	0	1	1

In those cases in which lesions occurred the entire CNS appeared to be susceptible though the lesions themselves were patchily diffuse and most readily observed in sections where numbers of fibres were cut longitudinally. The white matter contained abnormally numerous interstitial glia and affected nerve sheaths were twisted and showed irregular swellings with

decreased affinity for myelin stains (Figs. 1 and 2). The most severely affected sheaths contained no stainable myelin whilst in others discrete 'beads' of stained material were present, often situated in small lateral 'pouches' of the sheath (Fig. 3). In the dorsal funiculi of the cord where nerve fibres are small and more uniform in diameter the dilatation of the sheaths was most readily appreciated.

In shaker lambs up to about a month old considerable quantities of sudanophil and osmiophil lipid were found in the form of globules or crystals. This material occurred both within the fibres where it was associated with dilatations of the sheaths or lying free in the interstituum and perivascular spaces (Fig. 4). In hairy-coated non-shakers of the same age the myelin defects were less extensive and irregularly associated with a much smaller amount of sudanophil lipid. Slightly older hairy lambs (6–10 weeks of age), irrespective of whether they were shakers or not, never showed appreciable amounts of such lipids. At no time was a significant gitter cell reaction observed in relation to the lesions. Hydrolysed OTAN-stained sections, however, did show material with the tinctorial qualities of sphingomyelin within the cytoplasm of perivascular macrophages in quantities in excess of that found in the normal control lambs. In animals up to 11 weeks of age the PAN method also showed large globules of a cholesterol-like substance in the white matter (Fig. 5) in contrast to known normals in the same age groups (Fig. 6). Histochemically the distribution of cytochrome oxidase activity in grey matter showed no abnormalities and manometric assays gave results of the same order as have been encountered in the cerebral cortex of normal lambs (Howell and Davison, 1959; Barlow, 1963b). Slight cytochrome oxidase activity was, however, demonstrated by Burstone's method in the cerebellar white matter and the long tracts of the spinal cord of hairy lambs 1 to 3 months of age. This activity appeared to be associated with both the interstitial glia and the swollen sheaths (Fig. 7). In normal lambs activity in these areas has not been demonstrated later than about 5 weeks of age (Fig. 8).

In cases where the extent and degree of the myelin defect was small and difficult to determine using the Smith-Quigley method, these histochemical tests run in parallel with normal control tissue were of considerable help in reaching a decision. Cases in which an element of doubt remained were recorded as negative. In general, these difficulties were directly proportional to the age of the lamb.

The neuroglial changes also were most readily observed in sections where groups of fibres had been cut longitudinally, especially in the occipital white matter, cerebellum and in longitudinal sections of spinal cord. The abnormal cells occurred singly or in groups of up to 6 in between the fibres or as loose clusters near small vessels. They had apparently little cytoplasm and watery vesicular nuclei about the size of an astrocyte nucleus. The nuclei were ellipsoidal or irregular in shape and contained small amounts of chromatin but no nucleolus (Figs. 9 and 10). Such cells were observed in all the age groups examined but were most numerous in sheep under 6 months of age in which dividing forms were seen. In older animals not only was the frequency of these abnormal cells less but they were smaller and progressively more difficult to distinguish from normal neuroglial elements. Their origin and nature were not determined. From the morphology, position and frequency with which rod cells appear in their vicinity (Fig. 9), it would seem most likely that the abnormal neuroglia are derived from microglia. However, they have apparently little cytoplasm and few processes (Fig. 11) and gitter cells were not seen in association with them, so that the possibility of oligodendroglial origins cannot be dismissed. The two types of cells may be impossible to distinguish by staining reactions in pathological states (Tsujiyama, 1961).

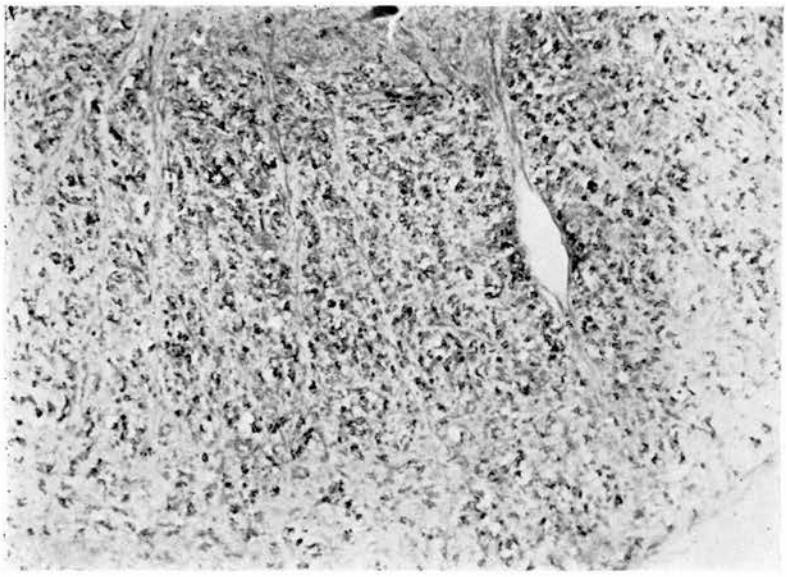

FIG. 1. Transverse section of spinal chord of shaker lamb showing the myelin defect in a portion of the ventral columns (Smith-Quigley, × 110, frozen section).

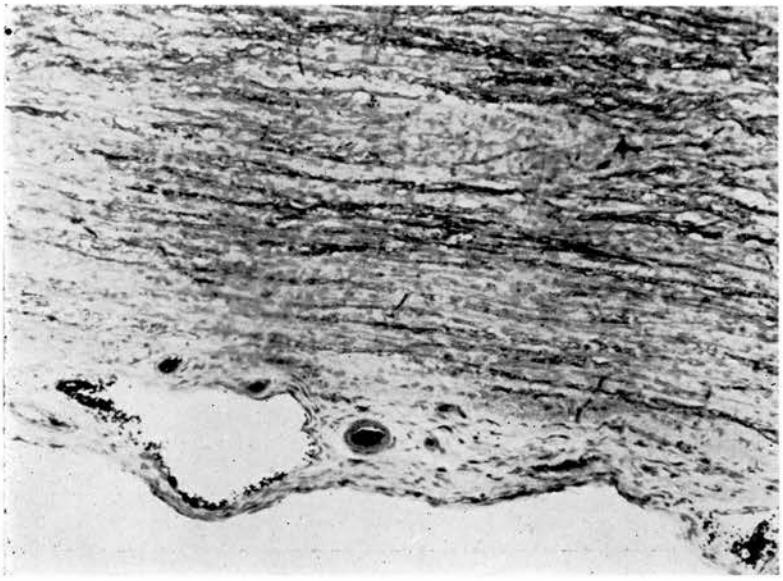

FIG. 2. Longitudinal section of the spinal cord of a shaker lamb showing the myelin defect in a portion of the ventral columns (Smith-Quigley, × 110, frozen section).

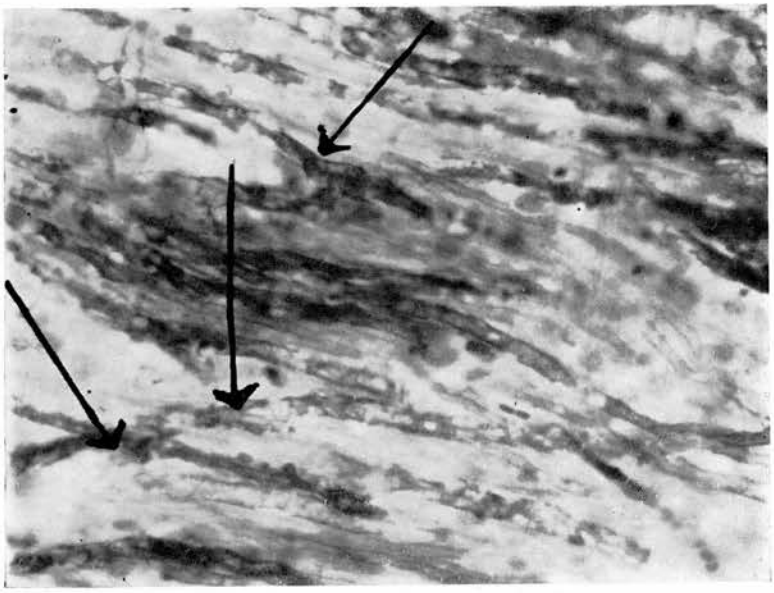

FIG. 3. Higher magnification of a portion of the same field as in Fig. 2, showing beading of myelin, dilation of the sheaths and 'pouchings' (arrowed) (Smith-Quigley, × 440, frozen section).

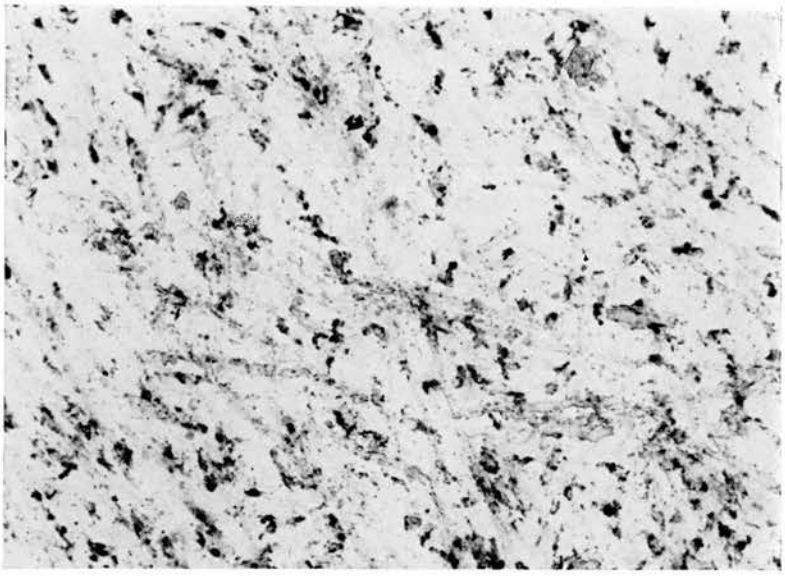

FIG. 4. Sudanophilic lipid in the spinal cord of a shaker lamb (Sudan IV, ×165, frozen section).

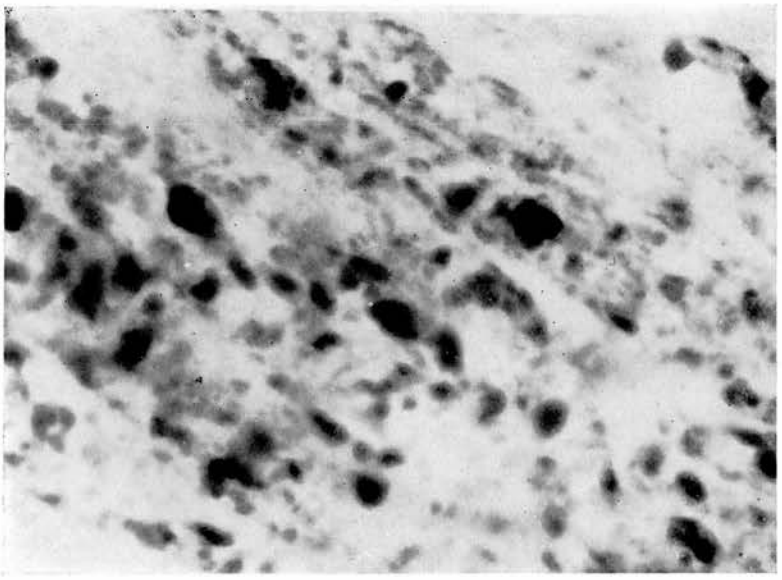

FIG. 5. Globules of cholesterol-like material in the myelin sheaths of longitudinal section of spinal cord of a shaker lamb (PAN, ×840, frozen section).

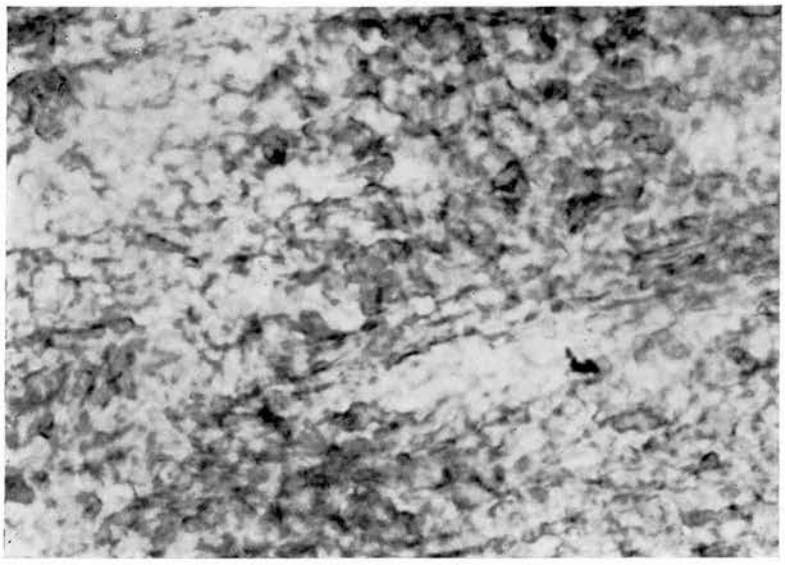

FIG. 6. A preparation similar to Fig. 5 from a normal lamb for comparison (PAN, ×840, frozen section).

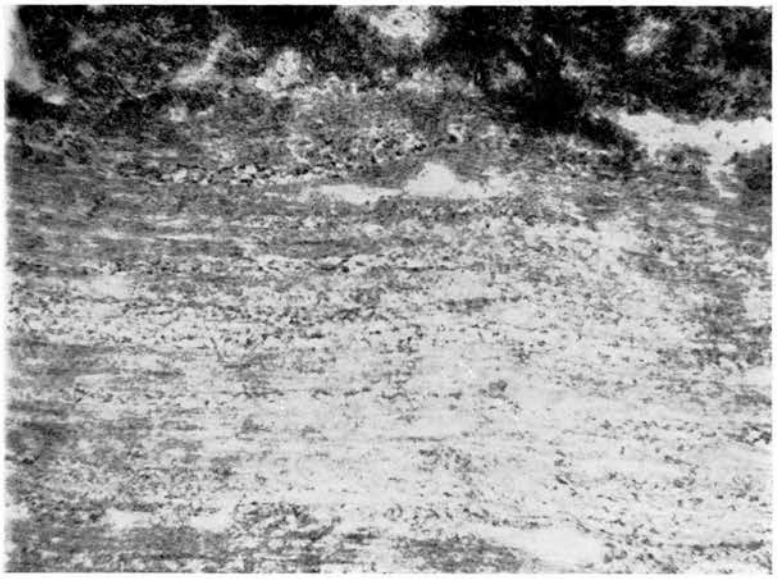

FIG. 7. Portion of a longitudinal section of spinal cord of a 9-week-old shaker lamb showing cytochrome oxidase activity in white matter (Burstone, ×120, fresh-frozen section).

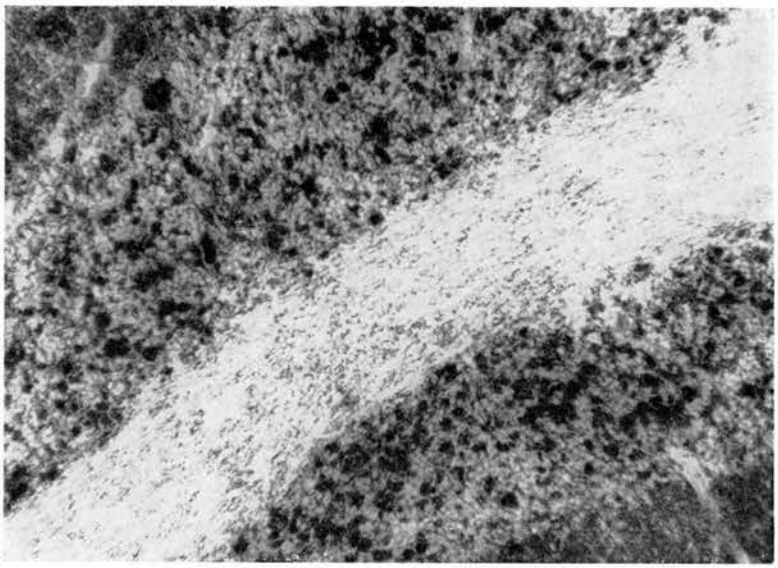

FIG. 8. Cerebellum of the same lamb as in Fig. 7, showing cytochrome oxidase activity in the white matter of a single folium (Burstone, ×135, fresh-frozen section).

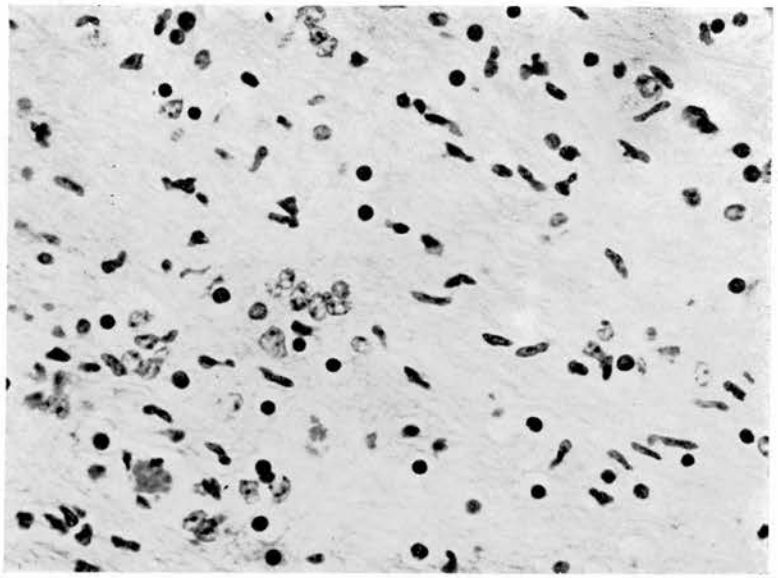

FIG. 9. Clusters of abnormal neuroglial cells in occipital lobe of cerebrum of a case of Border disease. Note the large numbers of stab cells in the vicinity (H. & E., ×440).

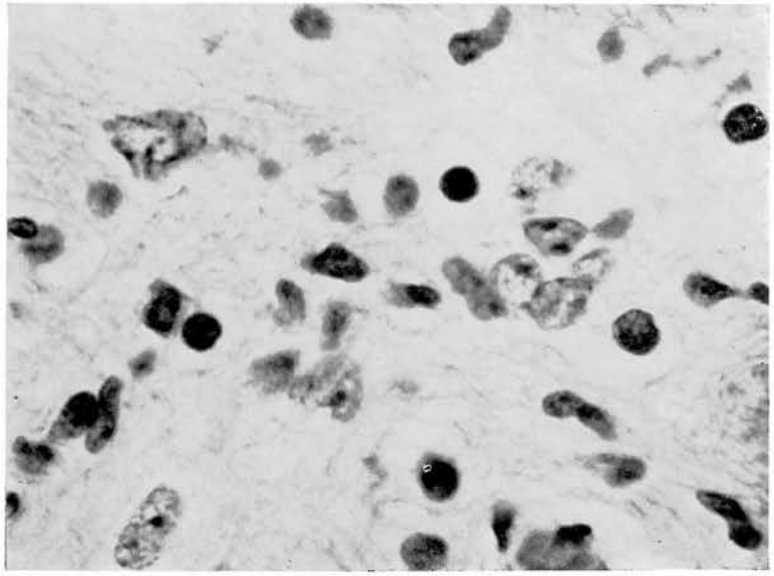

FIG. 10. Higher power view of a cluster of abnormal neuroglia in a portion of the same field as Fig. 9 (H. & E., ×1,200).

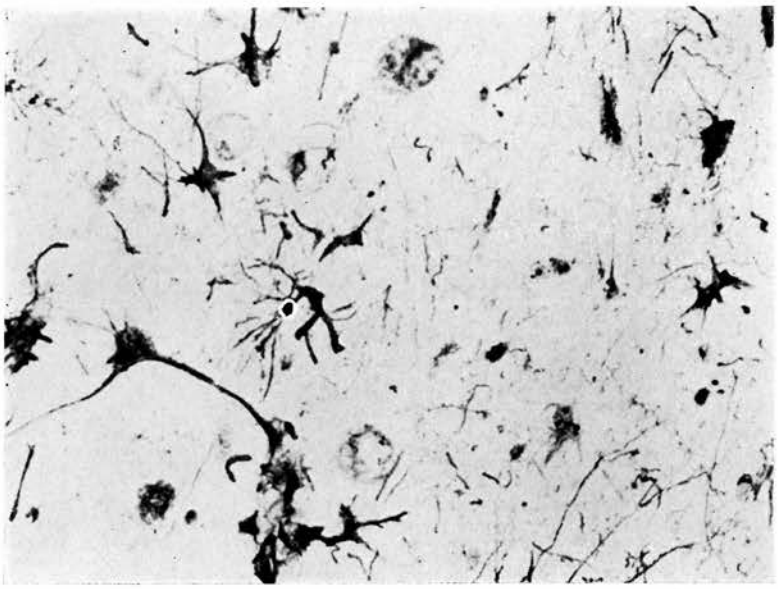

FIG. 11. Abnormal neuroglial cells in white matter of occipital lobe of a shaker lamb impregnated with silver (Tsujiyama, ×440, frozen section).

DISCUSSION

In sheep up to 9 months of age which had abnormally hairy fleeces for the breeds concerned, and which were considered on clinical grounds to be affected with Border disease, some degree of neuropathological change has been found consistently. The changes were present irrespective of clinical neurological disturbance but the myelin and neuroglial changes were generally most clear in animals less than 6 months of age especially in those lambs with rhythmic clonic spasm which have been called shakers. In those over 6 months of age the myelin defect was less regularly demonstrated and over 9 months of age the frequency with which neuroglial changes could be positively identified also decreased. However, the occurrence of both myelin and neuroglial changes in 28/29 such hairy sheep under 6 months of age and of neuroglial changes in all of 34 similar sheep up to 9 months of age strongly suggests that these changes are closely associated with Border disease. The absence of either myelin or glial changes in 9 sheep from birth to 3 years old whose fleeces were in the two least hairy grades, and from the 4 completely unconnected animals used for histochemical control, provides additional support. Four animals, of which the youngest was over 8 months old, were of intermediate fleece grade (III and IV). Of these, 3 showed neuroglial changes, but they would not have been suspected of Border disease solely on fleece criteria.

On the above grounds, a basis has been reached for the pathological confirmation of a diagnosis of Border disease in sheep up to 6 months old, but this has raised certain questions which require discussion but cannot be finally resolved here. The pathological examination has resulted in 3 older animals (Table I, numbers 32, 40 and 47) being suspected of Border disease which would not have been anticipated on the basis of their early fleece characteristics. It has become clear that there is no complete correlation between the degree of hairiness, the presence of neurological signs and the severity of the pathological lesions: shakers can display a variety of fleece types and non-shakers can have hairier fleeces than shakers. Therefore, it does not seem unreasonable that the disease could be present in a mild form without being manifest in the fleece. The occurrence of both myelin and neuroglial changes in 9 animals which did not shake has a bearing on the same issue as it indicates that shaking is not the result of lesions *per se*, but may be related to their extent or particular localization within the nervous system.

In hairy sheep more than 6 months of age, in which myelin defects were less regularly found, the diagnostic validity of a neuropathological examination is more doubtful. The swollen interstitial glial cells found in the majority of older sheep with fleeces of Grade III or hairier, but not in those of Grades I and II cannot be regarded as specific for Border disease. Similar cells have been observed in louping ill, swayback and certain forms of blindness in sheep. Although the present work contains only 6 animals which serve as controls for animals up to 6 months of age (Table I no. 12 and no. 13, plus the 4 lambs for specific histochemical tests), over 50 normal lambs of this age group and of several breeds have been examined in other investigations together with more than 250 lambs dying of natural causes without lesions being recognized in the central nervous system, and swollen neuroglia have not been seen in significant numbers in any of them (Barlow, unpublished). It is thus suggested that these cells are abnormal and may represent a response to a variety of stimuli.

With these considerations in mind it is possible to advance tentative diagnostic criteria for Border disease:

(i) Abnormally pigmented, hairy birthcoat or fleece in normally smooth-coated breeds.

(ii) A flock history of poor growth and viability of the lambs with no overt cause and/or a proportion of lambs showing rhythmic clonic spasms.

(iii) The occurrence in lambs under 6 months of age of myelin defects and clusters of swollen, interstitial glia. Over 6 months of age the presence of many abnormal glial cells may be also of some value in diagnosis in the absence of myelin defects, provided that other disease processes can be excluded.

That routine diagnosis may not be as simple as the third criterion implies is indicated by the failure to demonstrate myelin changes in one shaker lamb under 3 months of age. This could be explained by the patchy nature of the lesions which might have been missed in sampling for histology and also by some variability of the technical methods employed.

The variation in pathological changes with age shown in Table II suggests that the lesions in the nervous system may resolve with time and there is no evidence of irreversible damage or scar formation which would preclude this possibility. Such a view is supported by obser vations on a small number of severe shakers maintained indoors on *ad lib.* feeding for periods up to 18 months in which the gradual remission of signs was accompanied by progressively less severe pathological changes (Barlow, unpublished). However, it is possible that the older cases in this series only survived because they were less seriously affected initially. This second possibility also implies that the degrees of viability, hairiness and nervous tissue damage *at birth* are not closely correlated, but the data are insufficient for further analysis in this respect.

The histochemical findings with respect to fat, cholesterol, sphingomyelin and cytochrome oxidase activity in affected lambs in the first 2 months of life are insufficient to permit firm conclusions regarding the nature of the myelin defect. However, they do suggest that the lesions are regions of greater than normal metabolic activity in which there are abnormalities and rapid changes in the lipid composition of the myelin sheaths.

These considerations indicate that the neuropathological changes may be due to faults

TABLE III

NEUROPATHOLOGICAL, CLINICAL AND CHEMICAL COMPARISON OF BORDER DISEASE WITH SWAYBACK

*Border disease**	*Swayback*†
1. Congenital	1. Congenital or delayed
2. Offspring of hoggs and gimmers especially	2. Offspring of 2–3 crop ewes especially
3. Not associated with copper deficiency	3. Associated with copper deficiency
4. Not a clear-cut clinico-pathological entity	4. A clear-cut clinico-pathological entity
5. Tremor, fleece abnormality, reduced viability	5. Tremor, ataxia, reduced viability
6. ?Nervous system lesions inconstant	6. Nervous system lesions constant
7. Lesions not systematic	7. Lesions very systematic
8. Never cystic lesions of the cerebrum	8. Cystic lesions of the cerebrum occur in a variable proportion of cases
9. Nerve cell changes typically absent	9. Nerve cell changes constant
10. Axonal changes not seen	10. Axonal degeneration occurs
11. Myelin sheaths swollen and distorted—large amounts of fat present in younger cases	11. Myelin sheaths degenerate with gradual production and elimination of lipid
12. Lesions resolve. Associated with non-astrocytic neuroglial cells	12. Lesions repair by astrocytic fibrogliosis

* Hughes *et al.* (1959); Barlow and Dickinson, this report.

† Barlow *et al.* (1960); Barlow (1963 a and b).

in the process of myelination. The term congenital hypomyelinogenesis seems to be an appropriate description for them, since in sheep all regions normally become myelinated during intra-uterine life (Romanes, 1947).

Our findings, therefore, confirm and extend those of Hughes *et al.* (1959), who describe similar lesions of the myelin sheath in those cases of Border disease which exhibited nervous signs. In an addendum they considered that the central nervous symptoms and pathology were similar to those of hypomyelinogenesis congenita in sheep (Markson *et al.*, 1959). Markson and his colleagues and more recently Barr (1964) generally did not find fleece abnormalities in hypomyelinogenesis congenita. It may be that in certain breeds these are not evident because of the nature of the normal fleece and that hypomyelinogenesis congenita in sheep and Border disease are one condition, or that two distinct diseases exist in which the neuropathology is similar. However, neuroglial changes of the type found in this study have not been described previously.

Markson *et al.* (1959) indicated some of the differences between hypomyelinogenesis congenita and swayback and an extended list of comparisons between the latter and Border disease is now possible (Table III). Whilst both conditions appear to affect the formation and maintenance of myelin in young lambs and may involve common biochemical processes, profound differences are evident.

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The Demonstration of the Transmissibility of Border Disease of Sheep

Sir,—Border disease was first recognised as a clinical entity by Hughes, Kershaw and Shaw (1959). Barlow and Dickinson (1965) suggested the following diagnostic criteria as a result of detailed clinical and pathological examination of many cases:—

1. Hairy birthcoat, which may be excessively pigmented, in normally smooth-coated breeds of sheep.
2. A flock history of poor growth and viability of lambs, a proportion of which may show rhythmic clonic spasms (shakers).
3. The occurrence in lambs under six months of age of myelin defects (hypomyelinogenesis) and clusters of swollen interstitial glia.

For the investigation of Border disease an experimental flock of normal and affected Clun sheep has been established on an Animal Breeding Research Organisation farm for several years. Suspicion that an infectious agent was involved was aroused in 1963 when a Suffolk lamb with a hairy fleece and having hypomyelinogenesis was born to a ewe introduced into this flock from a source where the disease has never been found. This suspicion was reinforced in 1964 when an unrelated group of Suffolks, introduced the previous autumn, gave birth to lambs displaying the full range of defects found in Border disease and including hairy shaker twins with hypomyelinogenesis; a large number of control Suffolks, kept on farms where the disease has never occurred, produced normal lambs. A third importation of Suffolks in September, 1966, have had offspring, by a different ram, which again include apparently normal lambs, hairy lambs and hairy shaker lambs, while controls kept out of contact produced normal lambs. Histological examination remains to be carried out for the current lambing.

During 1965 a number of small scale inoculation experiments using a brain/spleen pool from shaker lambs were carried out. Various doses, concentrations and routes of administration were employed; abortion occurred in all these experiments. The only ewe to carry a foetus to late gestation, was one inoculated 60 days after mating which aborted a fresh 131 day purebred Dorset Horn foetus with an abnormally hairy fleece, a state of myelination inconsistent with its gestational age and with glial abnormalities similar to those in Border disease.

In 1966 a larger experiment was designed to test transmissibility. Dorset Horn sheep were chosen because of their consistently fine birthcoats. Forty two-, three- and four-year-old ewes and a ram were obtained from a flock with a history free from any suspicion of Border disease and were placed in a clean environment. Service dates were recorded and those ewes considered pregnant were divided into two groups of 15 and one of seven animals, balanced as far as possible for age and lambing date.

One of the large groups was inoculated intraperitoneally and subcutaneously with a total of 5 ml. of 5×10^{-3} saline dilution of the original 1965 brain/spleen pool, while the other was injected similarly with a suspension prepared from the aborted Dorset Horn foetus. The remaining seven ewes were uninoculated controls. Following inoculation the groups were isolated in separate concrete pens. The outcome is summarised in Table I. Microscopic examination has so far been carried out on only two lambs of the experimental groups; one was stillborn with a normal fleece, but both fulfilled the histological criteria of the disease.

TABLE I

	D/H Foetus	Brain/ spleen pool	Controls
Ewes giving birth to live lambs	10/15	8/15	4/6*
No. of ewes aborting	3	5	0
Live lambs born	11	9	5
No. of shakers	5	8	0
No. of hairy, dead or alive	8	10	0
No. weak but no definite signs	5	1	0
No. normal	0	0	5*
Mean gestation of live born lambs (days)	144·7	142·4	145·7

*In addition one ewe was killed for control purposes at 129 days gestation when carrying a live apparently normal foetus.

These results indicate that a transmissable agent is involved in the causation of this disease and that the agent reaches the embryo from an infected mother, since tissue from affected lambs is infective. Our experiences with the Suffolk sheep suggest that the disease can be transmitted by natural contagion and since subsequent matings usually result in the birth of normal lambs we suspect that some maternal immunity may occur. Full reports of this work are in preparation.

We wish to record our thanks to Mr. I. G. Shaw, M.R.C.V.S., for drawing our attention to this disease and locating the initial material.

May 17th, 1967.

Yours faithfully,
A. G. DICKINSON.
A.R.C. Animal Breeding Research Organisation, Edinburgh, 9.

R. M. BARLOW.
Animal Diseases Research Association, Moredun Institute, Edinburgh.

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22.

AN ELECTRON MICROSCOPIC STUDY OF THE SPINAL CORD IN BORDER DISEASE OF LAMBS

BY

P. A. CANCILLA and R. M. BARLOW

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An Electron Microscopic Study of the Spinal Cord in Border Disease of Lambs

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SUMMARY. The ultrastructure of the spinal cord in 2 cases of Border disease is described. The findings include delayed myelination, demyelination, cavitation of the myelin sheaths and extensive astrogliosis.

BORDER DISEASE WAS first recognized in neighbouring counties of England and Wales and affects lambs of different breeds (Hughes *et al.*, 1959). The criteria for diagnosis include hairy birth coat, low viability, rhythmic clonic spasms, congenital hypomyelinogenesis and abnormal neuroglia (Markson *et al.*, 1959; Barlow & Dickinson, 1965). Gradual remission of clinical signs is associated with resolution of the lesions in the nervous system, but death without overt cause may occur at any time. Recent biochemical studies have indicated a defect in central myelin akin to that of swayback and indicative of amyelination rather than demyelination (Davison & Oxberry, 1966). The present study was undertaken to characterize further the morphology of the myelin in Border disease, and more particularly to define the nature of the abnormal neuroglia described by Barlow & Dickinson (1965).

MATERIALS AND METHODS

Two Suffolk cross lambs one and 3 days old were used. The diagnosis of Border disease was based on the finding of hairy birth coats and rhythmic clonic spasms and was confirmed by the paucity of central myelin demonstrated by conventional staining methods. Five normal lambs of the same ages served as controls. The animals were killed and a portion of the spinal cords fixed *in situ* by perfusion of an isolated segment of the abdominal aorta (Cancilla & Barlow, 1966). The perfusate was 3% glutaraldehyde buffered to pH 7·4 with 0·067 M sodium cacodylate. Sections, 1μ thick, of epon-embedded spinal cord were stained with alkaline toluidine blue for light microscopy and thin sections were stained with uranyl acetate for electron microscopy. The grids were examined in a Siemens Elmiskop 1a. at 80 Kv.

RESULTS

Light Microscopy

The spinal cords of the normal lambs were well myelinated at birth. Each axon was surrounded by a thick, compact myelin sheath (Fig. 1). Occasional glial cells were present between closely approximated myelinated fibres. By contrast, the lambs with Border disease had axons that were unmyelinated, or surrounded by thin, ill-defined myelin sheaths (Fig. 2). Interfascicular glial cells were more numerous and frequently aggregated. Occasional deeply-stained myelin sheaths were devoid of axons and had collapsed. Large myelin-filled macrophages were not encountered.

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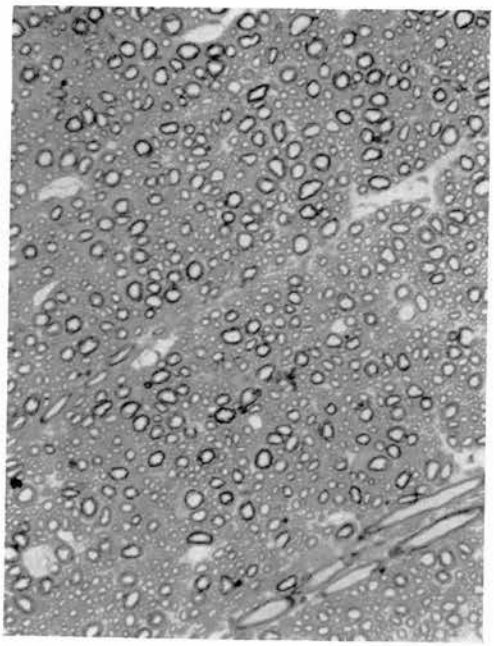

FIG. 1. Transverse section of spinal cord of normal one-day-old lamb. There is a thick, dark myelin sheath surrounding each clear axon. Occasional nucleated cells are present. Toluidine blue. × 300.

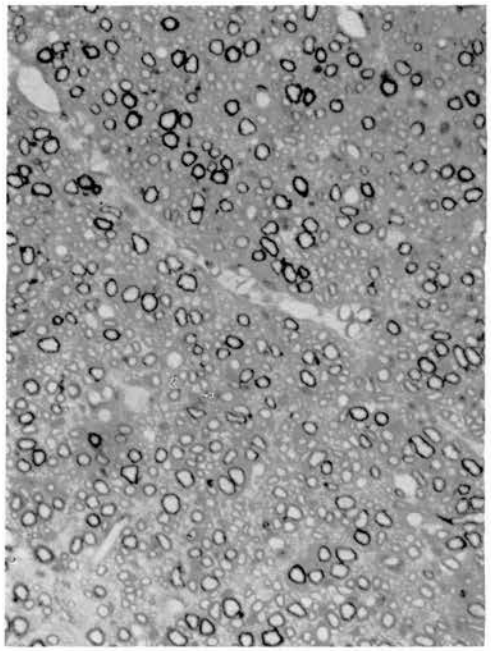

FIG. 2. Transverse section of spinal cord of lamb with Border disease. Compare with Fig. 1. There is a paucity of myelin and an increase in nucleated cells. Toluidine blue. × 300.

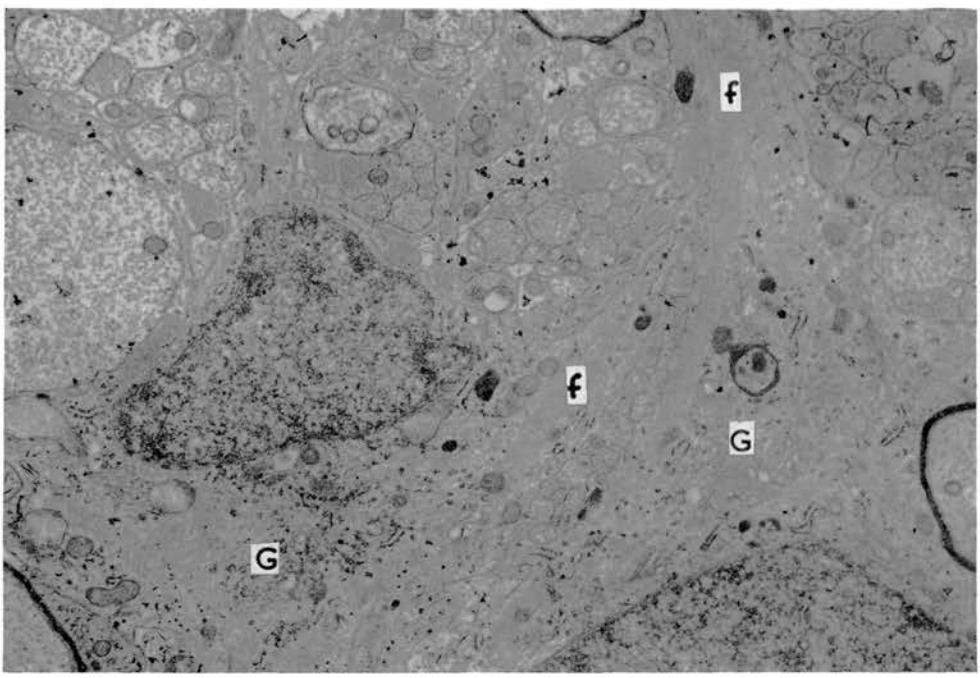

FIG. 3. Border disease; the 2 nucleated cells are astrocytes. Their perikarya have well-defined Golgi substance (G), fibrils (f), dense-bodies and prominent cisternae of rough endoplasmic reticulum. Note the numerous transversely cut axons and glial processes and the scattered myelinated axons. × 11,500.

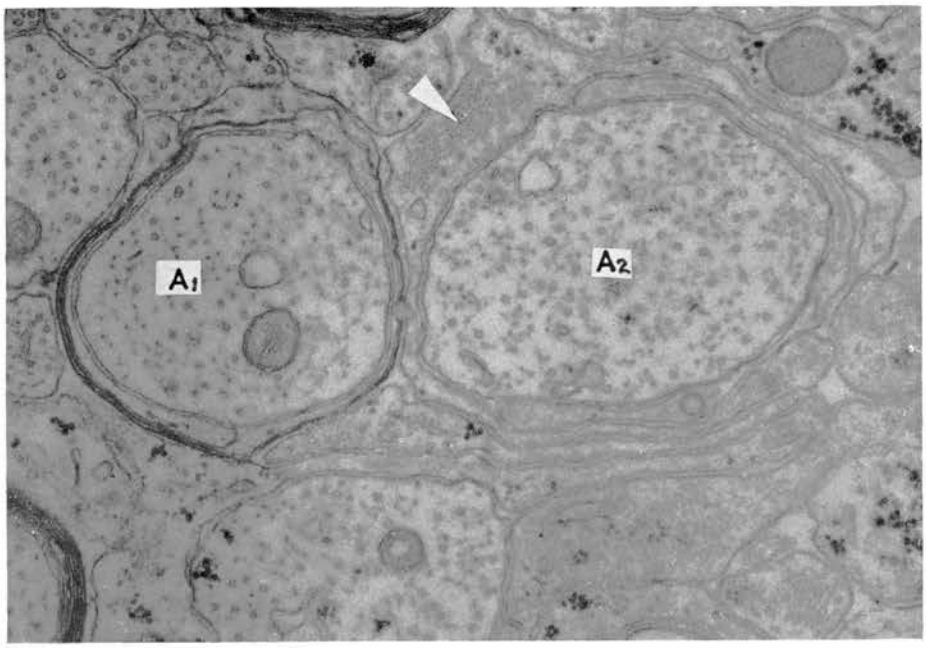

FIG. 4. Border disease; 2 axons (A_1, A_2) occupy the centre of the picture. A_1 is covered with a thin myelin sheath and A_2 is surrounded by layered cell processes some of which belong to astrocytes (arrow). × 40,000.

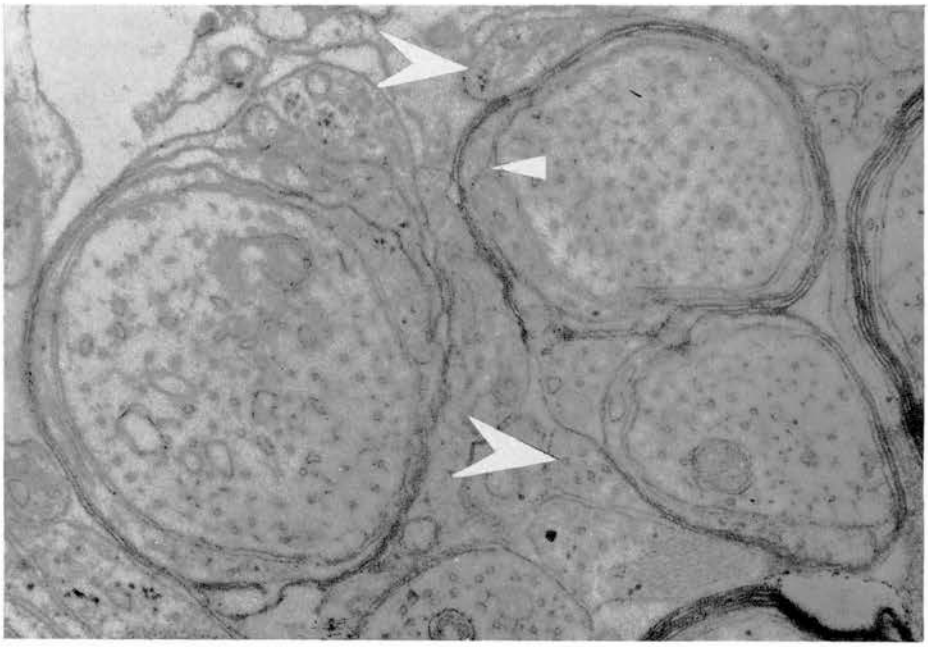

FIG. 5. Border disease; 3 axons are present with thin myelin sheaths. A mesaxon (small arrow) and outer loops (large arrows) are indicated. × 50,000.

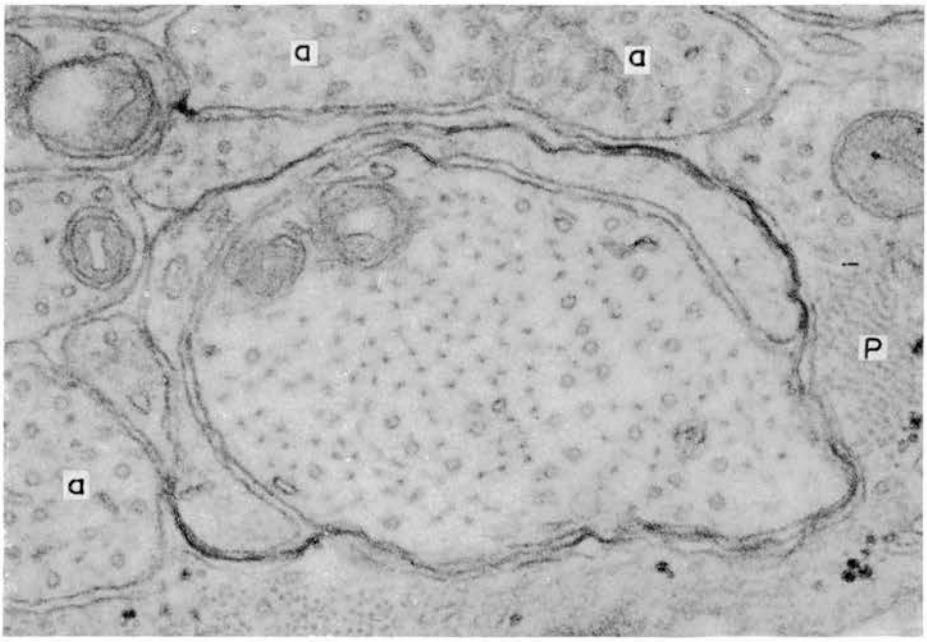

FIG. 6. Border disease; the axon in the centre has a thin myelin sheath. A closely applied astrocyte process (P) has fibrils and glycogen. Unmyelinated neural processes (a) are seen. × 80,000.

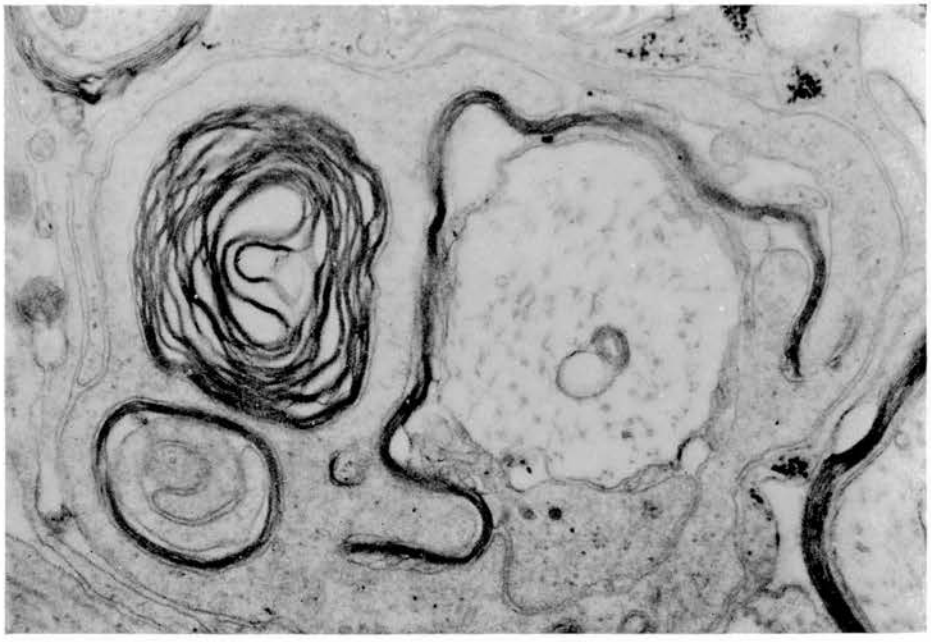

FIG. 7. Border disease; the process of a cell has separated a portion of a myelin sheath from its axon. The cytoplasm of the cell contains lamellar lipid profiles. × 40,000.

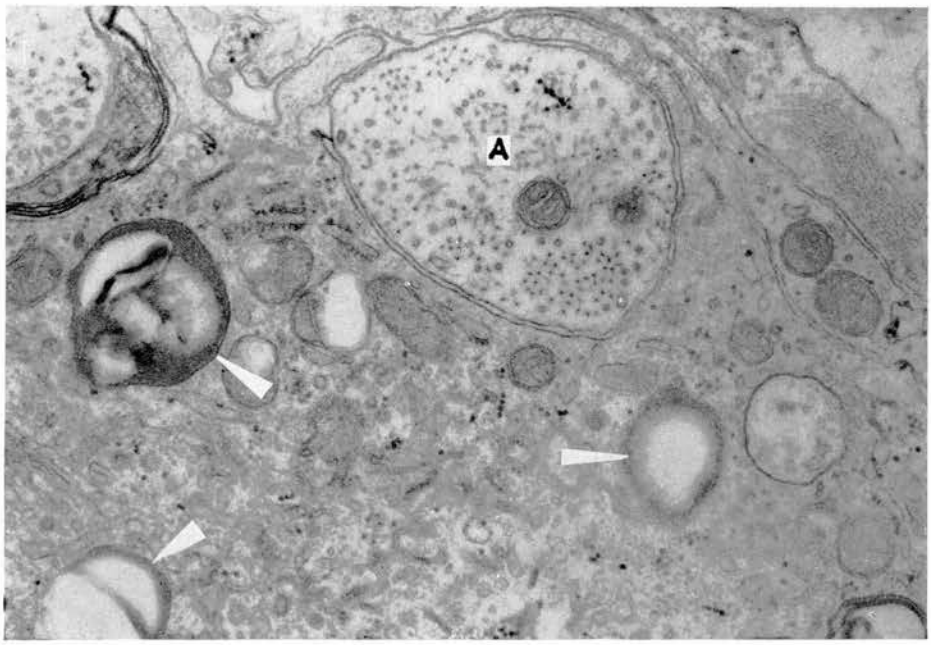

FIG. 8. Border disease; an axon (A) is partially surrounded by a cell containing lipid debris (arrows). × 40,000.

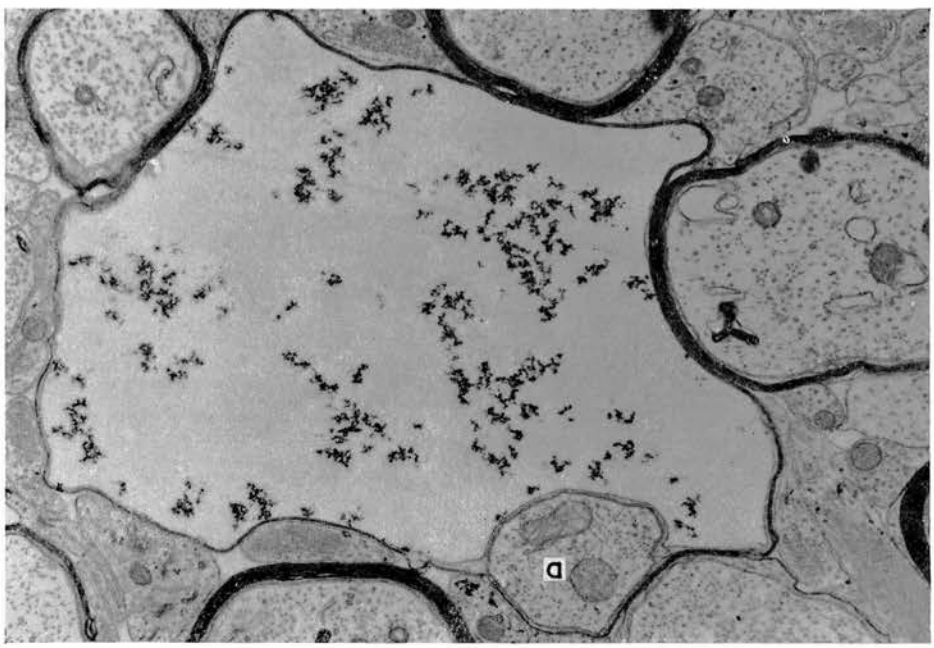

FIG. 9. Border disease; a large cavity with granular, electron-dense material has formed between myelin lamellae. The axon (a) is at one pole. × 20,000.

Electron Microscopy

The white matter of the spinal cords of the 2 affected lambs was composed of tightly packed cell processes of neurons and astrocytes (Fig. 3). Myelinated fibres were readily identified, but were fewer and smaller than in the controls. Bundles of myelinated fibres and cell processes were separated by groups of cells (Fig. 3) which were evidently the abnormal glial cells described in an earlier publication (Barlow & Dickinson, 1965). The cytoplasm of these cells contained granular electron-dense glycogen and bundles of fibrils 60 to 75 Å diameter and hence they were identified as astrocytes. They appeared active with a well-defined Golgi zone, dilated cisternae of rough-surfaced endoplasmic reticulum, numerous mitochondria and prominent dense-bodies.The nucleus of these cells was oval or elongated and contained condensed masses of chromatin; nucleoli were seen rarely. Extensions of the cell bodies cleaved between neural processes and were observed in transverse sections as oval or circular profiles containing fibrils, glycogen and mitochondria (Fig. 3).

The neural processes were identified as axons by the presence of tubules 150 to 200 Å diameter and fibrils approximately 100 Å diameter. Many of these axons were free of an enveloping myelin sheath, though frequently they were surrounded by successive layers of closely applied cell processes.The origin of these processes was not always determined, but clearly some were derived from astrocytes since they contained fibrils and glycogen (Fig. 4). Adjacent axons were observed with thin myelin lamellae and characteristic end loops (Figs. 4, 5 and 6). Often a localized bulge of cytoplasm was encountered on the segment of myelin immediately adjacent to the end loop (Figs. 4, 5 and 6) giving the appearance of a second loop. This bulge represented the residual cytoplasm of the enveloping cell and separated adjacent myelin lamellae. Membranes were shared at the junction of adjacent myelin sheaths (Figs. 5 and 9).

Axons were also encountered which were surrounded only by processes of cells the cytoplasm of which contained numerous lamellated membranes and electron-dense material (Fig. 7). Sometimes a process of such a cell was observed to separate a myelin sheath from its axon at a node of Ranvier (Fig. 8). The cytoplasmic inclusions of these invading cells were interpreted as phagocytosed myelin.

Cyst-like dilatations of portions of the circumference of myelin sheaths were observed also. They contained granular electron-dense material and occurred in the intraperiod region of the sheath (Fig. 9), or between the sheath and the axon.

DISCUSSION

Myelination of the ovine spinal cord occurs early in gestation (Romanes, 1947) and formed myelin has a characteristic mesaxon, outer loop and repeating period (Peters, 1960). In Border disease there is a deficiency of myelin (Hughes *et al.*, 1959; Markson *et al.*, 1959; Barlow & Dickinson, 1965) which at the ultrastructural level is seen to be due to the presence of morphologically normal myelin sheaths with less than the usual complement of lamellae. This finding is consistent with the biochemical observations of Davison & Oxberry (1966) of an overall decrease in the myelin lipids in Border disease.

Barlow & Dickinson (1965) were unable to classify the abnormal neuroglia which they observed in the white matter in Border disease. Though the nucleus was about the size of an astrocyte nucleus, with the light microscope they were unable to demonstrate nucleoli or cytoplasmic processes and concluded that these cells were not astrocytes. On the basis of the

interfascicular location of the cells these authors considered that such cells might be derived from microglia or oligodendroglia. The present study has shown that these cells are, in fact, astrocytes, since they contain the characteristic fibrils and glycogen. The abundance and evident activity of these cells is consistent with the finding of a raised cytochrome oxidase activity in the white matter in Border disease (Barlow & Dickinson, 1965). Although astrocytic processes extended around individual neural processes, there was no evidence of their participation in the formation of myelin. The proximity of the astrocyte processes to the axon may have retarded contact of the myelin-forming oligodendroglial cells with the axons resulting in a delay of myelinogenesis, or the extensive gliosis may have slowed the normal process of spiral wrapping.

In addition to retarded formation of myelin, there was evidence of some myelin destruction. There was invasion of the nerve sheaths by macrophages at the nodes of Ranvier and naked axons were surrounded by cells with sequestered myelin debris. This was probably the sudanophil lipid described by Barlow & Dickinson (1965) in lambs up to a month old.

The local dilatations and pouchings of the sheaths described by Barlow & Dickinson (1965) may be the homologues of the peculiar splits in the sheaths which occurred at the intraperiod line and which resulted in the formation of cavities containing electron-dense material. Apart from their granular content, these splits resembled those which occur in alkyl tin poisoning in the rabbit (Aleu *et al.*, 1963). Early splits or doubling of the space between major dense lines, such as have been described in the oedema which follows the cerebral implantation of silver nitrate (Hirano *et al.*, 1965), and in allergic encephalomyelitis (Lampert & Carpenter, 1965), were not observed in these 2 cases of Border disease.

ACKNOWLEDGMENTS

We wish to thank Dr. A. G. Dickinson of the Animal Breeding Research Organization, Edinburgh, for making the Border disease material available to us.

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EXPERIMENTS IN BORDER DISEASE

I. Transmission, Pathology and Some Serological Aspects of the Experimental Disease

By

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Moredun Research Institute, Gilmerton, Edinburgh

INTRODUCTION

The clinical entity of Border disease was first recognised by Hughes, Kershaw and Shaw (1959) and diagnostic criteria concerning certain aspects of flock history and abnormalities of the fleece and central nervous system were proposed by Barlow and Dickinson (1965). The experimental reproduction of Border disease by the inoculation of pregnant ewes with tissues from newborn lambs affected with the disease has already been reported in brief (Dickinson and Barlow, 1967; Shaw, Winkler and Terlecki, 1967). Seven S gamma globulin which is not normally present in precolostral lamb serum has been found frequently in affected lambs (Gardiner, 1967).

The purpose of the present communication is to report in detail methods and results of some of these experiments and to describe the pathology of the experimental disease.

MATERIALS AND METHODS

Sheep. Dorset Horn ewes were selected because their lambs normally have a fine white birth coat of considerable uniformity. Forty $1\frac{1}{2}$, $2\frac{1}{2}$ and $3\frac{1}{2}$-year-old polled ewes were purchased from a reliable source with no history of infertility or neonatal death. A proven Dorset sire was also acquired from the same source. On arrival the sheep were placed in a clean environment, bled to provide baseline sera, wormed and treated for foot rot. After mating, 37 ewes which were judged pregnant because they did not return to service were divided into 2 groups of 15 and one group of seven animals, the groups being balanced as far as possible with respect to age and parity of ewes and expected lambing date. Thereafter, the experimental groups were housed separately in indoor concrete pens guarded by antiseptic foot-baths. The ewes were observed daily for evidence of abortion. Aborting ewes were examined for evidence of the common abortion organisms viz. Vibrio, Toxoplasma and Bedsonia (enzootic abortion of ewes). Lambs born alive were examined for hairiness or coarseness of fleece and the presence of nervous symptoms. All lambs were killed at between 1 and 12 days of age for pathological examination.

Inocula. The inoculum for Group 1 was prepared from the brain and spleen of a 131 day Dorset Horn foetus with an abnormally hairy fleece and a state of myelination inconsistent with its gestational age. This foetus was from a ewe which had been inoculated at 60 days gestation with brain and spleen from a case of Border disease. Equal parts of the two tissues were ground in a Griffith's tube and the homogenate diluted with saline to a concentration of 5×10^{-3}. Five ml. of this suspension were injected partly intraperitoneally and partly subcutaneously into each ewe. The inoculum for Group 2 was prepared in the same way from pools of

TABLE 1

			Ewes				*Offspring*						
			Gestation				*Clinical signs*	*Pathology*					
Group	*Ewe No.*	*Age in years*	*Day of inoc.*	*Length (days)*	*Result*	*Age of lamb at P.M. (days)*	*Sum of fleece scores†*	*Clon. spas.*	*Hypomyel.*	*Gliosis with bizarre cells*	*Interfasc. lipid*	*C'bral cavitn.*	*7S γ glob.*
	1	3·5	71	148	ML	8	21	+	+	++	–	–	++
	2	3·5	67	143	ML	7	10	++	++	++	–	–	N.D.
	3	3·5	65	145	FL	1	22	+++	+++	–	+	–	N.D.
	4	2·5	61	145	ML	1	15	++	+++	–	+	–	±
	5	1·5	66	144	ML	4	18	++	+	+++	–	–	+
I.	6	1·5	58	143	ML	1	23	+++	++	+	–	–	–
Inoculum:	7	1·5	66	140	ML	1 (died)	9	–	++	++	–	–	++
Dorset	8	1·5	72	141	FL	1	16	+	++	+	–	–	±
Horn	9	1·5	66	141	FL	1	24	+	+++	+	+++	–	±
foetus					FL	1	10	+	+++	–	+++	++	+
	10	3·5	72	127	A				Unfit for examination				
	11	3·5	66	145	ML	1	9	+++	++	–	–	–	+
	12	1·5	63	No conceptus found									N/A
	13	2·5	63	” 77 ”	”A								N/A
	14	1·5	38	71	A				Unfit for examination				N/A
	15	1·5	27		A				” ” ”				N/A
	16	3·5	68	134	A				Unfit for examination				N/A
					FA		10		” ” ”				+
	17	3·5	71	147	FL	12	24	++	++	++	–	+	–
	18	3·5	65	144	MD	0	24	N/A	++	++	+++	–	N.D.
	19	3·5	71	142	ML	3	21	+++	+++	+	–	–	N.D.
					A				Unfit for examination				
II.	20	3·5	70	136	MD	0	10	N/A	+++	–	–	–	+
Inoculum:					FL	1	24	++	++	++	–	–	+
brain/	21	3·5	66	145	FL	1 (died)	19	–	+++	–	+	–	±
spleen	22	2·5	61	129	FD	0	N.D.	N/A	+++	+	–	++	–
pool.	23	2·5	65	144	ML	2	24	+++	++	++	–	–	+
Field cases	24	2·5	68	142	FD	0	9	N/A	+++	+	–	–	N.D.
	25	1·5	66	142	ML	1	13	+++	+++	+++	+++	–	–
	26	1·5	63	144	ML	4	24	++	+++	++	–	–	+
					FL	4	23	+	+++	–	–	++	+
	27	1·5	70	143	FL	1	21	+	++	–	–	+++	–
					A				Unfit for examination				
	28	1·5	58	115	A				” ” ”				N/A
	29	1·5	64	131	A				” ” ”				N/A
	30	1·5	72	114	A				” ” ”				N/A
					A				” ” ”				N/A
	31	3·5	N/A	129	FL*	N/A	9	N/A	–	–	–	–	–
	32	1·5	”	145	ML	2	9	–	–	–	–	–	–
III.	33	3·5	”	145	ML	N.D.	9	–			N/A		–
Uninoculated					ML	1	9	–	–	–	–	–	–
control	34	1·5	”	146	FL	N.D.	9	–			N/A		–
Sheep					FL	1	9	–	–	+	–	–	–
	35	2·5	”	145	ML	N.D.	8	–			N/A	–	–
	36	3·5	”	148	ML	N.D.	9	–			N/A		–
	37	1·5	”	146	FL	N.D.	9	–			N/A		N.D.

KEY. I. – Live born lamb D – Stillborn full term lamb M – Male F – Female N.D. – not done * – Killed for examination N/A – Not applicable
A – Abortion (foetal death before term, with or without expulsion of the conceptus or foetal mummification without expulsion).
– No spasms, hypomyelinogenesis, gliosis, interfascicular lipid, cerebral cavitation or 7S γ glob. ± Weak line, 7S γ globulin.
+ Fine tremor; mild patchy or diffuse hypomyelinogenesis; slight gliosis; traces of interfascicular lipid or microscopic cavities.
++ Moderate tremor, able to stand and suckle; severe hypomyelinogenesis; moderate gliosis, interfascicular lipid; focal cerebral cavities.
+++ Very severe tremor, unable to stand unaided; almost total lack of myelin; severe gliosis with numerous bizarre cells; masses interfascicular lipid; massive cerebral cavitation.
† For details of fleece assessment see materials and methods.

brain and spleen taken from severe newborn cases of natural Border disease the previous spring and stored at −20°C. for 8 months. Ewes were inoculated between 27th and 72nd days of gestation as detailed in Table 1.

Pathological methods. Post-mortem examination was carried out as soon after death as possible. The lamb skins were pinned out, dried and treated with formalin on the dermal surface. The fleeces were assessed by an independent observer using a modification of the method of Dry (1955). The birth coat of the body and breech was scored on a scale 1 (fine) to 7 (hairy) and the degree of halo hairiness of the back and belly each scored on a scale of 1 to 5. The fleeces of all lambs were assessed on a single occasion. When fleeces were presented to the observer for a second or third time during his examination, his evaluation remained the same.

Representative blocks of all organs and tissues were fixed in Zenker formol, formol saline or Baker's formol-calcium. Particular attention was given to the central nervous system (CNS) from which 21 blocks representative of all regions of these areas were taken for paraffin sections. A second comparable series of blocks was retained for frozen sections. Histological methods included haematoxylin and eosin, Luxol fast blue, Giemsa, Sudan IV, Smith Quigley, OTAN (Adams, 1959), phosphotungstic acid haematoxylin, P.A.S., toluidine blue, Cajal's gold sublimate, and Holmes silver method for neurofibrils.

Immunological methods. Sera were collected from aborting ewes and paired with preinoculation samples, for use in the CF test for enzootic abortion of ewes and for the Toxoplasma dye test.

Blood samples were also taken from lambing ewes and newborn lambs prior to suckling. Samples of cardiac blood were obtained from stillborn lambs and where possible also from aborted foetuses. Sera from lambs and foetuses were examined by immunoelectrophoresis for the presence of 7S γ globulin. After electrophoresis, troughs in the gel were filled with rabbit antiserum prepared by multiple inoculations of whole sheep serum. Precipitation patterns were allowed to develop for up to 72 hours. If no gamma globulin was detected under these conditions or if only a faint trace of reaction was visible the sera were re-run against a range of dilutions of the rabbit antiserum. Decisions as to the presence and origin of any gamma globulin detected in this manner were made as described previously (Gardiner, 1967).

Gel diffusion and complement fixation tests were used in preliminary attempts to demonstrate the presence of Border disease specific antibodies in ewe and lamb sera. Simple homogenates of brain, spleen, lymph node and placenta were used as antigens.

RESULTS

The data presented in Table 1 describe the material used in the experiment and give clinical and pathological details of the fate of all pregnancies in each of the 3 groups of sheep. No consistent difference between Groups 1 and 2 attributable to the source of inoculum is apparent and they may be considered as one for the purposes of description. The mean gestation of inoculated animals was 2·8 days less than the mean for uninoculated sheep, but this difference is not significant statistically.

Abortion

With the exception of ewe 31 which was killed to provide a control foetus all uninoculated ewes carried their young to term and produced live healthy lambs. In the inoculated groups, however, 9/30 (30 per cent.) aborted or delivered mummified foetuses at term and a further 2 failed to lamb though showing no evidence of abortion. Inoculated ewes at no time suffered malaise or loss of appetite even whilst aborting. Foetuses were aborted at various times between 71 and 143 days from conception and in all stages of preservation. The shortest period from inoculation to expulsion of the conceptus was 42 days (30) but foetal

death had clearly occurred some time earlier. Mummified foetuses were shrivelled and brown with adherent stringy membranes. The body cavities contained sero-sanquinous fluid and there was a variable amount of putrefaction. In better preserved foetuses the skin, usually with wool attached, was stained a dirty brown by the foetal fluids. There was generalised anasarca, often blood-tinged, which affected both the foetus and its membranes. It was particularly evident over the poll and associated with early maceration of the membranous bones of the head. The placental attachments were brown, greyish white, or cherry red with pin-point white foci. No cellular thickenings of the intracotyledonary areas were noted and microscopic and cultural examinations of placental or foetal tissues failed to reveal any evidence of vibrios or other pathogenic bacteria, Toxoplasma or Bedsonia species. No cytopathogenicity was observed on tissue cultures prepared from aborted material.

Liveborn Lambs

As can be seen from Table 1 the offspring of control ewes had very consistent and low fleece scores (Mean of sums 8·9). None showed any neurological derangement at birth or subsequently and 3 only were killed for histological examination. In the inoculated groups, however, all the liveborn lambs, with 2 exceptions, showed clinical disturbance of the locomotor system from birth (Fig. 1). One of these exceptions (21) had a hairy birth coat, and one (7) had a coat of similar quality to the controls, but both died in less than 24 hours and showed neuropathological changes consistent with Border disease. Also worthy of note is the offspring of ewe 11 which showed severe nervous symptoms, but whose birth coat gave a total score of 9 compared with the mean of 17·5 for lambs born to the inoculated groups.

Clinical Signs

The severity of neurological signs varied greatly between individuals and where possible assessment was delayed until the day following parturition (Table 1). The most severely affected lambs (+ + +) were unable to rise and showed slow rhythmic movements of trunk and limbs, or were so convulsed by clonic spasms that they were unable to maintain the standing position and suckle unaided. Less severely affected lambs (+ +) also showed rhythmic clonic spasms, but were able to undertake voluntary movements and suckle. In the most mildly affected lambs (+) only fine tremors of ears and tail were present and at birth these were masked by physiological motor incompetence.

Pathology

Macroscopic appearances. Lambs from inoculated ewes showed no consistent gross lesions at post-mortem. A few showed renal hypoplasia characterised by slight residual lobulation of the surface, a narrow cortex and abundant peri-pelvic mesenchyme. In some affected lambs the brain was small with a degree of ventricular dilation and the spinal cord was narrow and unusually firm. In 5 lambs cystic transformation of the cerebral white matter was present; in 4 this was confined to small areas in the parahippocampal and inferior temporal gyri, but in the 5th it was more generalised. Grossly these lesions resembled those seen

in some congenital swayback cases. In the remaining lambs no gross lesions were recognised.

Histology of the nervous system. The neuropathological histology of natural Border disease has been described in detail (Barlow and Dickinson, 1965) and consists of 3 components, namely hypomyelinogenesis, hypercellularity of white matter with a proportion of abnormal glia and, in the youngest animals, interfascicular accumulations of lipid. In the present experiment all the lambs and foetuses from the inoculated groups showed one or more of these abnormal components each of which was scored on an arbitrary scale of increasing severity from + to + + + (Table 1). The lesions affected all levels of the CNS and were either diffuse or patchy. For diffuse lesions the scoring system was adequate, but where the lesions were patchy it was less satisfactory and the latter were accorded a + score regardless of their local severity. In brief, the lesions consisted of twisted and distorted fine or swollen nerve fibres with a reduced affinity for all the myelin stains used which gave them a beaded or ghostlike appearance depending upon the severity of hypomyelinogenesis (Fig. 2). Axons were apparently spared. Interstitial glial nuclei were more numerous than the normal. A proportion were swollen and hydropic, often with a folded nuclear membrane and finely divided chromatin, and lacked a clearly defined nucleolus. Fibrillary gliosis was not evident. Though compound granular corpuscles were not seen in the interstitium, aggregations of lipid droplets were present in some cases (Table 1). This lipid was not readily soluble in alcohol or xylol, was partially sudanophilic and gave variable red-black/brown reaction with OTAN. Other larger globules and amorphous masses were stained by PAS, but failed to show metachromasia with toluidine blue or cresyl violet. None of this material was birefringent.

The cystic lesions observed in some of the lambs from inoculated ewes (Fig. 3) require more detailed description as such lesions have not been reported in naturally occurring Border disease. In the live offspring of ewe 27, which was the most severely affected lamb, the septum pellucidum was bifid and gelatinous lesions or frank cavities replaced most of the temporal and frontal white matter. Microscopically, the lesions appeared as rarefactions, splits or actual softenings in a densely cellular tissue containing small numbers of delicate myelinated nerve fibres. Around the blood vessels there was frequently a complete absence of myelin (Fig. 4). Within the lesions and nearby leptomeninges there were light perivascular cuffs of lymphocytes, monocytes and occasional plasma cells. Oligodendroglial nuclei and fibrous astrocytes accounted for the hypercellularity of the lesions, whilst in the softened regions compound granular corpuscles and PAS positive, non-argentophilic plaques were also numerous (Figs. 5 and 6). In less severely affected cases softening was not evident, the lesions appearing as densely cellular foci in the para-hippocampal and inferior temporal gyri. In no case did the cortical grey matter appear to be affected. Typically, neuronal degeneration at subcortical levels was also absent. In one case (25), however, severe chromatolysis was present amongst the Purkinjé cells, neurons of the medial vestibular nucleus, the large motor neurons of the ventral horns of the spinal cord and in the dorsal root ganglia. Though no cerebral lesion was found in this lamb, hypo-myelinogenesis and hypergliosis were both extremely severe.

Histology of the placenta. Despite the variation in the gross appearance of the cotyledons, the histological picture was remarkably uniform. The changes seen in arborted placenta were non-specific in character and mimicked the degenerative changes seen in the normal full term placenta. There was necrosis of chorionic villi with desquamation of the trophoblast and haemorrhage into the maternal/foetal space. The latter was also lightly infiltrated with polymorphonuclear leucocytes. In fresher specimens the membranes were oedematous.

Following lambing or abortion 6 inoculated ewes were killed for examination and compared with one newly lambed control ewe and one killed on the 129th day of gestation. In 2 of the 6 inoculated animals perivascular infiltrations of plasma cells were found in the uterine cotyledons, but otherwise the changes observed appeared to be of a physiological nature and varied only in the degree of degenerative and regenerative change.

Serology

Twenty samples of precolostral serum were obtained from lambs born from inoculated ewes and in 14 of these 7S gamma-globulin was detected. In the remaining 6 sera and all 8 sera from control group lambs this globulin was not found. Serum complement fixation titres with ovine abortion virus antigens were of a low level as were the toxoplasma dye test titres. These findings indicate that neither of these agents was involved in the experimental disease. Preliminary attempts to demonstrate antibody-antigen reactions specific to Border disease were unsuccessful.

Culture

Attempts to demonstrate the agent of Border disease in eggs for other tissue cultures have been unsuccessful.

DISCUSSION

The results of this experiment demonstrate unequivocally that a condition satisfying the defined diagnostic criteria of Border disease has been provoked by the inoculation of pregnant ewes with a crude brain/spleen suspension from lambs with the natural disease and from an experimentally infected foetus. The agent can evidently retain its potency for several months at −20°C., but preliminary attempts to identify the agent have not been successful.

Infection in the ewes was clinically silent. Although 30 per cent. of pregnancies terminated in abortion or foetal mummification, at no stage was malaise observed in any of the ewes. Shaw *et al.* (1967) have reported temperature rises up to 1·3°C. following inoculation. Border disease would, therefore, appear to be a condition like rubella in man (Gregg, 1941; Medearis, 1967) and panleukopaenia in the cat (Kilham, Margolis and Colby, 1967) in which an infection during pregnancy causes mild or inapparent maternal disease, but which crosses the placenta to cause serious dysmorphogenesis. Once the foetus has become infected, the skin and the nervous system would appear to be the principal target organs. Whereas the fleeces from control lambs consistently gave low scores, those from affected lambs were, in general, much higher indicating coarseness of the birth coat and

the presence of considerable halo hair. Great variability was evident amongst the fleeces of affected lambs and this was not apparently related to the severity of clinical signs or pathological changes elsewhere.

In the CNS the interstitial glia of the white matter appeared to be most affected; they were hyperplastic, defective in their myelin-forming function and frequently their nuclei had a hydropic appearance and a bizarre shape. The nature of these bizarre glia has been discussed elsewhere (Barlow and Dickinson, 1965; Cancilla and Barlow, 1968); it was suggested that they have morphological features of both oligodendroglia and astrocytes.

The experimental disease was associated with cerebral softenings in a small number of cases. These lesions have not been reported in Border disease hitherto and grossly they resembled those which occur in swayback (Innes and Shearer, 1940; Barlow, Purves, Butler and Macintyre, 1960; Cancilla and Barlow, 1960). Histologically, however, they were distinguishable from those of swayback. They consisted of hyperplastic and bizarre glia similar to those found elsewhere in the CNS, upon which the focal necrotising or inflammatory processes had been superimposed. In this respect they had features in common with the lesions produced by foetal infection with blue tongue vaccine virus (Schultz and Delay, 1955; Young and Cordy, 1964) and the single case of congenital encephalitis and axial necrosis in a newborn lamb reported by Sigurdsson, Palsson and van Bogaert (1961). The lesions, unlike those in the cases of Young and Cordy, were not mineralised. These authors attributed the softenings to hypoxia consequent upon occlusive vasculitis, but there is little to suggest that such may be the case in our lambs.

There is no evidence to suggest that copper deficiency is involved in Border disease (Hughes *et al.*, 1959), but in view of the occurrence of lesions of the cerebral white matter it is unfortunate that copper assays were not performed on the tissues. Since all the sheep were maintained indoors on hay and concentrates, a type of management conducive to copper storage (Allcroft and Lewis, 1957) and since control lambs remained normal, it seems unlikely that swayback complicated the experiment. Furthermore, significant neuronal degeneration, a constant feature of swayback (Barlow *et al.*, 1960) was observed in only one case, and not one of those with cerebral cavitation. In this instance generalised hypomyelinogenesis and glial hyperplasia were so severe as to result in axonal destruction and the neuronal changes have been interpreted as secondary to this axon loss.

The findings of substantial amounts of interstitial lipid requires discussion. Such lipid has been found previously (Barlow and Dickinson, 1965) in Border disease in very young animals, but its inconsistency amongst the youngest and most severe cases in the present series is puzzling. With the light microscope its exact location is also obscure. Its histochemical properties, however, resemble those of the tiny lipid droplets present in the interstitium of normal myelinating white matter in the sheep foetus (Barlow, 1969). This lipid may represent an accumulation of myelin precursors, the utilization of which has been impaired by the disease.

The histological appearances of the placentae in the cases of experimental Border disease so far examined indicate that this organ is not directly affected by the disease, but may undergo premature involution as a consequence of foetal ill-health. In the normal sheep it is not thought that placental transfer of immuno-

globulins occurs (Barboriak, Meschia, Barron and Cowgill, 1958), but it is conceivable that the superficial villous necrosis and separation of foetal and maternal cotyledons in Border disease might allow the passage of maternal serum proteins to the lamb in utero. In these circumstances a pre-colostral lamb serum would be expected to have a immuno-electrophoretic pattern very similar to that of the ewe. As this is not the case (Gardiner, 1967) it is suggested that the gamma globulin which is found in 70 per cent. of the pre-colostral lamb sera in the inoculated group, is foetal in origin. Similar situations have been shown to exist in the pig, and the experimental production of immuno-globulins by foetal lambs and pigs has been clearly demonstrated (Silverstein, Thorbecke, Kraner and Lukes, 1963; Šterzl, Kostka, Řīha and Mandel, 1960; Binns, 1967). The gamma globulin in question here may be non-specific in character or it may be produced in direct response to an antigen or antigens of the Border disease agent or indirectly to antigens arising from tissue damage. Further information is, however, required on the nature of the Border disease agent before a satisfactory answer can be given to these questions.

The experimental findings, as a whole, confirm and extend an earlier view (Barlow and Dickinson, 1965) that there is no complete correlation between the degree of hairiness, the presence of neurological signs and the severity of the pathological changes in Border disease. In the present experiment the age of the foetus at infection does not appear to have influenced the outcome (over the range 27 to 72 days). In part, however, this lack of correlation may be a reflection of the rather arbitrary methods of scoring some of the observations. It is clear that the diagnosis of Border disease has inherent difficulties and that more precise diagnostic criteria are urgently required.

SUMMARY

Border disease of lambs has been reproduced experimentally in very high incidence by the inoculation of pregnant ewes with a brain/spleen suspension from affected lambs. The causal agent has not been identified.

Abortion was a significant component of th experimental syndrome, but live-born lambs showed the typical clinical and pathological changes associated with the natural disease. In addition, cystic lesions of the cerebral white matter were found. Grossly these resembled the cystic lesions in swayback, but were distinguishable microscopically. Precolostral sera of 14 out of 20 affected lambs and foetuses contained 7S gamma globulin.

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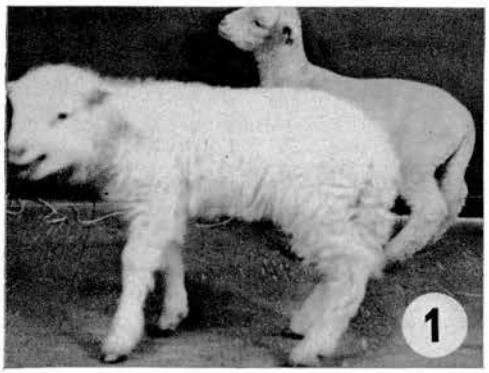

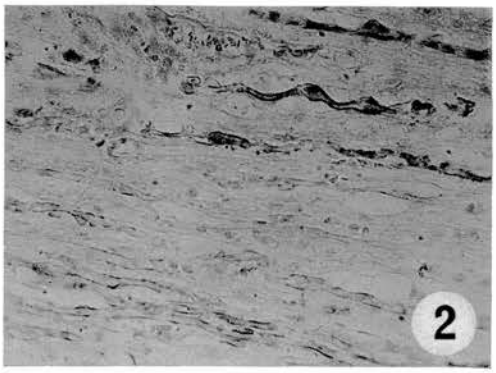

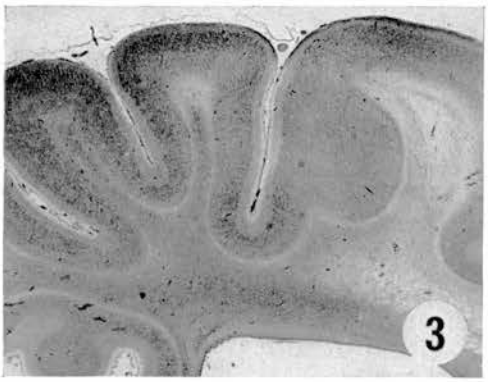

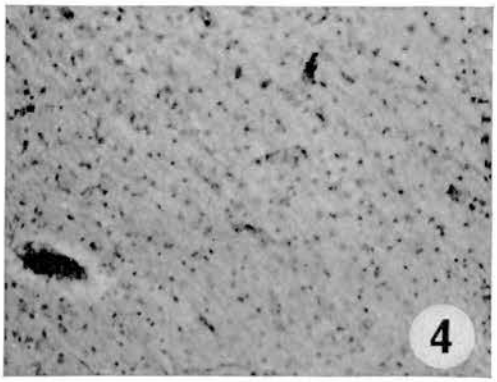

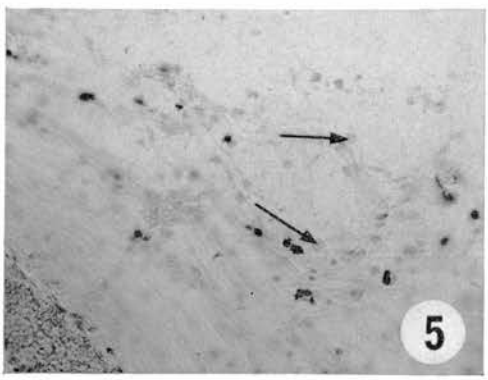

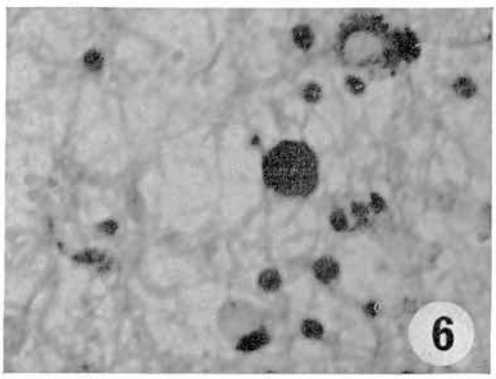

LEGENDS

Fig. 1. 4 day old Dorset Horn lamb with experimental Border disease. The fuzzy outline is a result of the tremor and the coarse halo hair. In the background is a normal 4 day old D.H. lamb.

Fig. 2. L.S. spinal cord of lamb with experimental Border disease showing beaded staining and ghost-like appearance of myelin sheaths. Smith Quigley × 190

Fig. 3. Gelatinous softening of subgyral white matter in experimental Border disease. H. & E. × 2.5

Fig. 4. Edge of gelatinous lesion. The hypercellular nature of the tissue is evident, capillary endothelium and pericytes are prominent and there is perivascular rarefaction of myelin. PAS × 80.

Fig. 5. Edge of gelatinous lesion showing several heavily stained compound granular corpuscles and other non-lipid containing macrophages (arrow). Sudan IV × 190.

Fig. 6. Edge of gelatinous lesion showing a PAS +ve plaque and 2 macrophages. PAS × 320.

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Congenital Heart Disease in the Fowl

In this paper on congenital heart disease (CHD) in fowls, special attention is paid to ventricular septal defects (VSD) (Siller 1958) and the aortic dextroposition complexes (Siller 1967).

In commercially reared fowls, CHD occurs with an incidence of about 0·6% of autopsied birds (Siller & Hemsley 1966) and VSD is the most common form. The low muscular type of defect causes severe clinical symptoms and affected birds do not generally survive for more than a few days. The prognosis is equally poor for birds with large subaortic defects, unless the left ventricular orifice of a subaortic defect is surrounded by 'lips'. These lips consist of proliferated endocardial tissue and appear to form a compensating mechanism whereby, during ventricular systole, the left-to-right shunt may be prevented or minimized. These structures seem to occur in no other animals.

There is statistical as well as anatomical evidence to indicate that a VSD in the fowl can close during post-embryonic life. In view of the very high population incidence of VSD in certain inbred lines, ranging from 3·6–84%, a hereditary ætiology is strongly suggested. The embryological development of these spontaneous defects was studied by Rychter *et al.* (1960).

Overriding aorta is the most common form of aortic dextroposition encountered in fowls. Complicated forms such as Fallot's tetrad have not been seen in birds and, with the exception of one case of complete transposition, the pulmonary artery is always normal in size and position. Incomplete transposition, in which both the aorta and pulmonary artery arise from the right ventricle, can usually be recognized when the thorax is opened because the apex of the heart is dextrorotated by up to 90 degrees. A fairly normal circulation may be maintained in some cases because the aorta lies directly over the orifice of the VSD, thus the phenomenon of 'streaming' may prevent any significant mixing of venous and arterial blood.

In a recent, as yet incomplete, study (Siller & Carr, unpublished) involving more than 6,000 embryos and newly hatched chicks from seven different breeds and lines, an association was often found between sternal fissure and CHD. Only about a third of the 72 birds affected with sternal fissure had normal hearts. The aorta was transposed to the right ventricle in 25 others while in the remainder other forms of CHD were present. It has not been possible, so far, to implicate incubational or other environmental factors in the development of this syndrome, nor is there as yet any substantial evidence to suggest a hereditary basis. This work is being continued.

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Influence of Environmental Agents on Mammalian Fœtal Development

The production of new structures through complex chemical processes during prenatal development is the result of a constant interplay of hereditary and environmental factors. The expression of the inherited genes is dependent on the environmental conditions which can modify or prevent the development of genetically determined structures. The same pathological end results can arise from hereditary and from environmental disturbances.

Among the agents capable of modifying fœtal development are physical factors like X-rays and anoxia, infections such as rubella, toxoplasmosis and rickettsia, endotoxins and a very large variety of chemicals. Only for a few of them has it been possible to give proof of their noxious effects in the human embryo.

The influence of environmental conditions is dependent on the developmental stage of the embryo, on its genetical susceptibility and on the physiological or pathological condition of the mother.

The reaction of the embryo to a specific compound varies not only between different animal species, but also within a given species, between strains and even between individuals of the same strain.

The difference in reaction to teratogenic agents in various animal species can be related to the particular metabolic pathways involved.

Influence of drugs: Drugs which can act on general growth, leading to fœtal death, include antimitotics, antimetabolites, long-acting sulphonamides, dicumarol and quinine. Other compounds, like actinomycin D, inhibit the growth potential of the fœtus, leading to congenital malformations.

Aminopterin and x methyl-folic acid are embryotoxic and have a teratogenic activity in

several species including the human fœtus. The size and weight of the fœtus are generally much below normal.

In humans marked size differences have been reported after aminopterin and after busulphan treatment. Infants born of mothers who are smokers weigh less than those of non-smokers at any stage of their development. The prematurity rate is also twice as high when the mothers are smokers.

Nicotine is teratogenic in the mouse and particularly affects the skeleton.

Variations of the morphological type of malformation: Experiments in rodents with various drugs show that the reaction of the fœtus varies in relation to the developmental stage. For example, between day 6 and day 9, 6-mercaptopurine determines central nervous malformations in the rat, while after day 11 leg anomalies are produced. These anomalies are similar to those produced in humans by thalidomide.

Relation to chemical structure: Results obtained with purine analogues and oral hypoglycæmic agents are significant examples of the fact that neither the chemical structure nor the pharmacological properties of a drug allow one to predict their possible action on the fœtus.

Remote detectable effects: Certain drugs or environmental factors do not produce obvious effects during fœtal development but striking examples of remote drug action have been observed. For example, after administration of high doses of cortisone to pregnant rats (20 mg/kg daily from day 6 to day 16) there is no apparent deleterious effect on the evolution of pregnancy and the fœtuses are normal in appearance though they are extremely frail and the majority die in the second or third week of post-natal life.

Interesting adjustment disturbances have been reported in manganese-deficient rats and guinea-pigs. The newborn have a normal appearance but they develop ataxia due to malformation of the vestibular component of the inner ear. It has also been observed that, in the mouse, manganese deficiencies produce anomalies of equilibrium which are real phenocopies of the genetical ataxia known in these species. If these genetically abnormal mice are fed an extra amount of manganese during pregnancy the occurrence of ataxia can be prevented.

Pseudo-dysmorphogenesis: Somatotrophic hormone (STH) has been considered by many biologists as a stimulant of embryonic growth. However, from studies in rats it can be concluded that STH administered to the mother does not stimulate embryonic growth but inhibits it. The overweight of the newborn is related to a prolongation of the gestational period.

Haloperidol, a tranquillizing drug, when administered in doses of 2–10 mg/kg in pregnant rats or mice determines in 30% of litters a striking inhibition of growth. Fœtuses are apparently normal but are undersized. By exploratory examination of the uterus as well as by unilateral hysterectomies it could be demonstrated that the growth inhibition of haloperidol is due to delayed implantation.

Conclusion

In comparison with the present-day safety of the newborn, the unborn has to face dangers which are greater than at any previous period of time. Disinfectants, pesticides and many drugs harmless to the mother can have deleterious effects on the fœtus and determine impairment of prenatal growth, congenital malformations or remote disorders which account for postnatal mortality.

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Disease and Development of the Nervous System

The developing organism is susceptible to as wide a range of disease-producing factors as the adult. In mammals the uterus may protect the fœtus from some of these factors but under certain circumstances, e.g. isoimmunization, it may also provide a hostile environment.

A disease-producing factor can act upon a developing tissue in three ways: it may destroy it; it may depress cell function or division thereby arresting development; or it may stimulate cell division and result in exuberant growths of normal or aberrant tissue. The susceptibility of the tissue to disease-producing factors will vary according to the rate of cell division and the degree of differentiation reached, and is influenced by factors such as 'substrate' competition and the functional competence of the developing organism's defence mechanisms.

The interactions of disease and development are thus complex. Most embryonic organs arise with initial moments of intense activity and brief encounters with disease at these moments can result in death or gross deficiencies which may preclude further development. Morphogenesis is more gradual and an encounter with disease during the various phases of this process can lead to a variety of malformations. Development of the nervous system is especially protracted and interaction with disease can produce a range of lesions, the final appearance of which may obscure the actual pathogenesis.

Two diseases of newborn lambs – swayback and border disease – illustrate this concept. In both there is a myelin defect. Swayback is associated with copper deficiency and has been regarded as a demyelinating disease. Serial pathological examinations, cytological histochemistry and electron microscopy have recently yielded information which suggests that failure of the neurone to sustain its axon is the basic lesion. The effect on the myelin sheath, if such is present, is a consequence of this and thus swayback is not a demyelinating disease.

Border disease is caused by a transmissible agent and congenital hypomyelinogenesis is the main neuropathological manifestation. Preliminary ultrastructural studies indicate that faulty differentiation of spongioblasts into myelin-forming oligodendroglia and astrocytes may occur. In this event both border disease and swayback can be regarded as malformations.

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Experimentally-induced Hypomyelinogenesis and Cerebellar Hypoplasia in the Pig

Outbreaks of congenital tremor in pigs in Britain may be classified pathologically (Done & Harding 1967) as:
Type A: Cases with lesions of the central nervous system: I Cerebellar hypoplasia, usually with spinal hypomyelinogenesis. II Spinal hypomyelinogenesis without cerebellar hypoplasia.
Type B: Cases without observed lesions of the central nervous system.

The following evidence suggested that congenital tremor type AI was the result of transplacental infection with particular strains of swine fever virus:
(1) In Great Britain the disease was found to be almost constantly related to antecedent swine fever infection of the dam (Harding *et al.* 1966).
(2) After the eradication of swine fever by slaughter no further cases of congenital tremor type AI have been seen.
(3) Although infection of pregnant sows with swine fever virus was not rare prior to June 1965 (Campbell 1965), congenital tremor occurred as an epidemic in some herds but not at all in others. Typically it was not associated with malformations other than of the central nervous system.
(4) Workers in several countries have produced experimental transplacental infection of pig fœtuses with strains of swine fever virus but none has reported the occurrence of congenital tremor in the progeny of experimentally infected sows.
(5) Isolations of clinically mild strains of swine fever virus, but of no other virus, have been made from pig fœtuses showing cerebellar hypoplasia before birth.

Growth studies on the fœtal pig cerebellum (Done & Hebert 1968) had indicated that the probable period of maximum vulnerability was after about sixty-two days' gestation. In an attempt to infect the fœtal CNS before this stage, Done & Harding (1968) inoculated 9 pregnant primiparous sows at 10, 15, 20, 25, 30, 35, 40, 45 and 50 days of gestation with a strain of swine fever virus which had been isolated from pig fœtuses in a naturally occurring outbreak of congenital tremor with cerebellar hypoplasia. Another similar sow was retained as an uninoculated control. None of the sows showed any clinical abnormality during pregnancy, though all but the control developed precipitating antibodies to swine fever by the time of parturition.

All the experimentally infected sows farrowed litters of normal size without significantly raised pre- or intra-partum mortality. Some or all of the piglets from all the infected sows showed symptoms of congenital tremor; all the piglets of the control sow were clinically and morphologically normal.

Piglets in affected litters showed varying degrees of cerebellar hypoplasia and dysplasia, with retarded myelination of the brain and spinal cord and reduction of spinal cord size. All the piglets which showed symptoms had lesions of the brain and/or spinal cord, but a few animals with definite lesions of the central nervous system were clinically apparently normal.

The developmental defects in the cerebellum and spinal cord were more severe in the clinically affected progeny of sows infected earlier in pregnancy but there was considerable variation within litters. However, the defects were qualitatively similar in all cases and not referable to the stage of development of the fœtal central nervous system at the time that the sows were exposed to infection. It is considered that this strain of swine fever virus exerts specific effects on particular cells, and that, provided infection occurs before their phase of maximum vulnerability is past, the actual time at which infection of the fœtus occurs is relatively unimportant.

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Parental Injuries to Offspring

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Parental Injuries to the Offspring in Various Animal Species

It is only within recent years that more attention has been paid to maternal behaviour patterns in animals; previously workers were more interested in studying other kinds of behaviour such as dominant-subordinate relationships, courting, mating and nest-building.

Great care should be taken when comparing reports on maternal behaviour in animals and man since in animals information is obtained by observation and experimentation whereas in humans subjective information can be obtained.

Classification
Maternal injuries to the offspring in animals generally occur accidentally or as a result of maternal inexperience, but aberrant maternal behaviour may account for some injuries. It is preferable to group injuries according to the signs shown by the offspring and to recognize that different ætiological factors may be responsible. In animals the following injuries may be inflicted: cannibalization, crushing and laceration.

Cannibalization
Pigs: True cannibalism occurs quite commonly in pigs, especially under those systems of management where cross-suckling occurs.
Dogs: Quite frequently puppies may be accidentally cannibalized and this is especially so in the prognathic breeds as the mouth formation makes severing of the umbilical cord difficult. The cord may be chewed in such a way that the surrounding abdominal wall is removed and the pup is eviscerated.
Cats: Accidental cannibalization is rare, but cases of true cannibalization in the post-partum rest period have been recorded.

Crushing
Puppies of the larger breeds of dog and also piglets are often accidentally crushed by their mothers whereas in kittens this seldom occurs. Accidental crushing may occur because the mother does not respond to the cries of the young, but other contributing factors are high mother/offspring weight ratio, large litters, and restricted parturition space. Increased maternal anxiety resulting from disease or a number of external stimuli may result in abnormal behaviour causing the mother accidentally to stand or lie on her offspring.

Laceration
These injuries are most commonly caused accidentally during play, as a result of corrective training or when the offspring which have strayed from the nest are retrieved too roughly. Cats rarely intentionally injure their offspring, but puppies and piglets may be deliberately attacked by their mothers. In puppies severe head injuries resulting from maternal aggression have been recorded.

Apart from these injuries some offspring may die as a result of hypothermia caused by the mother neglecting, rejecting or scattering her young. Others may die as a result of the mother licking the offspring excessively immediately after birth. The reasons for these actions on the part of the mother are not understood.

It is impossible to estimate the number of offspring maimed or killed in these ways since no established channels for reporting and recording exist.

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Maternal Diet and Perinatal Death

As it is impossible in a short communication to review all maternal dietary deficiencies, I shall restrict my remarks to protein-calorie deficiency, a common condition which can be brought about by underfeeding or by the consumption of diets inadequate in quantity or quality of protein (Platt *et al.* 1961).

The requirements change with the developmental and physiological state of the individual. In addition, stresses such as infection and trauma increase the requirements for protein, so that a diet which is just adequate for a healthy individual can, during episodes of stress, become frankly deficient. Dietary requirements can best be defined by the single term of Net Dietary-protein Calories per cent (NDpCal%), that is the proportion of dietary calories which, as protein, is fully utilized for anabolic purposes. The suggested

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HYDROCEPHALUS IN CALVES ASSOCIATED WITH UNUSUAL LESIONS IN THE MESENCEPHALON

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INTRODUCTION

Hydrocephalus in cattle is not uncommon. It has been described as a single entity (Houck, 1930; Cole and Moore, 1942); in conjunction with chondrodystrophy (Seligman, 1904; Crew, 1923–24; Wriedt, 1930; Gregory, Mead and Regan, 1942; Berger and Innes, 1948) and associated with acroteriasis by Dyrendahl and Hallgren (1956). In these cases a hereditary basis was suggested, a recessive gene being most usually implicated.

The purpose of this paper is to report the results of an investigation into an outbreak of hydrocephalus in calves, the aetiology of which was obscure, but in which a characteristic and possibly unique histological lesion was found.

RESULTS

History

The outbreak occurred in 2 successive years in a well-managed herd of 82 Ayrshire cows. Calving took place between August and February and in the first year 10 calves were hydrocephalic. All calves were born alive and at full term, though retained placenta was not an uncommon sequel. In this season also a calf was born which showed torticollis and outward rotation of the right forefoot but no hydrocephalus. In the second year 5 animals aborted and 3 calves were stillborn. Four liveborn and 1 of the stillborn calves showed lesions of the brain disorder. Two cases were from cows which had produced affected calves the previous year.

Cases occurred in calves of both sexes and amongst the offspring of heifers and cows up to 6 years of age. The female stock was all home-bred with the exception of 6 heifers, all of which bore normal calves. All the dams of affected calves may have been the progeny of a single bull, but the records were not sufficiently complete to confirm this. Two Ayrshire bulls, aged 3 and 5 years and purchased from different sources, sired all the calves born in the first season and most of those in the second year. Cases occurred among the offspring of both these bulls, but not among the progeny of a third Ayrshire bull purchased in October of the first year. In March of the second

year a Shorthorn bull was also acquired and no cases occurred among the 60 calves born to him.

Management was good and had remained unchanged for a number of years. The cows grazed outside from April to November and were housed in courts during the winter. Young stock and dry cows were fed turnips and straw, and milking cows received a ration of dairy nuts, oats, draff, silage, hay, straw and a magnesium rich mineral mixture. Normal fertiliser practice was carried out, but, since soil analyses performed during the second year of the outbreak had revealed that 9 out of 9 samples were low in cobalt, the young grass was dressed with cobalt sulphate at 2lb/acre in the following spring. Six out of the 9 samples were also on the borderline of copper deficiency.

Clinical Signs

Parturition was uncomplicated though in almost every case there was an excessive volume of foetal fluids which were white and cloudy and of an unusually slimy consistency. With 1 exception the hydrocephalic calves were born alive, but were unable to stand and usually died within 48 hours. The frontal region was domed, the eyes protruded and there was usually nystagmus. Rectal temperatures were normal. In addition to the 15 cases, a calf was born which showed no craniomegaly, exophthalmos or nystagmus, but which had torticollis and outward rotation of the right forefoot. This calf was able to walk only with difficulty.

Pathology

Of the 16 abnormal calves, only the last 10 were available for examination. One of these was stillborn, 6 died and 3 were killed within 48 hours of birth. No gross lesions were found in the viscera, musculature or skeleton apart from the head. The cranium was enlarged and domeshaped. In the more severe cases the fontanelles were open. In 1, the angle of the mandible was unusually acute (Fig. 1).

The meninges appeared normal. The cerebral hemispheres were dilated and fluctuant, the dorsal surface being longer and more convex than normal. This distention was accompanied by thinning of the brain substance of the hemispheres which was especially evident in the occipital poles. The gyri were for the most part narrow and shrunken, though ventro-laterally some were broad and flat. The sulci were shallow. The posterior extremities of the occipital lobes tended to overlie the cerebellum to a greater extent than normal (Fig. 2). In 2 cases the optic nerves were narrowed and in 1 of these the infundibulum was not apparent.

Coronal sections revealed massive dilatation of the lateral ventricles, the brain substance of the cerebrum being reduced to a thin rind (Fig. 3). The cortex was narrowed and in 2 cases was a dirty yellow colour and showed a laminar zone of separation from the underlying white matter. The latter showed varying degrees of thin-

ning and in most cases was abnormally tough and resilient to the touch. The septum pellucidum was fenestrated or represented by a vestigial fragment hanging from the roof of the cavity. The hippocampi were thinned in all cases and in the 2 most severely affected brains the fornix was entirely membranous. The 2 halves of the diencephalon were widely separated and usually showed some degree of dorsoventral compression—presumably the result of pressure within the ventricles. The third ventricle was also dilated.

In each specimen there was pronounced lateral narrowing of the mesencephalon and overlying of the posterior colliculi of the corpora quadrigemina by the anterior colliculi. The aqueduct of Sylvius was represented by a vertical slit which was extremely narrow at the isthmus beneath the anterior colliculi, but allowed the passage of a sound. The brain substance of the mesencephalon was markedly firmer than usual and the vessels appeared very numerous and prominent, especially in the more peripheral parts dorsally and ventrally.

The cerebellum appeared structurally normal in all cases, though in 1 it was reduced in size. There was no evidence of the Arnold-Chiari malformation; the foramina of Luschka appeared to be patent, and the choroid plexuses normal. The medulla and spinal cord exhibited no unusual features.

Histopathology

The pia-arachnoid was substantially normal, except for a patchy fibrous thickening without cellular infiltration over the parietal lobes and brain stem. In all specimens the cortex was compressed and a number of neurons were shrunken and atrophic, and occasionally necrotic. In the 2 cases showing yellow discolouration, necrosis was a more pronounced feature (Fig. 4). The zone of separation from the underlying white matter in these 2 calves was located in the region of the "U" fibres. This zone appeared relatively acellular and myelinated fibres were very few in number (Fig. 5). The remaining white matter in all cases had a porous, spongy appearance and was highly cellular. It consisted of a network of fine fibres in which myelinated nerve bundles were present but widely separated. Sudanophilic fibres, fatty globules, or gitter cells were not observed in the centrum semiovale. A somewhat flattened but intact ependyma was present. The hippocampus showed marked thinning, atrophy and compression of the neuronal and granular elements. The same picture of hypercellularity, pericellular haloes and neuronal shrinkage was observed throughout the brain stem, though both these features and the wide separation of myelinated nerve bundles became progressively less noticeable caudally.

In the thalamus and midbrain a proportion of the larger neurons showed margination of the nucleus, chromatolysis and granular disintegration. These changes were particularly severe in the red nucleus of 1 animal where in addition there were focal microglial

accumulations. There was no evidence of neuronophagia. Throughout the mesencephalon and extending a variable distance into the thalamus and pons there was a distinct proliferation of the small peripheral arteries. Each vessel had a broad cuff of proliferating fibrillary astrocytes, which gave the section a ragged, scarred appearance (Fig. VI). Usually the scarring was most prominent beneath the corpora quadringemina and in the region of the trochlear nerve roots. In no case, however, did the astrocytosis involve the immediate periaqueductal tissue. The ependyma of the slit-like aqueduct was normal except at the commissures where it showed slight fragmentation.

Though fully myelinated fibres were present in the optic nerves their general appearance was similar to that of the centrum semiovale in that there appeared to be an overall paucity of myelinated fibres. No consistent histopathological changes were recognised in the cerebellum, medulla or spinal cord.

Investigations

Bacteriological examination of brain and other organs from each calf gave consistently negative results, cultures being negative after 48 hours incubation under aerobic conditions. Cultures made from the stomach contents of the stillborn calf revealed no significant organisms after 6 days incubation in an atmosphere containing 10 per cent. CO_2. Mice inoculated intracranially with brain suspension from a typical case were alive and healthy 4 weeks after injection and showed no macroscopic brain lesions post-mortem. Blood from 1 calf was examined with the following results: P.C.V.—53 per cent; total R.B.Cs.—7·14/cu.mm.; haemoglobin—120 per cent. (16·6 g/100 ml.). Total W.B.Cs.—5,200 cu.mm. The P.C.V. is high.

Liver copper values of the 3 calves examined were within the normal range (470, 390 and 600 p.p.m. on a dry matter basis). Blood samples taken from 6 cows that had had abnormal calves within the period of a week to 3 months previously, showed normal copper values (0·08 to 0·10 mg./100 ml. blood). Blood taken from 1 calf gave the following results: calcium 8·64 mg. per cent. serum; magnesium 2·13 mg. per cent. serum; inorganic phosphate 2·7 mg. per cent. blood. The inorganic phosphate value is below the normal range.

DISCUSSION

According to Russell (1949) "the common background of hydrocephalus (in man) is formed by the single factor of obstruction, and the immense variety of pathological lesions productive of hydrocephalus have this single feature in common; all create an obstruction at some point in the pathway of the cerebrospinal fluid". In animals the pathogenesis is less well documented.

In cattle congenital hydrocephalus has been described along with various cranial manifestations of chondrodystrophy (Seligman, 1904; Crew, 1923—24; Wriedt, 1930; Gregory *et al.*, 1942; Berger and

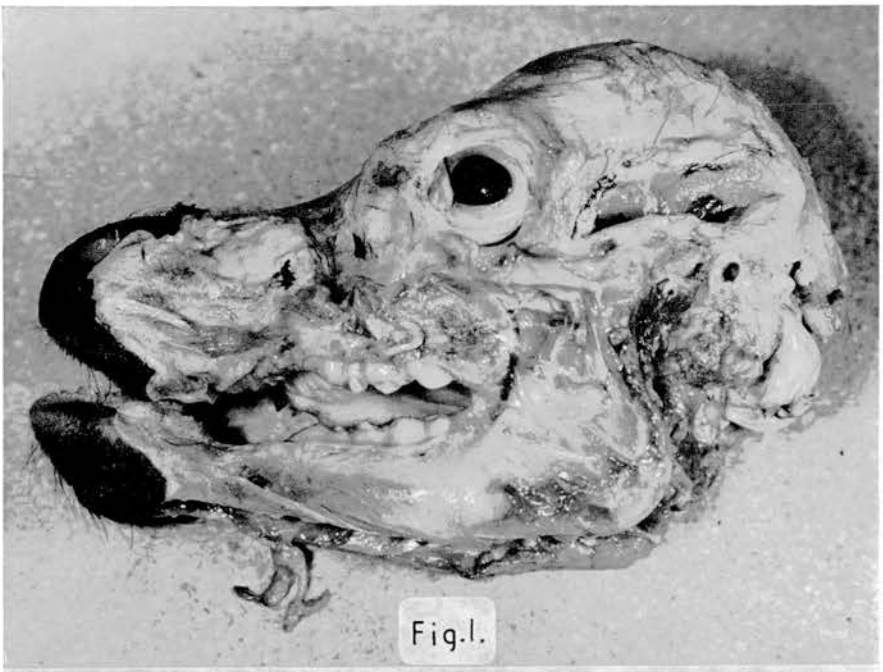
Innes, 1948). Ely, Hull and Morrison (1939) noted its presence in 1 out of 4 cases of agnathia in Jersey calves. Cole and Moore (1942) concluded that the abnormally ventral position of the foramen magnum might have resulted in compression closure of the foramina of Luschka in the cases they described.

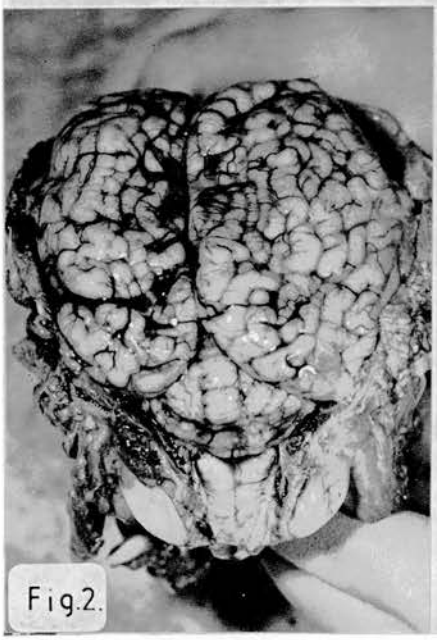
In the present outbreak skeletal deformity other than of the frontal region was not observed apart from 1 case in which the angle of the mandible was unusually acute. The occurrence of lateral narrowing of the mesencephalon with peripheral perivascular gliosis in all 15 cases of hydrocephalus, and more mildly in another calf which showed postural defects, suggests that these lesions may be of primary significance. Though the slit-like aqueduct was mechanically patent at post-mortem examination, the shape is such that in life the lumen could readily be occluded, especially once the hydrocephalus was established when pressure from the dilated cerebral hemispheres would tend to produce lateral compression of the midbrain. It is possible, however, that the entire midbrain deformity was due to compression by the cerebral hemispheres.

The severe perivascular gliosis which was present in the more peripheral regions of the mesencephalon does not appear to have been reported previously. It seems doubtful whether such scarring would necessarily result in slit-like deformation of the aqueduct, though its presence in all cases examined may indicate a primary role. The perivascular distribution of the glial scars is suggestive of a post-inflammatory state of haematogenous origin operating in the latter part of pregnancy. The discoloured, abnormally viscous excess of foetal fluid with which each case was associated would support a theory of intrauterine infection or toxaemia. Malformations may result from maternal virus infections in the human subject (Gregg, 1945) and Cordy and Schultz (1961) have reported ataxia in lambs which may have been the result of infection with the virus of bluetongue in early pregnancy. However, the absence of clinical illness in the dams and the fact that cases occurred over a 6 month period in 2 successive years, without any evidence of immunity, does not support the theory of an exotic infection. Furthermore, the only stock introduced immediately prior to the outbreak were virgin heifers from a farm with no history of a similar condition, and none of these gave birth to affected calves.

Since all the dams involved may have been the progeny of 1 sire, and since 2 bulls (which also may have been distantly related) sired all the affected calves, whilst 2 other completely unrelated bulls left no cases, there may be a case for considering a genetic aetiology. However, the records are too incomplete to confirm or refute this.

Millen, Woolam and Lamming (1954) have shown that hydrocephalus in rabbits may result from maternal deficiency of vitamin A. Hypovitaminosis A is unlikely to have been implicated in this outbreak since the cows spent the preceding months at grass. Assuming that the defect originated at approximately the same stage of preg-





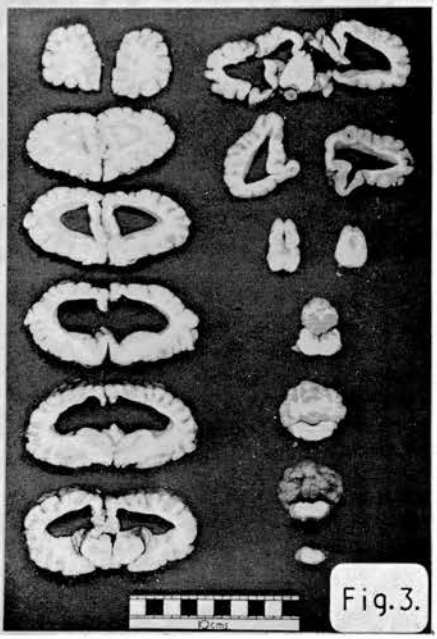


Fig. 1. Note the slight doming of the roof of the cranial vault and the acute angle of the mandible.
Fig. 2. View of the distended brain from above and behind.
Fig. 3. Coronal sections of hydrocephalic brain. Note the gross dilatation of the lateral ventricles, the lateral constringency of the mesencephalon and the slit-like aqueduct.

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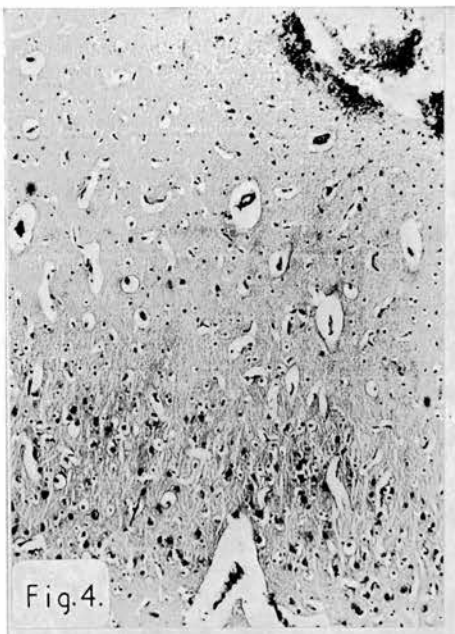

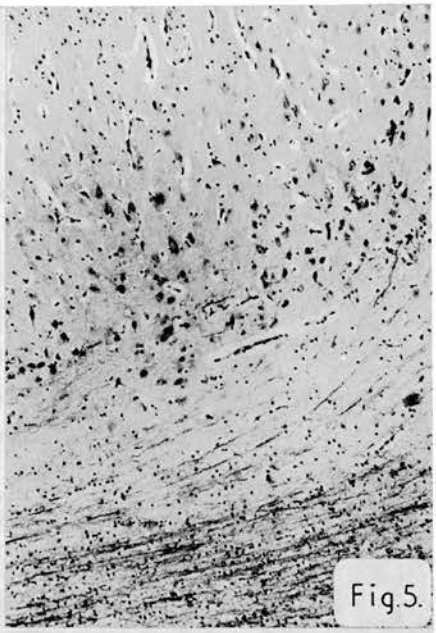

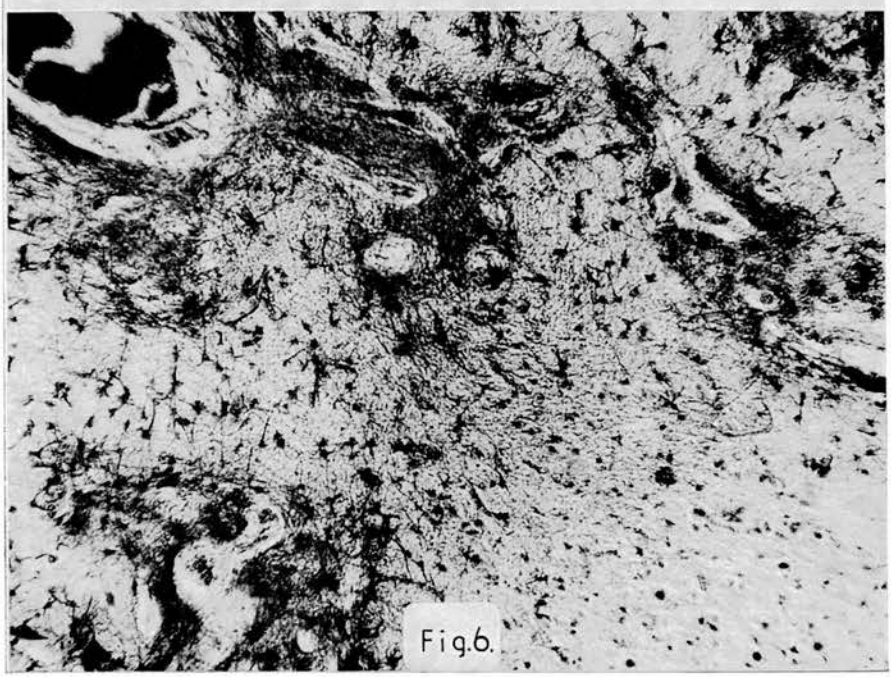

Fig. 4. A portion of the cerebral cortex from a case of hydrocephalus, showing slight round cell infiltration of the meninges, dilatation of the perivascular spaces and neuronal pyknosis and necrosis (H. & E. × 70).

Fig. 5. Cerebral subcortex from a case of hydrocephalus showing defective myelin staining in the region of the "U" fibres. This region is also relatively acellular (Luxol Fast Blue × 70).

Fig. 6. Midbrain from a case of hydrocephalus showing severe perivascular gliosis (Cajal × 90).

nancy in each case, several animals must have been grazing when foetal malformation commenced. Liver analyses are no help in this respect as vitamin A is not present in the liver of the newborn calf.

The hypercellularity and deficiency of myelinated fibres in the remaining cerebral white matter of the hydrocephalic calves is reminiscent of certain cases of swayback in lambs. However, the hyaline necrosis of multipolar nerve cells and myelin degeneration in the long tracts of the spinal cord found in virtually all cases of the latter condition (Barlow, Purves, Butler and Macintyre 1960) were not observed in any of the calves. Furthermore, there was no evidence of copper deficiency in the affected calves or their mothers.

CONCLUSIONS

Internal hydrocephalus is reported as a congenital condition in calves. Fifteen cases occurred during a 2 year period. The condition appeared to have resulted from obstruction of the cerebrospinal fluid pathway due to a slit-like deformation of the aqueduct of Sylvius. This was associated with lateral narrowing of the midbrain, the peripheral areas of which showed vascular proliferation and severe perivascular gliosis.

The aetiology was not determined, though various possibilities are discussed.

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AN ATAXIC CONDITION IN RED DEER (*CERVUS ELAPHUS*)

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INTRODUCTION

According to Cameron (1923), Parnell (1929-36), Darling (1937) and Whitehead (1950), red deer (*Cervus elaphus*) are hardy creatures reaching their prime at about 10 years of age and have a life span of 25 years or more. Apart from old age and injury, parasitic diseases are recorded as the main causes of death. Whitehead (1950), however, mentions an ataxia occurring in deer parks in red deer, wapiti, Pekin sika and yak, and implies that the condition is not uncommon. He notes that the ailment is commonly called "rickets", but does not himself subscribe to this view since the disease affects mature animals and is unaccompanied by bony deformities of the limbs.

Since the description of the gait of affected animals was reminiscent of swayback in lambs, and as no reference to a pathological evaluation of this condition was found, the opportunity to visit an affected herd was taken in August, 1962. This paper records the clinical findings and some information on the pathological and biochemical features of the disease.

MATERIALS AND METHODS

The deer park. The area was an enclosure of 232·5 acres of rough grazing and woodland situated in N.W. England at an approximate height of 150 feet above sea level. It was generally flat except for steep slopes where two confluent streams traversed the park. Woodland covered nearly 50 acres of the total area and consisted of mixed hardwoods and softwoods of poor quality intermingled with a fair number of shrubs, particularly rhododendron.

The pasture was rough and varied greatly in quality, and in places it was wet and rushes grew abundantly. About 10 years previously several hundred tons of low grade lime were spread over the whole area and in 1962, 4 areas varying between 7½ and 11 acres were dressed with basic slag and lime, or nitro chalk. This resulted in a general improvement in the grazing.

The population. The deer, prior to 1941 totalled about 300 and grazed over 1890 acres of parkland. In that year, due to the exigencies of war, the herds were severely culled and confined in the smaller area already described. The population distribution at the termination of our visit was as follows:-

	Red Deer (*C. elaphus*)	*Fallow Deer* (*Dama dama*)
Stags/bucks	5	13
Hinds/does	52	63
Followers	7	29
Total	64	105

Both types of deer grazed freely over the whole area but normally did not graze together. A small number of St. Kilda and Soay sheep were also maintained in the park.

The ataxic condition had been recognised for at least 50 years and the morbidity estimated at about 5 per cent. per annum. The condition was regarded as a slowly progressive disease of adult red deer, fallow deer being unaffected. Sometimes affected animals were found dead, but the majority were destroyed at an earlier stage.

Methods. A Land-Rover was used in order to approach the animals closely and a marksman with a small bore rifle was stationed amongst straw bales in a trailer. After a few hours the deer became used to the presence of the vehicle and could thereafter be approached from down wind within 30/40 yards for observation. Selected specimens were then shot at a point just above the elbow. As the animal went down it was approached on foot and where possible a blood sample taken from the jugular vein for copper analysis (Butler, 1962). The carcase was then removed to a nearby farm building for post-mortem examination, and the collection of tissues for microbiological, histological and biochemical investigation. A total of 8 affected red deer were examined, together with one apparently normal red stag and one 3 months old stag calf whose mother was severely ataxic, though the calf itself appeared normal. Six apparently normal fallow bucks were also killed and similarly examined.

The deer park was divided into 15 arbitrary areas according to local geography and landmarks, and herbage and soil samples were collected from each area for trace element analysis according to the modified procedure of Calder and Voss (1957) as described in earlier work on swayback in lambs (Barlow, Purves, Butler and Macintyre, 1960a). In view of the duration of the field study and the distance of the park from the laboratory all specimens for biochemical and microbiological examination were stored in an insulated cabinet with solid carbon dioxide (I.C.I. Drikold). Tissues for histological examination were placed in 10 per cent. formol-saline.

The laboratory methods were substantially the same as those employed in previous work with sheep (Barlow *et al.* 1960a and b). Certain minor modifications to histological techniques were made in an attempt to improve the quality of preparations from this unfamiliar species. In some cases, too, attempts were made to estimate cytochrome oxidase and succinoxidase activity in portions of frontal lobe which had been deep frozen, using the methods of Gallagher, Judah and Rees (1956) and Schneider and Potter (1943). Iron in liver and brain was estimated by the method of Trinder (1956).

RESULTS

Clinical Signs

The inability to approach closer than 30 to 40 yards precluded a detailed examination and no opinion regarding nystagmus, tactile reflex activity, or fine muscular tremors was formed; nor was any assessment of visual, olfactory, or auditory acuity possible, as the deer were always found in groups and showed a group response to the approach of the observer. When grazing undisturbed there was little evidence of disease amongst the red deer. Though the general condition of the herd was suboptimal there was little to distinguish the affected animals from the healthy in terms of size, conformation or bodily condition. At the time of the visit the antlers were fully grown and velvet was being shed. Stags with more than 10 points were not observed.

On approach the group would cease grazing and raise their heads and it was then possible to see which members of the group were affected. Small movements of the head appeared to throw the hind quarters off balance, and the animals would sag to one side or the other with little apparent attempt at postural correction. Sometimes this led to the animal pivoting about its forelegs. With movement, the defective posture usually became more apparent and though co-ordination of limb movements was not lost affected deer tended to trot with their hind quarters leaning, and the long axis of the body at an angle to the direction of movement. There was sometimes slight spasticity and such animals were inclined to stumble. If they fell, they appeared apprehensive and had difficulty in regaining their feet. At no time did affected animals appear to be in pain. Exercise did not cause any appreciable exacerbation of the ataxia. Clinical signs were not seen in fallow deer or red deer less than 2 years of age.

Pathology

Gross and histological examinations were made on 16 deer, of which 8 were ataxic and the remainder clinically normal. No consistent lesions were observed on gross examination of the viscera, musculature, skeleton, or nervous system, and evidence of intercurrent disease such as helminthiasis was insignificant.

The only consistent lesions were microscopic and situated in the spinal cord. They were present to a variable degree in all the clinical cases of ataxia and also in the non-clinical adult red deer, but were not found in the red calf or the fallow bucks.

Degenerate fibres were found scattered throughout the ventral and lateral columns, but were most numerous in the peripheral regions adjacent to the dorsal nerve roots and the ventral median fissure where the most advanced lesions were also found. The relative severity of the pathological changes varied not only from case to case, but also from one level of the spinal cord to the next. In almost every case the most advanced and widespread lesions were found in the

lateral funiculi in the cervical region, whereas in the lumbosacral enlargement they occurred in the ventral funiculi. Degenerating fibres were also traced rostrally through the lateral parts of the medulla into the restiform body.

In most instances examination of H. and E. stained paraffin sections was disappointing and showed only variable numbers of widely dilated fibres. Frozen sections stained with Sudan IV or by the methods of Glees or OTAN (Adams, 1959) however, clearly revealed the extent of the damage, variable amounts of free fat being present, both within affected fibres, and as globules within gitter cells or lying freely in the interstitium. Two animals showed particularly clearcut demyelination in these regions (Figs. 1, 2, 3) Coincident with the demyelinating process there occurred a fibrous gliosis which appeared to be more properly the result of proliferation of cell processes than of cell division (Figs. 4, 5). Though axons were ultimately lost their destruction was a relatively late phenomenon (Fig. 6). Since axonal swelling was less common than shrivelling and fragmentation, the loss of axons was attributed to crushing by the glial reaction.

Frank degeneration of nerve cells at any level of the C.N.S. was rarely observed. Large multipolar cells at all levels frequently appeared shrunken and eosinophilic and lay in retraction spaces, but the Nissl granules and neurofibrillary apparatus remained demonstrable. No clearcut lesions were found in the ventral horn cells or dorsal nucleus of the spinal cord (Clarke's column), dorsal motor vagus, lateral cuneate, dentate and cerebellar roof nuclei, Purkinjé cells, tectal or cortical neurons. In the brain stem, however, cells of the red, medial vestibular and olivary nuclei showed abnormal fineness of Nissl granules, or occasionally actual chromatolysis with fragmentation of neurofibrils. The nucleus of such cells was frequently pyknotic but rarely displaced to the margin of the cell. Necrotic cell fragments and empty cell spaces were found only in the red nucleus of the oldest case. A feature of the nerve cells of all the older deer (5 to 10 years) was the quantity of lipofuchsin contained in the cytoplasm. The amount of pigment seemed more closely correlated with the age of the individual than with the disease, as considerable quantities were also present in the cells of the fallow deer. The granules were most clearly demonstrated by Luxol fast blue (Figs. 7, 8 & 9).

In deer of all ages, whether diseased or not, hyaline or granular eosinophilic "bodies" were found displacing fibres in the vicinity of nerve cells. These bodies were particularly numerous in the region of the lateral cuneate nucleus and in H. and E. paraffin sections deceptively resembled the "hyaline" necrotic cells of swayback (Fig. 10). Their failure to stain by other methods strongly suggested that they were not nerve cells or fibres, but their true nature was not determined. Lesions of peripheral nerves were not observed, nor were abnormalities recognised in dorsal root ganglia, though

occasional fibres with dilated weakly myelinated sheaths were observed in the dorsal root.

Lesions of the cerebrum and cerebellum were inconstant and minor in degree. Occasional vessels in the white matter showed small zones of état criblé and small numbers of gitter cells in the Virchow-Robin spaces. In the cerebellum fat globules were inconstantly found in the granular layer in sites close to the Purkinjé cells or adjacent to the white matter.

All the deer except the 3 month old calf showed deposits of haemosiderin in the spleen. Those with most haemosiderin in their spleens showed smaller amounts in the liver and sometimes also in the kidneys (Figs. 11 and 12). No significant microscopic lesions were observed in other organs or tissues.

Bacteriological and virological examination of brain tissue from 4 cases failed to reveal any pathogenic organisms in culture or on inoculation into mice.

Biochemistry

The values obtained for the copper and iron content of the blood, liver and brain of affected and normal adult deer are summarised in Table I. Only a limited interpretation of these data can be made at the present time since there is no information in the literature on normal values for red deer.

It is possible to assess the results for adult animals by comparing those for the affected deer with those for the normals, but it should be borne in mind that the former were all red deer, whereas the latter were all fallow deer which belong to a different genus. If it is assumed that this distinction has no appreciable effect on the values certain conclusions may be drawn. As is indicated in Table I the copper content of the blood and liver of affected animals tended to be higher than that of the normals. In the case of the blood levels the difference is statistically significant ($P = 0{\cdot}01 — 0{\cdot}001$), but comparison of the liver values is complicated by the fact that the variance of the affected animals is much greater than that of the other group ($P = 0{\cdot}01 — 0{\cdot}001$). When a modified "t" test was applied (Fisher and Yates, 1948) the mean values did not show a significant difference. The values for the iron content of the liver show a similar but more exaggerated pattern and in this case it was possible to demonstrate that the difference between the mean values is significant ($P = 0{\cdot}01 — 0{\cdot}001$).

The levels of copper and iron in the frontal lobe of the cerebrum were similar in both groups, but the variance of the iron values was again significantly greater in the affected group ($P = 0{\cdot}01 — 0{\cdot}001$). The relationship between the copper and iron content of the liver suggested by these results was examined further by calculating correlation coefficients. Values of —0·66 and —0·16 were obtained for affected and normal animals respectively, but only the former is statistically significant ($P = 0{\cdot}5 — 0{\cdot}01$) indicating that iron tended

TABLE 1

COPPER AND IRON CONTENT OF WHOLE BLOOD, LIVER AND BRAIN (FRONTAL LOBE) OF ADULT DEER

Description		*Blood*	*Liver, p.p.m. D.W.*		*Brain, p.p.m. D.W.*	
		μg. Cu/100 ml.	*Cu*	*Fe*	*Cu*	*Fe*
Affected (Red deer)	Range	28·4 — 45·3	7·01 — 20·1	503 — 1736	2·19 — 4·93	72·4 — 125
	Mean ± S.D.	36·1 ± 6·4	10·9 ± 4·1	922 ± 430	3·85 ± 0·93	90·1 ± 18·3
	No. of samples	7	9	9	7	7
Normal (Fallow deer)	Range	16·2 — 28·9	6·74 — 9·59	212 — 505	3·30 — 5·26	79·2 — 83·9
	Mean ± S.D.	22·3 ± 4·4	8·57 ± 0·97	296 ± 105	4·22 ± 0·83	82·6 ± 2·3
	No. of samples	6	6	6	4	4
†Statistical significance of difference between mean values		**	N.S.	**	N.S.	N.S.

† The levels of significance are indicated thus:—
N.S.—Not significant (P = >0·05) **—Very significant (P = 0·01 — 0·001)

to accumulate in the liver as the copper content was reduced. This may be a sign that the copper status of these animals was low enough to depress haemopoiesis, but on this basis it is difficult to explain why a significant correlation was not found in the normal deer which had similar concentrations of copper in their livers and significantly lower concentrations of iron.

No succinoxidase or cytochrome oxidase activity could be demonstrated in any of the 6 brains tested, but this was regarded as a technical failure due to prolonged storage or insufficiently rapid and complete freezing after death.

Herbage and Soil Analysis

The results of the analyses carried out on the herbage and soil samples are summarised in Table 2. The levels of copper in both herbage and soil do not suggest a straightforward copper deficiency and there are no abnormally low values. Although the total copper content of soil is of limited value in assessing whether or not it is copper deficient, all the total copper levels are high compared with Scottish soils (Purves and Ragg, 1962).

As regards molybdenum, all the herbage levels are normal and much lower than those of teart pastures (20 – 100 p.p.m. D.M.) which cause a conditioned copper deficiency in cattle (Lewis, 1943). The cobalt levels in both herbage and soil are generally inclined to be low, and the whole area can be regarded as having a borderline or deficient cobalt status on the basis of the soil analysis.

TABLE 2

TRACE ELEMENT CONTENTS OF HERBAGE AND SOIL SAMPLES

Area	*Herbage* (*p.p.m. D. W.*)				*Soils* (*p.p.m.*)		
	Co	Mo	Pb	Cu	Co	Pb	Total Cu
1	0·12	0·8	3·9	7·6	0·34	4·4	16
2	0·08	0·9	4·4	5·2	0·29	1·8	14
3	0·10	0·8	0·4	6·2	0·27	1·1	17
4	0·15	2·4	1·8	10·9	0·26	1·1	13
5	0·12	1·7	1·3	6·5	0·36	1·0	18
6	0·12	0·8	3·4	7·7	0·31	1·0	18
7	0·05	1·2	2·9	6·2	0·33	0·9	21
8	0·05	1·6	3·0	4·9	0·29	1·0	16
9	0·07	1·3	3·7	7·3	0·27	2·1	21
10	0·07	2·0	3·6	5·9	0·19	3·3	25
11	0·10	2·0	2·8	7·5	0·23	1·5	22
12	0·29	2·5	1·9	7·0	0·29	1·3	23
13	0·37	1·7	3·2	8·0	0·17	3·4	20
14	0·15	1·4	2·4	11·4	0·28	1·2	23
15	0·09	0·8	1·8	8·0	0·34	0·9	13

Note:- Figures for cobalt and lead in soils represent acetic acid extractable contents.

The values of acetic acid extractable lead on the other hand are rather higher than is normal for Scottish soils (ca 0·5 p.p.m.). For example, in one hundred representative soil samples from arable fields selected at random in S.E. Scotland one of the authors (D.P.) found only one sample with an acetic acid extractable lead content greater than 3 p.p.m., whereas in this population of 15 soils there are 3 such samples. However, the lead contents of the herbage samples are not abnormally high, but these results are possibly less important because of the tendency of the lead content of herbage to vary with the time of year.

The results of the routine soil analyses are given in Table 3. Low phosphate levels are present in most of the areas. The pH values are surprisingly low in view of the liming history. Spectrographic analysis of the water samples showed insignificant quantities of cobalt, copper, lead, molybdenum, chromium and nickel.

TABLE 3

PH., LIME REQUIREMENTS, POTASSIUM AND PHOSPHORUS AVAILABILITY OF SOIL

Area	*pH*	*Lime requirement (cwt $CaCO_3$)*		*Available (lb./acre)*	
		pH 6·0	*pH 6·5*	*K*	*P*
1	4·5	H	H	101	5
2	5·4	30	70	105	5
3	6·2	—	20	96	3
4	4·3	H	H	145	5
5	5·4	40	70	145	2
6	4·6	90	H	160	3
7	6·3	—	15	165	2
8	5·6	20	60	188	2
9	5·4	30	60	110	3
10	5·8	20	60	105	3
11	5·3	40	90	155	3
12	4·9	80	H	96	3
13	4·4	H	H	125	6
14	4·3	H	H	188	6
15	5·2	H	H	135	3

H—denotes a lime requirement too high for normal application.

DISCUSSION

Since the work was undertaken because of a resemblance of the ataxia to swayback in lambs emphasis will be placed on a comparison of these two conditions. The diagnostic criteria of swayback have been defined as follows (Barlow *et al.*, 1960a):- ataxia of the newborn or young animal; cavitation or gelatinous lesions of the cerebral white matter and/or a characteristic picture of chromatolysis, neurone necrosis and myelin degeneration in the brain stem and spinal cord; low copper status.

In the present investigation ataxia was observed only in adult and adolescent animals and a 3 month old calf from a severely affected hind was both clinically and histologically normal. Though inconclusive in view of the small numbers involved, these findings indicate that the condition is not primarily one of new born and young deer calves. The abnormalities of gait were strikingly similar to those of swayback with the exception that in deer they did not become more apparent with exercise, but the impossibility of making observations at close quarters precluded detailed clinical comparisons.

Cerebral lesions of the type found in swayback were wholly absent from the deer, a finding which is consistent with the theory that the former are developmental in origin (Barlow *et al.*, 1960b). The degenerative changes of the brain stem and spinal cord were remarkably similar to those of swayback, both in distribution and the sequence of events occurring in the myelin sheaths, but they differed in two very important respects. In the first place chromatolysis and necrosis of large motor nerve cells were virtually absent. Secondly axons persisted in demyelinated regions until apparently crushed by proliferating glial fibres whereas in swayback sheath degeneration is accompanied by irregular axonal swelling which appears to be unrelated to the formation of the glial scar (Barlow, 1959).

Comparison of the copper content of blood, liver and brain with values obtained for swayback lambs must be made cautiously in view of the possible influence of species and age differences, but it is relevant to note that the values obtained for both affected and normal deer are similar to those observed in cases of swayback (Howell and Davison, 1959; Barlow *et al.*, 1960b). However, the copper content of the pasture in the deer park was not low and there was no history or clinical evidence of swayback in the sheep concurrently grazing it. It is evident from these considerations, that despite certain similarities, this condition in deer is not swayback as previously defined.

The accumulation of haemosiderin in the livers and spleens of the deer coupled with the discovery of low levels of cobalt in the soil and herbage is suggestive of a blood dyscrasia due to cobalt deficiency. Unfortunately the methods of catching and killing the animals were quite unsuitable for haematological studies. It is, therefore, interesting to compare the neuropathology with that of the subacute combined degeneration accompanying pernicious anaemia in man. In the latter, the damage commences in the mid-thoracic region, the dorsal funiculi are seriously affected, axonal degeneration occurs early and gliosis probably only takes place during remissions (Greenfield, Blackwood, McMenemy, Meyer and Norman, 1958). Thus it has little similarity to this degenerative condition of ventral and lateral tracts in which gliosis occurs early and axons tend to persist, and which may be defined provisionally as a ventro-lateral sclerosing myelopathy.

The clinical signs are not unlike those of Phalaris staggers in cattle which can be prevented by the administration of cobalt

(McDonald, 1942, 1946). Further comparison is not possible since the detailed pathology of the latter has not yet been described. The toxic species of Phalaris have not been found in Britian.

The white matter changes are similar to those of amyotrophic lateral sclerosis in man, but this ataxic condition of deer cannot be regarded as a system degeneration analogous to progressive muscular atrophy in man unless nerve cell loss and muscular atrophy appear very late in the disease and so have escaped detection in this series of cases.

The discussion has been confined to a possible nutritional aetiology, but the evidence presented does not exclude the possibility of infective or genetic causes.

CONCLUSIONS

An investigation has been made into a hitherto undescribed ataxia of adult red deer. Although it bears some clinical resemblance to swayback in lambs and is associated with similar levels of copper in the blood and tissues, the condition does not fulfil the diagnostic criteria for swayback

Comparison with other diseases of animals and man reveals some similarities, but the pathology appears to be unique and has been defined as a ventro-lateral sclerosing myelopathy. The condition is associated with a low cobalt status of the soil and herbage, but the significance of this finding has not been determined and the aetiology of the disease is obscure. Infective or genetic factors cannot be excluded at this stage.

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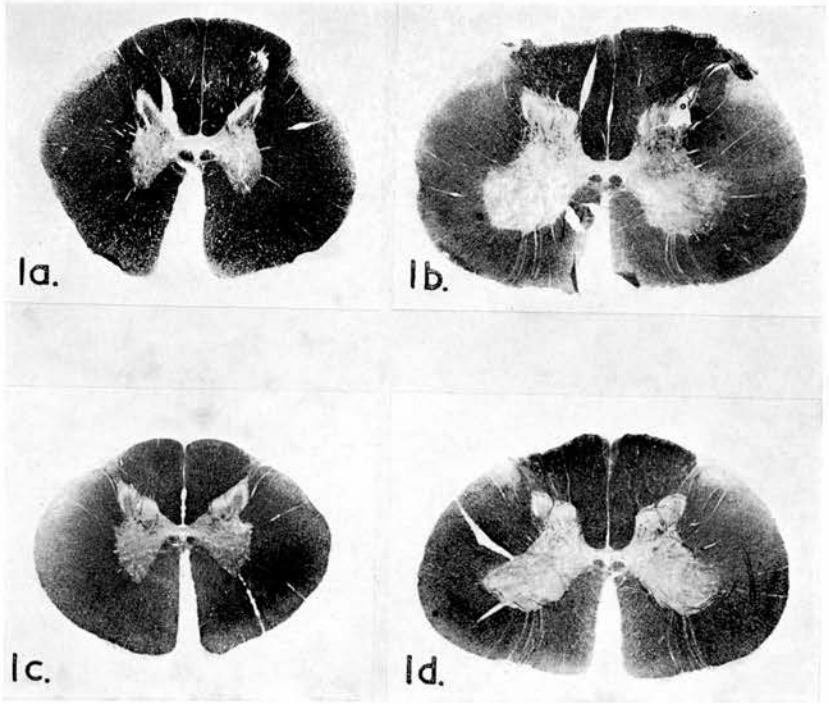

Fig. 1. T.S. spinal cord at different levels showing varying degree of demyelination (Smith Quigley × 3).

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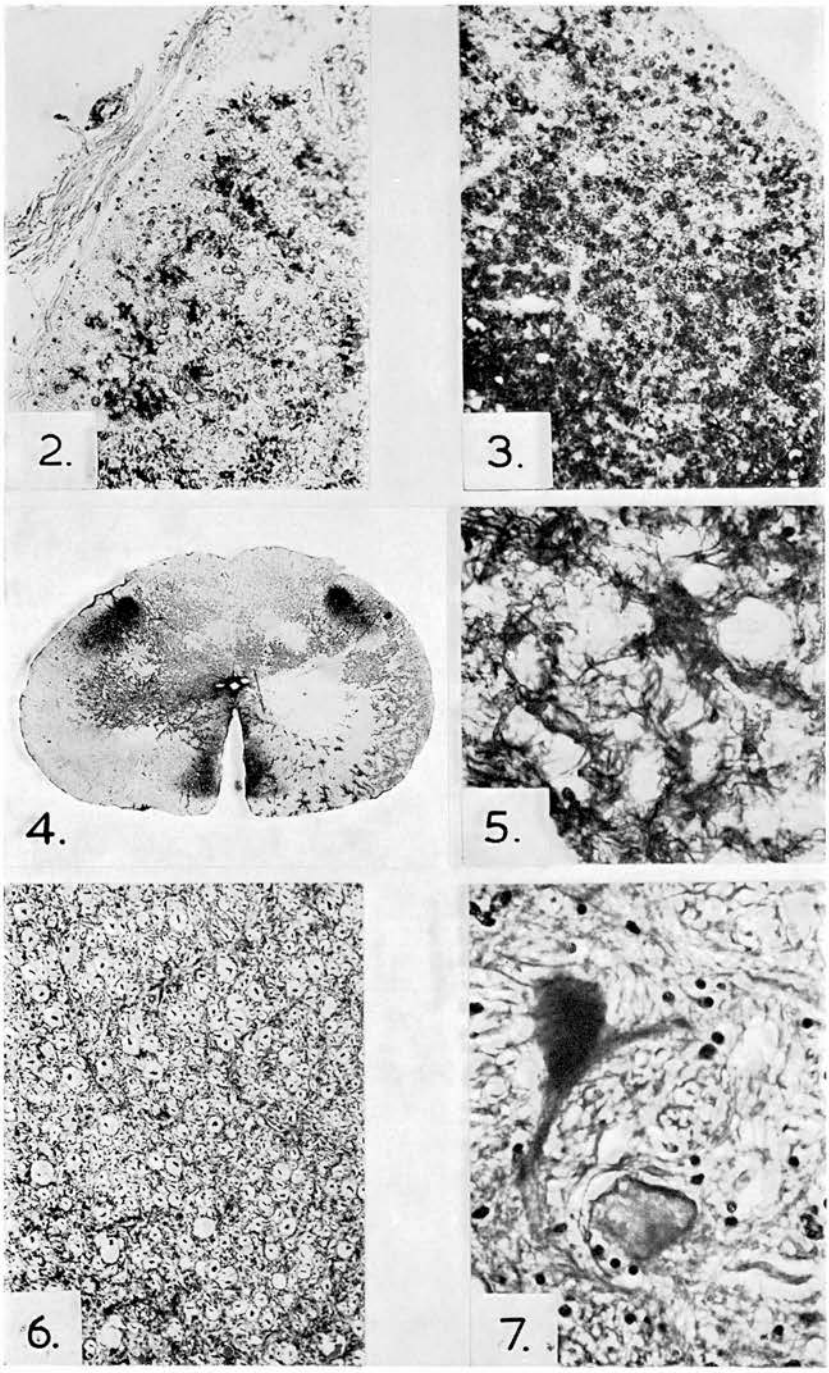
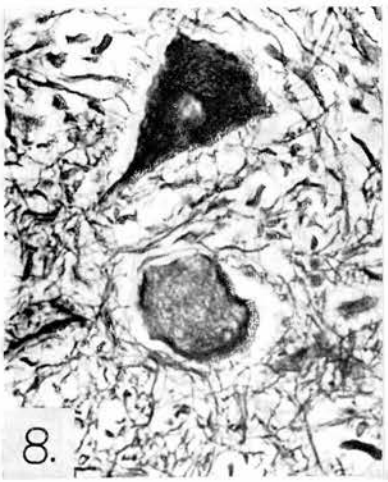
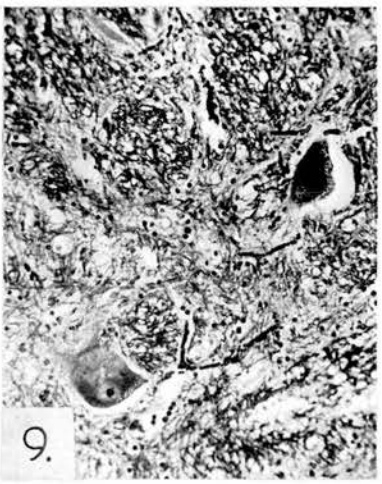
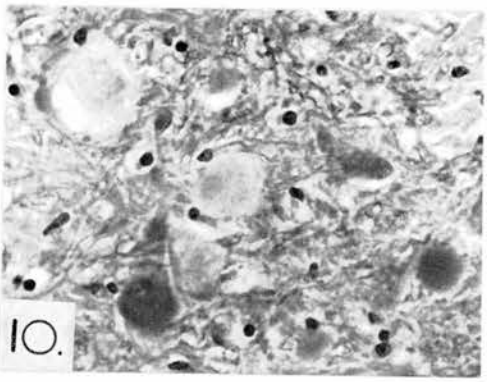
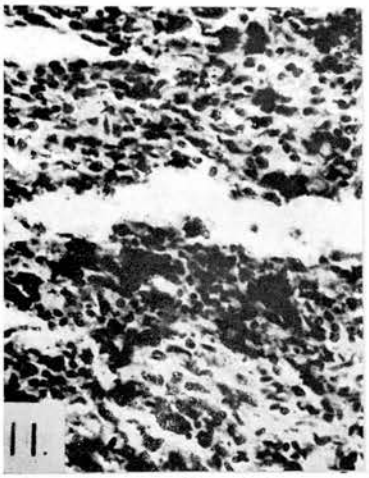


Fig. 2. T.S. spinal cord in dorsal part of lateral funiculus showing myelin degeneration and accumulation of free fat (Sudan IV × 100).
Fig. 3. T.S. spinal cord. Same area as Fig. 2. showing degenerating myelin and demyelination (OTAN × 70).
Fig. 4. T.S. spinal cord showing gliosis in demyelinated areas (Holzer × 3).
Fig. 5. T.S. spinal cord. High power view of a portion of the lateral funiculus. Same section as Fig. 4. showing gliosis (Holzer × 400).
Fig. 6. Same area as Fig. 5. showing general persistence of axons despite gliosis (Holmes × 100).
Fig. 7. Red nucleus of 10 year old ataxic deer showing chromatolysis (H & E × 400).









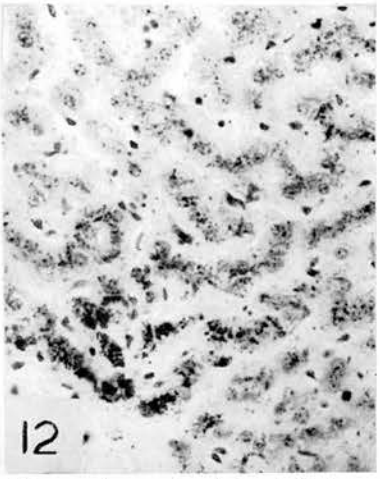


Fig. 8. Adjacent section to Fig. 7. showing disintegration and peripheral displacement of neurofibrils (Holmes × 400).

Fig. 9. Red nucleus of ataxic deer showing chromatolysis and intracellular accumulation of lipofuchsin granules (Scarba red, Luxol fast blue × 130).

Fig. 10. Hyaline and granular eosinophil bodies in lateral cuneate nucleus (H & E × 400).

Fig. 11. Haemosiderin accumulations in spleen of aged ataxic deer (Perle × 300).

Fig. 12. Haemosiderin accumulations in liver parenchyma and von Kupffer cells (Perle × 300).

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ADDENDUM

Since the manuscript of this paper was submitted an apparently identical condition under the title "Enzootic ataxia of red deer" has been published by Terlecki, Done and Clegg (1964). *Brit. vet. J. 120,* 311. The main findings of these authors are similar to our own though nerve cell and axonal degeneration are also described, and they considered that the disease is pathologically similar to swayback.

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Bright Blindness—A Condition Prevalent in Yorkshire Hill Sheep

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SUMMARY.—A previously undescribed blindness of sheep is recorded

This condition characterised by progressive degeneration of the neuroepithelium of the retina is widely distributed in hill flocks in the West Riding of Yorkshire. The incidence of the condition appears to be increasing and the disease is of considerable economic importance in certain flocks.

The similarities of the changes seen in the eyes to those seen in other types of retinal degenerations are discussed.

It is suggested that sheep grazing bracken (*Pteris aquilina*) on moors with a high proportion of bracken are particularly likely to become blind. The reason for the apparent association between blindness and bracken is not yet known.

Introduction

BLINDNESS in sheep has been reported in many parts of the world in association with lesions of the central nervous system (Hartley, 1956; Jensen, Griner & Adams, 1956; Mullins, Hartley & Salisbury, 1958; Hartley & Kater, 1959; Zlotnik, 1960; Wensvoort & van den Akker, 1960; Terlecki & Markson, 1961; Spence, Stevens, Saunders & Harris, 1961; Hartley, Kater & Andrews, 1962; and Zlotnik, Nisbet & Campbell, 1963). Affected sheep showed other central nervous signs and usually died rapidly. In all cases the primary lesions were in the brain and in these reports no mention is made of any histopathological examinations of the eyes from affected animals, and where ophthalmoscopic examinations were performed these showed no abnormalities.

Congenital blindness with obvious microphthalmia has been described in Texel lambs by Zwiep (1958) and Hanset (1961).

Contagious agalactia in sheep and goats (Zavagli, 1946), causes characteristic lesions in the eye, udder and joints.

Under experimental conditions a reduction in the vitamin A intake of sheep may result in night blindness (Guilbert, Miller & Hughes, 1937; Hart & Miller, 1937; Peirce, 1945, 1954; Dutt & Sawhney, 1962). Adequate proof that sheep suffer spontaneously from avitaminosis A under field conditions is still awaited (Moore, 1960).

It is also known that sheep suffering from pregnancy toxaemia may become blind (Fertig, 1956).

The most commonly recognised form of blindness in sheep in Britain is that resulting from infectious keratitis (Blakemore, 1947). This condition which is readily diagnosed in the live animal, usually affects several sheep in the flock.

The present paper records the occurrence of a previously undescribed disease of sheep characterised by blindness. This condition, which appears to be increasing in incidence, is widespread in Yorkshire hill flocks.

Materials and Methods

Prior to 1963 four flocks had been brought to our notice in which a number of sheep were said to become permanently blind each year.

In 1963 a letter was sent to 2,824 sheep farmers in the northern part of the West Riding of Yorkshire requesting details of any blindness in their flocks. Replies totalling 1,057 were received. Later investigations were concentrated upon the Wharfedale and Nidderdale areas where blindness was found to be most prevalent. All the hill sheep farms in 30 parishes were visited and affected sheep were examined.

Thirty-five sheep with a history of blindness were collected for more detailed ophthalmoscopic, *post-mortem* and histopathological examinations.

In the letter of enquiry farmers were asked if the eyes of affected sheep had been normal and clear in appearance, as distinct from the cloudy eye seen with infectious keratitis. Other questions on the breed and age of affected animals and incidence of blindness were also included but the answers to these proved unreliable.

In an attempt to define the types of blindness all the farmers reporting blind sheep without an obvious cause were visited, when it became apparent that the condition known as "bright blindness" was limited to sheep in hill flocks grazing open moor or hill allotments. The majority of affected flocks were in Wharfedale and Nidderdale where all the 217 farmers claiming hill sheep subsidy in 30 parishes were visited by one of the authors (W.A.W.). Information was obtained on the type of flock, the area of moor grazed, the breed, the number blind in 1961, 1962, and 1963, the usual age and time of year when blindness was first observed and the degree of blindness and recovery rate. Note was taken of the type of vegetation available and the grazing habits of the flocks. Details from two flocks with blind sheep are not included as the sheep were blind when purchased.

Blind sheep available were examined clinically and many of these were obtained for laboratory examinations.

The Collection of Sheep for Further Examination

Thirty-five sheep were collected from 30 flocks reporting blindness. One of these, a Rough Fell hogg nine months of age, had been blind for a few weeks when four months old but had recovered. The remainder were partially or completely blind. Some of the sheep were in good physical condition, others were poor.

Examinations of Materials

(i) *Biochemical.* Blood samples were taken from 20 sheep on arrival at the laboratory for calcium, magnesium, phosphorus, acetone, copper, and vitamin A estimations. The livers from 10 sheep were examined for vitamin A and copper content when the sheep were killed.

(ii) *Serological.* The sera were tested for *Toxoplasma* cytoplasma-modifying antibodies by Beverley and Beattie's (1952) modification of Sabin and Feldman's (1948) method.

(iii) *Ophthalmoscopic.* The eyes of the sheep were examined ophthalmoscopically and retinal photographs of a series of blind and normal sheep were taken.

(iv) *Pathological.* Nineteen Swaledale or Swaledale X ewes aged two to five years, all of which had a history of blindness extending from a few days to two years, were available for pathological examination. There was also the Rough Fell hogg which had the unusual history of recovery from blindness. Some of the sheep were retained for several months before slaughter. All were fully examined *post mortem* but satisfactory examination of the eyes were limited to the last nine animals in the series as suitable technical methods had to be developed.

(v) *Histopathological Methods.* Though some of the cases died from other causes the majority were killed by decapitation through the atlanto-occipital space. The brain and proximal portions of the optic nerves were removed and placed in 10 per cent. formol saline. After, primary fixation blocks were cut, post-fixed in corrosive sublimate and embedded in paraffin. The blocks were representative of all divisions of the brain, but the optic tracts were examined most intensively; coronal and horizontal slices of the occipital pole to include the cuneus and lingual gyrus, and serial transverse slices from the diencephalon and mesencephalon were taken so as to include the visual cortex and geniculo-calcarine tract, the proximal parts of the optic nerve, lateral geniculate body and optic striation. Material from these regions was also retained for frozen sections.

The eyes and the remaining portions of the optic nerves were dissected out. In earlier cases the eyes were sliced following formol saline fixation of the intact globe, or were deep frozen and the blocks sawn out with a fine-toothed saw and allowed to thaw in formol saline. The blocks were taken vertically and parallel to the optic axis of the eye. All these methods led to varying degrees of retinal separation either by unequal shrinkage of the tissues during fixation or trauma when taking the blocks.

Zenker-acetic fixation and slow dehydration of the intact eye were found to give satisfactory preparations, the blocks being returned to alcohol and double-embedded by a slight modification of Peterfi's method. In a few cases whole head perfusions *via* the carotid artery were carried out using either 10 per cent. formol saline post-fixing in Zenker, or 3 per cent. glutaraldehyde buffered to *p*H 7.4 with sodium cacodylate. Good perfusion of the eyes was not consistently obtained and the results with the former method were inferior to those obtained by immersion fixation with Zenker alone. Glutaraldehyde, when satisfactory perfusion was obtained, however, yielded excellent preparations almost equal in quality to those fixed by Zenker. These had the added advantage of remaining soft and easy to section.

In a few cases frozen sections for lipid demonstrations were cut from material which had been soaked in a sucrose-gum acacia mixture after fixation.

Eyes from a number of sheep unaffected by blindness were used as controls for each method.

Results

Field Investigation

It was necessary to examine blind sheep not affected with keratitis in detail in order to reach a diagnosis. Blindness was observed associated with coenurus infestation and also with pregnancy toxaemia. In two other sheep a lens opacity of unknown aetiology was present. Three types of flock were recognised in which blindness occurred of a type distinguishable from the above:—

A. Flocks in which sheep became blind and never recovered.
B. Those in which some sheep remained blind while others recovered.
C. Those in which all the blind sheep recovered their sight within a few weeks.

The three types of flock are considered separately, as it is not known if they were affected with different conditions or were showing various manifestations of the same condition.

Of the 1,057 farms from which replies were received 44 (4.2 per cent.) were type A. 4 (0.4 per cent.) type B, 10 (0.9 per cent.) type C, and 999 (94.5 per cent.) were of none of these types.

Of the 217 hill flocks visited in Wharfedale and Nidderdale 79 (36.5 per cent.) were type A, four (1.8 per cent.) type B, nine (4.2 per cent.) type C, and 114 (52.5 per cent.) were not of these types. Details were not obtainable from 11 of these flocks.

Fig. 1 shows the distribution of the flocks in the main area surveyed.

Fig. II shows the distribution of affected and unaffected hill flocks in the areas of Wharfedale and Nidderdale in which all hill flocks were visited.

The farms themselves are shown on the maps although in some instances the sheep grazed on a moor some distance away.

The incidence of sheep known to have become blind during 1961, 1962 and 1963 in all the affected flocks and in the hill flocks in Wharfedale and Nidderdale is shown in Tables I and II. The number of sheep at risk refers to all female sheep over one year

of age. The total of these was known for 1963 but not accurately for 1961 and 1962. The figures for 1961 and 1962 are an approximation based on the 1963 population.

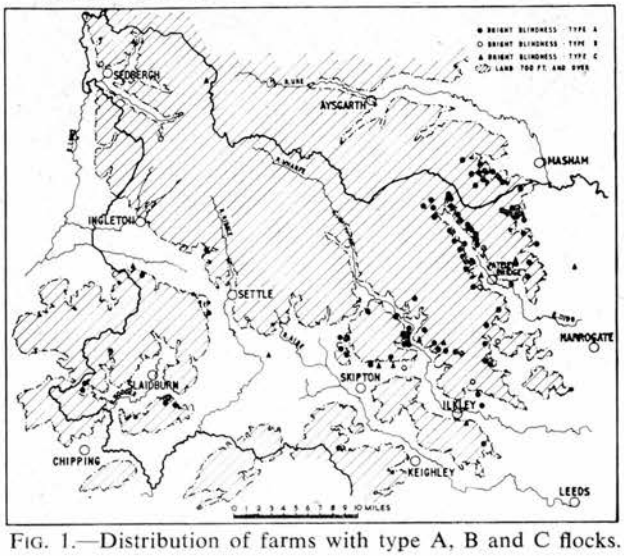

FIG. 1.—Distribution of farms with type A, B and C flocks.

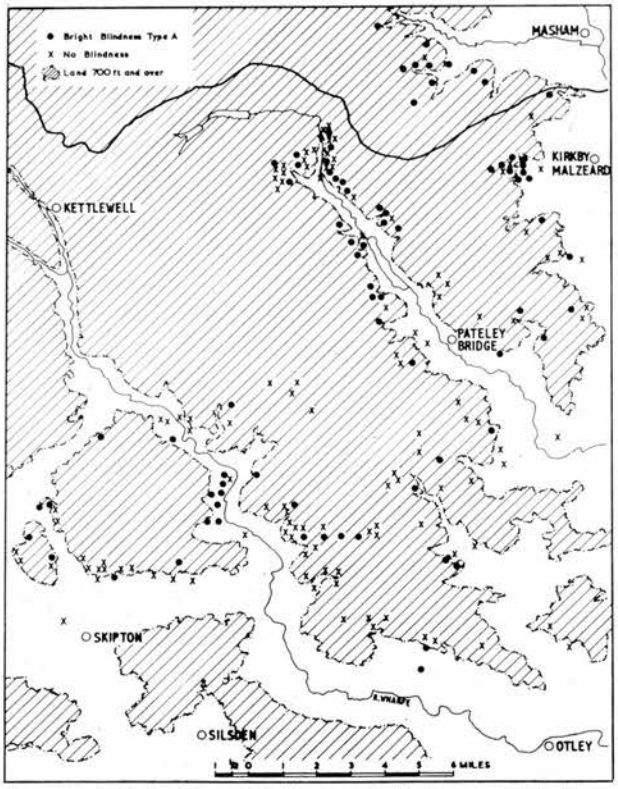

FIG. 2.—Distribution of type A hill flocks and hill flocks in Wharefdale and Nidderdale with no history of bright blindness.

The distribution of blind ewes in affected flocks during 1963 is shown in Table III.

The flocks with blindness were predominantly type A flocks in which the sheep never recovered their sight. This may be taken as the most typical group.

Enquiries on type A farms showed an apparent increase in incidence of the syndrome over the past three years with an average incidence of 1.29 per cent. in 1963. Certain flocks had more than 5 per cent. of sheep blind, making this the most important disease in these flocks.

The blind sheep are first detected in the autumn (September, October and November) in 83.3 per cent. of the type A; 83.3 per cent. of the type B; and 69.2 per cent. of the type C flocks, although the sheep are not seen more at this time of year than at any other time. According to the farmers these sheep were not seen to be blind during the previous lambing season.

Table IV shows the distribution of blindness in various age groups.

Cases of blindness are fairly evenly distributed between the groups of two-, three- and four-year-old sheep. Shearlings are seldom affected and although cases have been reported in sheep under one year of age none of these have been confirmed as bright blindness.

TABLE I

THE INCIDENCE OF SHEEP BECOMING BLIND DURING 1961, 1962 AND 1963 IN ALL KNOWN AFFECTED FLOCKS

(Percentages are shown in brackets)

	Number of sheep at risk	Type A	Type B	Type C
1961	20,563	181 (0·89)	7 (0·03)	14 (0·07)
1962	20,563	226 (1·05)	14 (0·07	15 (0·07)
1963	20,563	265 (1·29)	19 (0·09)	27 (0·13)

TABLE II

THE INCIDENCE OF SHEEP BECOMING BLIND DURING 1961, 1962 AND 1963 IN THE HILL FLOCKS IN 30 PARISHES IN THE WHARFEDALE NIDDERDALE AREA

(Percentages are shown in brackets)

	Number of sheep at risk in 206 flocks	Type A	Type B	Type C
1961	37,919	169 (0·45)	6 (0·02)	7 (0·02)
1962	37,919	212 (0·56)	14 (0·04)	9 (0·02)
1963	37,919	243 (0·64)	14 (0·04)	19 (0·05)

TABLE III

THE DISTRIBUTION OF BLIND EWES IN THE AFFECTED FLOCKS IN 1963

Percentage of ewes blind	Number of flocks		
	Type A	Type B	Type C
0—1	43	3	3
1—2	20	0	3
2—3	12	1	2
3—4	6	2	2
4—5	3	0	0
5—6	3	0	0
6—7	2	0	1
7—8	0	0	0
8—9	1	0	0
9—10	0	0	1
10—11	0	0	1
Total	90	6	13

TABLE IV

DETAILS OF THE AGES OF THE SHEEP WHEN FIRST SEEN BY THE FARMER TO BE BLIND

(Percentages are shown in brackets)

Age at which blindness is observed	Type A	Type B	Type C
Birth/3 months	0 (0)	1 (16·6)	0 (0)
3 months/1 year	2 (2·2)	0 (0)	1 (7·7)
1 year/2 years	12 (13·3)	1 (16·6)	2 (15·4)
2 years/3 years	52 (57·8)	0 (0)	6 (46·2)
3 years/4 years	61 (67·8)	3 (50·0)	8 (61·5)
4 years/5 years	38 (42·2)	5 (83·3)	3 (23·1)
Over 5 years	8 (8·9)	1 (16·6)	2 (15·4)
Not known	2 (2·2)	1 (16·6)	0 (0)

The number of each age group at risk is not known but approximately similar numbers of one-year-, two-year-, three-year- and four-year-old sheep were kept in each flock.

Examinations of the types of vegetation available for grazing by these flocks showed an apparent association between the distribution of bracken and the incidence of blindness. Forty-one (45 per cent.) of the type A flocks grazed heavy bracken while only 14 (12.3 per cent.) of the 114 flocks without blindness in Wharfedale and Nidderdale were on heavy bracken. All the 90 type A flocks in this area had access to bracken while 33 (29 per cent.) of the flocks without blindness had no access to bracken.

Closer investigation indicated that on poor moors where a high proportion of the available grazing is bracken certain sheep in a flock will graze only in the bracken beds and it is these sheep which are said by the shepherds to become blind. The bracken is eaten particularly in June when the fronds appear and in late August and September when the bracken commences to die back. The bracken is denuded of fronds or eaten to the ground (Figs. 3 & 4).

Although more Swaledale flocks were affected than those of other breeds blindness was seen in Dalesbred, Rough Fell and crossbred flocks. Swaledale and Dalesbred sheep comprise most of the flocks in Wharfedale and Nidderdale but the same breeds are common in Airedale and Ribblesdale where bright blindness is seldom seen.

Biochemical and Serological Examinations

Biochemical examinations of blood samples from 20 blind sheep revealed calcium, magnesium, phosphorus and acetone levels within the normal range. The blood copper levels of eight of the sheep were well below 0.1 mg. per cent. but this is not uncommon in hill sheep in this area and is not considered to be of significance in the present investigations. In only one animal was the blood vitamin A level of 14.3 iu per 100 ml. below the normal range.

In only one sheep (15 iu per g.) was the liver vitamin A level low and all the liver copper levels were normal.

Four sheep had significant titres to Toxoplasma but such results would be expected in any similar group of ewes with normal eyes (Beverley & Watson, 1959).

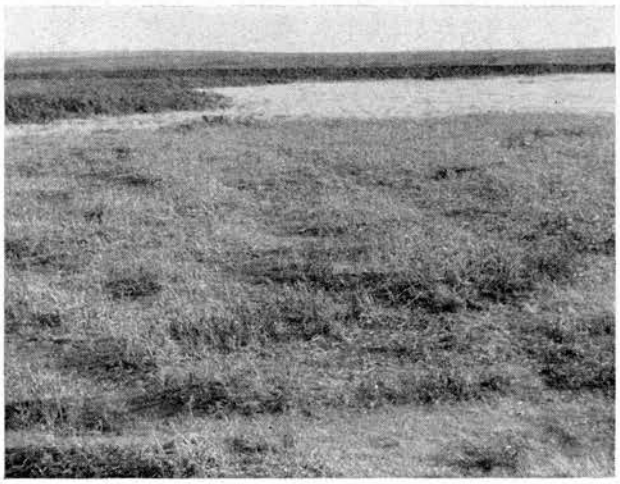

FIG. 3.—Denuded bed of bracken with normal bracken growing in the background.

FIG. 4.—Bracken eaten by sheep leaving only stalks and a few fronds.

Pathological

The general bodily condition of the blind sheep was below average. During the time the sheep were kept alive for observation five developed neurological symptoms. All of these were found later to have coenuriasis.

Parasitic pneumonia with the characteristics of Protostrongylus infestation occurred in 13 while small, circumscribed, pearly lesions of atheroma were found in the aortic or pulmonary arches of three cases. Examinations of other tissues did not reveal any significant lesions.

Ophthalmic

Many of the blind sheep can be identified in a group of normal sheep by their increased alertness, their erect attitude (Fig. 5) and by a characteristic glass-eyed appearance (Fig. 6).

The pupils of affected animals are dilated and therefore more circular in shape than the oval pupil of normal sheep. The pupillary reaction to light is poor and may be almost absent in advanced cases, the

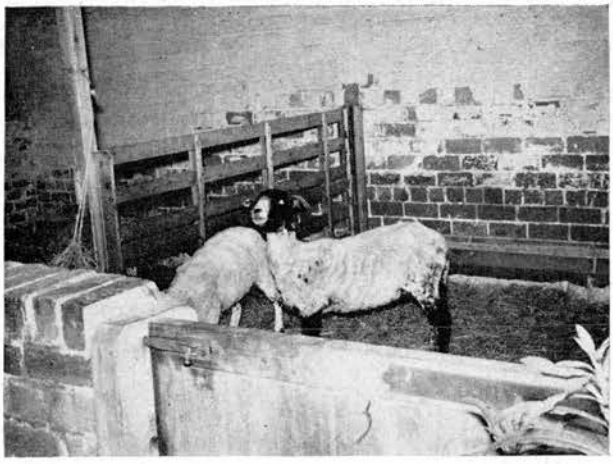

FIG. 5.—Typical alert attitude of sheep affected with bright blindness.

FIG. 6.—Dilated pupil and increased tapetal reflection giving the characteristic glass-eyed appearance.

pupil constricting only in bright sunlight. The cornea and aqueous and vitreous humours are clear; the lens is also clear and shows no evidence of cataract even in advanced cases. There is no conjunctivitis, keratitis or sign of any other inflammatory lesion of the eye.

Ophthalmoscopic examination of the normal fundus of the sheep shows a pink optic disc partially obscured by the large roots of the main retinal blood vessels. These vessels usually arise in three main groups. The distinction between artery and vein is clear, the vein being of greater calibre and more blue-red in colour. Smaller blood vessels from the optic disc are also visible on the retina, together with side branches from the main groups, particularly the group at about 12 o'clock. The tapetum lucidum is green to blue in colour. The tapetum nigrum is dull and dark greyish-brown.

Affected sheep have an optic disc paler than normal, the colour in advanced cases becoming grey-pink. The edge of the disc in all cases is clear and the blood vessels pass straight over this edge on to

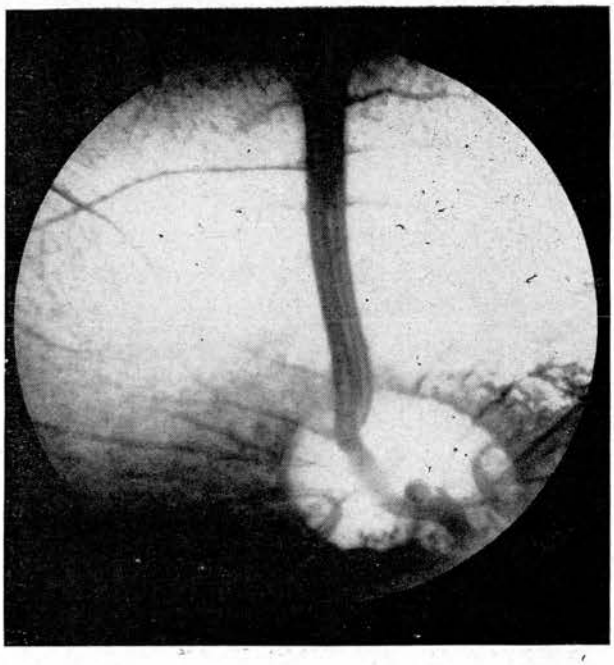

FIG. 7.—Normal eye.

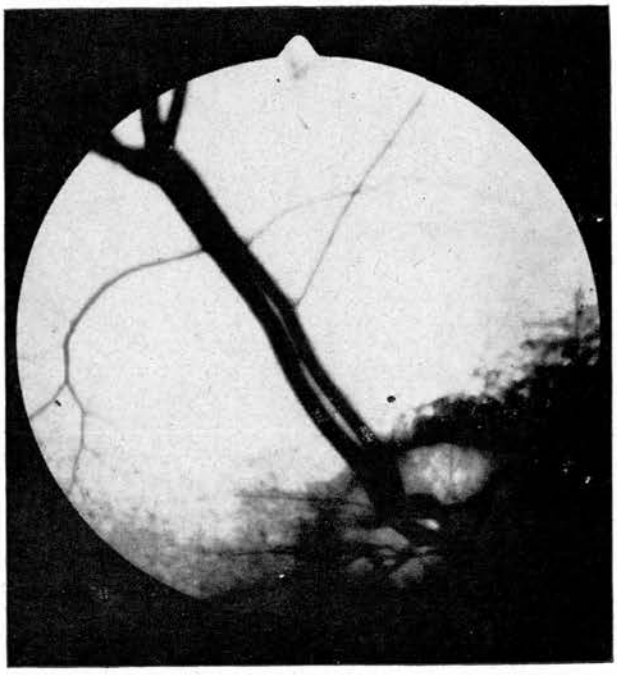

FIG. 8.—Normal eye.

the retina. Both arteries and veins are narrower than normal. This narrowing occurs in all the blood vessels so that the smaller ones are difficult to follow away from the disc. The main groups are still clearly visible over the fundus but their side branches are not so obvious. The artery and vein in the 12 o'clock group may be of almost equal calibre in some cases, but in others the vein is still larger than the artery. This slender pair of vessels appears more

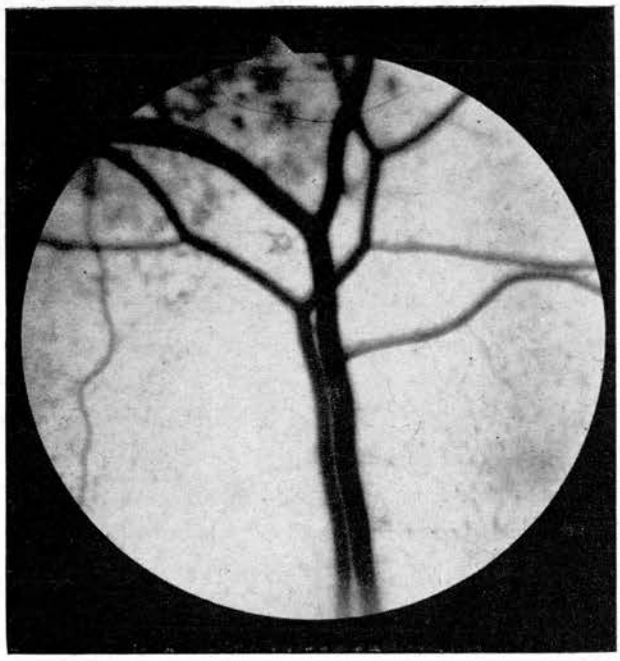

FIG. 9.—Normal eye.

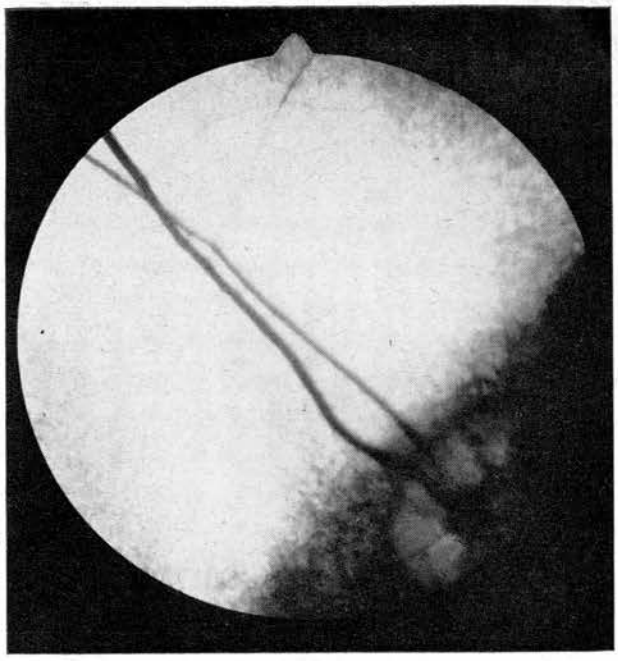

FIG. 11.—Affected eye.

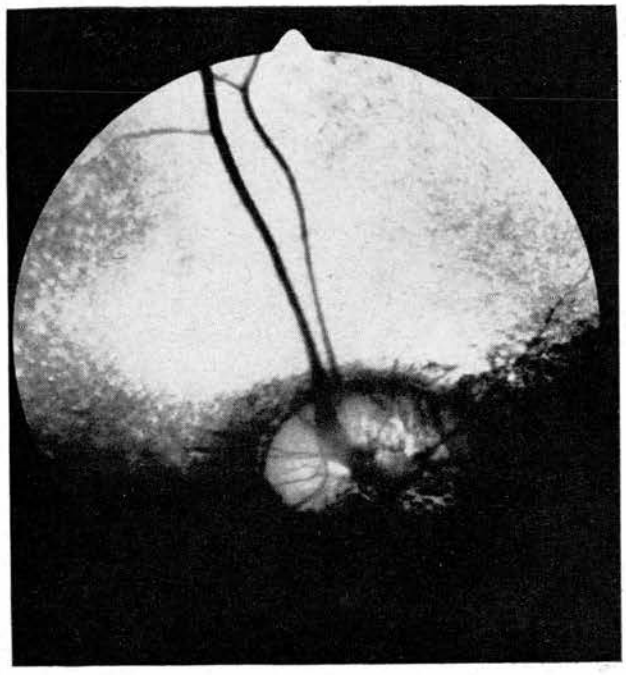

FIG. 10.—Affected eye.

FIG. 12.—Affected eye.

separated than in the normal eye. The roots of the main vessels may not show any diminution in width on the disc itself but they are obviously narrower when on the tapetum. This is not so noticeable in the more advanced cases. There is an increased reflection from the surface of the tapetum lucidum and this is perhaps the most noticeable change. The surface appears very shiny and smooth and almost mirror-like to the ophthalmoscope beam. The colour is mainly green to blue often with a yellow to orange sheen. In advanced cases the tapetum nigrum is paler than normal. This is due to fine cracks and small spots where the surface is shiny and the colour a very pale grey. In some parts the choroidal blood vessels may be visible (Figs. 7, 8, 9, 10, 11 and 12).

Twenty-nine of the 35 sheep collected had these changes affecting the eyes. Of the other six, five were later found to be affected with coenuriasis and one was the hogg with a history of recovery from blindness.

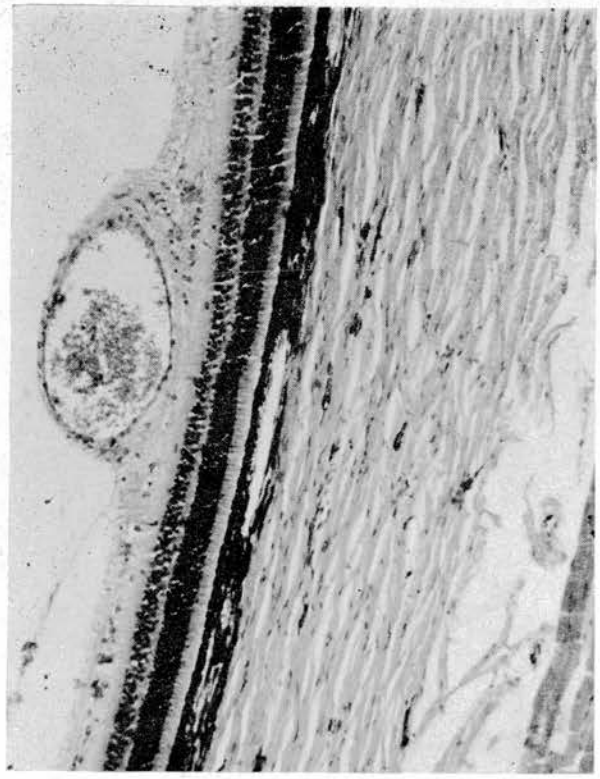

FIG. 13.—Vertical section through the eyes near the posterior pole showing normal retinal structure (H. & E. x 120).

Histopathological

In the nine cases in which satisfactory histological preparations of the eyes were obtained it was found that the main pathological changes were in the bacillary and outer nuclear layers of the retina (Figs. 13, 14).

In the most advanced cases the bacillary layer (the layer of rods and cones) and outer nuclear layer were completely atrophied together with portions of the inner nuclear layer, so that a flattened pigment epithelium abutted directly onto the remains of the inner nuclear layer the cells of which showed karyorrhexis (Figs. 15 and 16).

The inner plexiform and ganglion cell layers appear to be unaffected and no changes were recognised in the layer of nerve fibres or the optic nerve itself. While all parts of the bacillary and nuclear layers were involved the lesions were most advanced towards the posterior pole of the eye. Both eyes of a pair were affected to a similar degree. In earlier cases areas of retina were present in which the inner nuclear and plexiform layers remained intact and which showed karyorrhexis of the outer nuclear layer and fragments of degenerating rods and cones. It was not possible to determine whether the rods and cones had a differential susceptibility to degeneration. There was no evidence of inflammation and the products of degeneration were only visible in the earliest stages. The blood vessels appeared normal.

In some cases (notably, but not solely those fixed by perfusion) the lamina vitrea choroidea showed intense swelling (Fig. 17).

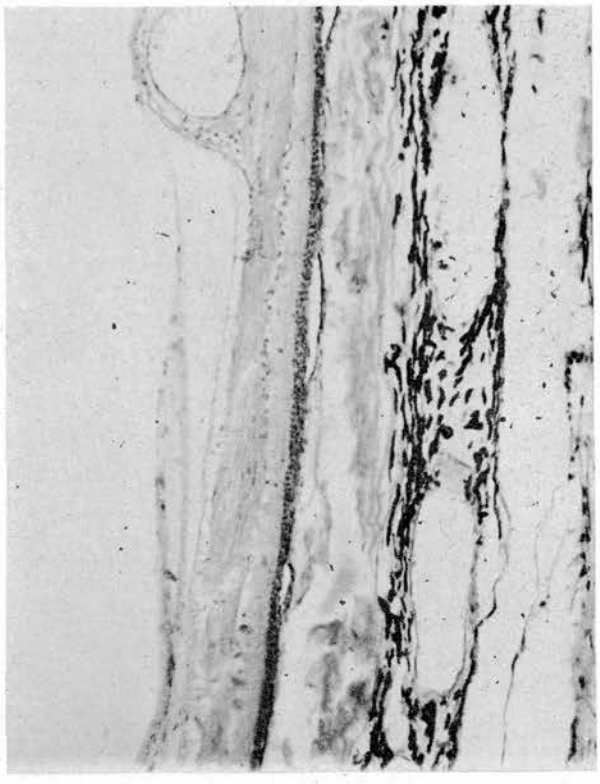

FIG. 14.—Similar region to Fig. 13 from a ewe affected with clear blindness showing advanced atrophy and loss of layer organisation (Perfused H. & E. x 120).

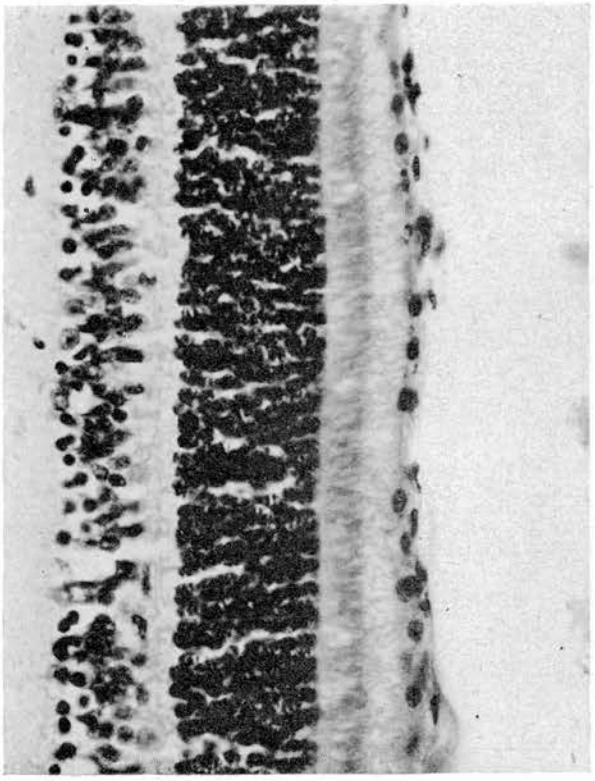

FIG. 15.—Eye of normal sheep showing the retina with exception of the layer of nerve fibres. The choroid is to the right (H. & E. x 440).

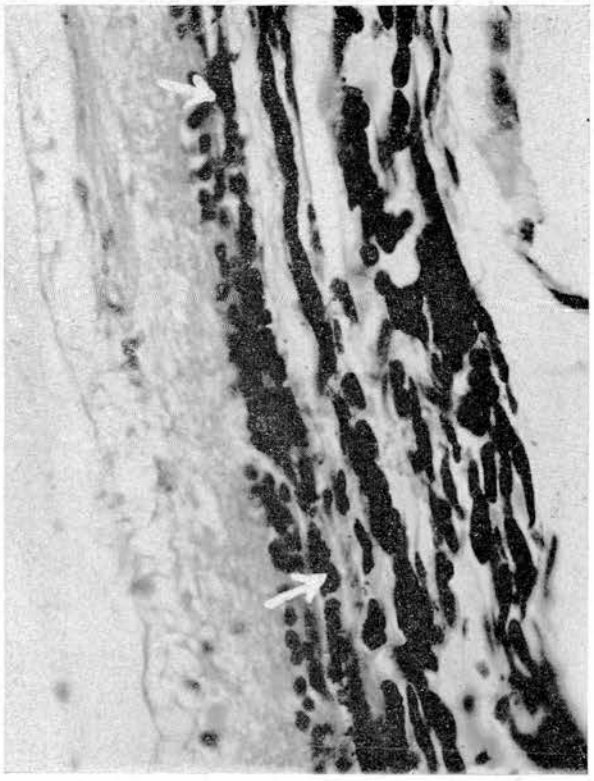

FIG. 16.—Retina from a long standing case of clear blindness, showing advanced destruction of the bacillary and outer nuclear layer. The flattened pigment epithelium (arrowed) can be seen adjacent to the remains of the inner nuclear layer (H. & E. x 440).

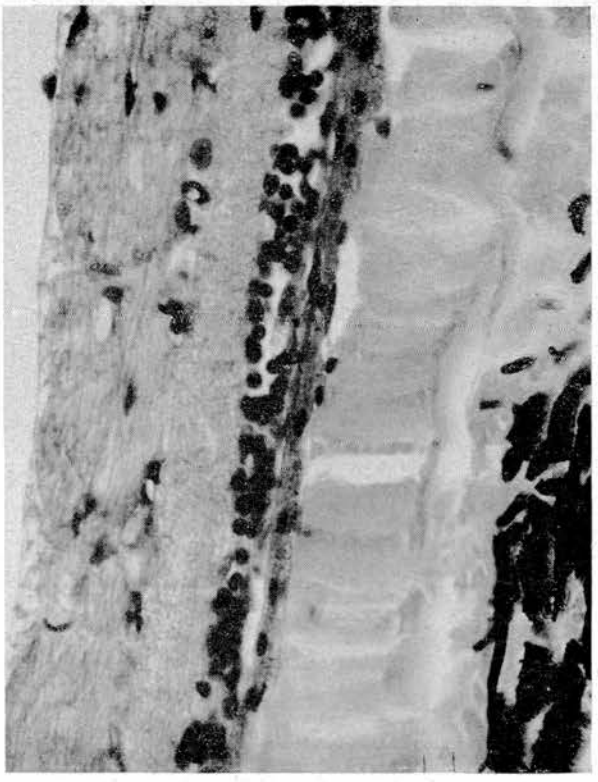

FIG. 17.—Retinal vestiges and portion of the choroid of a severe case of clear blindness showing intense swelling of the lamina vitrea choroidea (H. & E. x 440).

No specific changes were observed in other parts of the visual apparatus or in any other division of the brain. In four of the oldest cases there was slight endothelial swelling and light lymphocytic cuffing of vessels in the region of the geniculo-calcarine tract. No demyelination was observed and no degeneration of neurones in the cuneus, lingual gyrus or elsewhere. In cases of up to six months duration chains and clumps of swollen interstitial neuroglia similar to those described in Border disease (Barlow & Dickinson, 1965, in press), were consistently observed in the cerebral white matter (Fig. 18).

Discussion

The sheep affected with this condition are known as bright blind, clear blind, or glass-eyed by the farming community. The term "bright blindness" has been adopted in this paper as it is most descriptive of the affected live animal. The disease has been recognised by hill sheep farmers in Yorkshire for at least 50 years.

The characteristic eye lesions have only been seen in hill sheep and affected hill flocks are concentrated in mid-Wharfedale and upper Nidderdale (Figs. 1 & 2).

Tables I and II suggest that the three types of blindness have increased in incidence over the past three years. Discussions with sheep farmers have confirmed that there has been a marked increase during

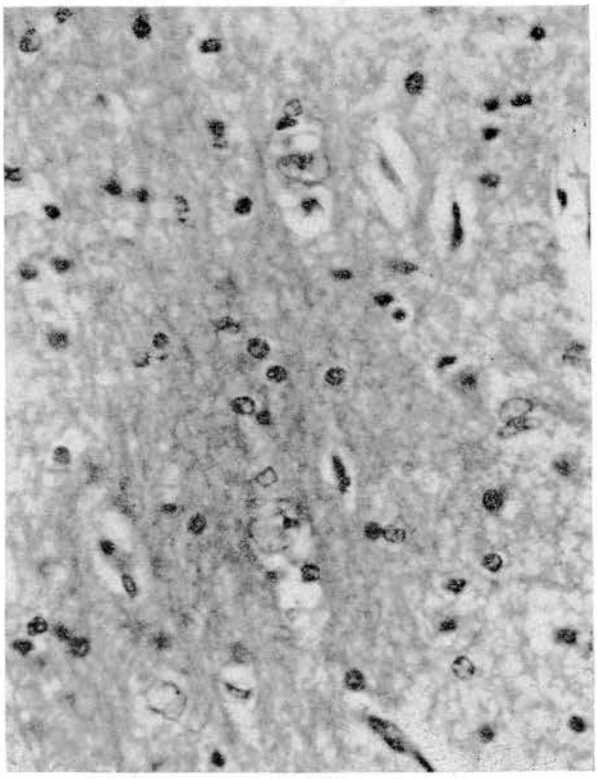

FIG. 18.—Swollen interstitial neuroglia in brain (H. & E. x 440).

the past 20 years and before 1945 few flock owners had seen the condition. In flocks which now have an annual incidence of 5 per cent. (Table III), cases were seldom seen 20 years ago.

All the cases confirmed have been in sheep over one year of age. Two-, three-, and four-year-old ewes are those most frequently affected (Table IV) but occasionally the disease is seen in older ewes and shearlings. Many of the hill flocks in this area sell their ewes after the third crop of lambs. No cases have been confirmed in rams although there are occasional reports of wethers being affected.

The lesions in the eyes of the blind sheep would appear to be a progressive degeneration or atrophy of the outer layers of the retina, but there is little evidence as to the rate of progress of the lesion. It seems that the products of degeneration must be rapidly removed, possibly by liquefaction, the integrity of the tissue being maintained presumably by the radial fibres of Müller.

The pathological findings in tissues other than the eye are so inconsistent, with exception of parasitic pneumonia, that it is unlikely that they play any rôle in the development of blindness. Though pneumonia occurred in over half the animals any possible relationship between it and the blindness must be extremely doubtful in view of the frequency of pneumonia of this type in this class of sheep and the relatively low incidence of bright blindness.

We have been unable to find an account in the literature of any similar type of blindness occurring naturally or experimentally in sheep but since the lesions affect both eyes equally, and there is no evidence of any inflammatory condition, a nutritional, toxic or genetic aetiology must be considered.

It is of interest to compare the ophthalmoscopic and histopathological findings in this condition with those of similar retinal degenerations in other species.

A similar spontaneous degeneration of the retina in certain breeds of dog, due to a generalised progressive atrophy of outer layers of the retina, has been described by Magnusson (1909, 1911, 1917), Hodgman and others (1949); Parry (1951, 1953); and Barnett (1962). This syndrome which was similar to that described by Bourne, Campbell and Tansley (1938) in the rat, and by Tansley (1951) in the mouse, was shown to be hereditary.

Parry (1953) discussed the similarity of this progressive retinal atrophy to human retinitis pigmentosa concluding that it is wise not to press the analogy between the dog disease and retinitis pigmentosa in man.

Tansley (1933) and Johnson (1943) have shown that severe vitamin A deficiency results in neuroepithelial degeneration of the retina in rats the entire course of which has been followed by Dowling and Wald (1958, 1960). There is a similarity between the most severe lesions of avitaminosis A in rats and those of bright blindness in sheep.

Experimental vitamin A deficiency in sheep is characterised by night blindness (Guilbert, *et al.*, 1937; Peirce, 1945) but the histopathological changes associated with this have not been described. With naturally occurring hypovitaminosis A in cattle, attention has been drawn to the changes in the optic nerves (Moore, Huffman, & Duncan, 1935; Blakemore, Ottaway, Sellers, Eden & Moore, 1947; and Abrams, Bridge, Palmer & Spratling, 1961) and not to retinal degeneration.

Several compounds are known, which, used experimentally, will cause degeneration of the intra-ocular tissues (Jubb & Kennedy, 1960). One of them, diphenyl thiocarbazone, is presumed to act by chelating zinc in which the retina is rich and which may be the prosthetic group of enzymes concerned with vitamin A metabolism. Some of these drugs are said to cause proliferation of the pigment epithelium but such pigmentation was not observed in these cases of blindness in sheep. The lack of pigment deposition is also in marked contrast to retinitis pigmentosa in the human in which this is a feature.

The field investigations do not implicate a simple genetic basis for the disease. Indeed its occurrence in several breeds or crosses, the tendency for affected flocks to be concentrated on certain moors and even certain areas of a particular moor (Figs. 1 & 2), the marked seasonal incidence, and the frequent purchase of rams from many sources with no close in-breeding, suggest that this is unlikely. In these flocks affected ewes are seldom used for breeding but are usually sold for slaughter in the autumn in which they are seen to be blind. Those which are bred produce apparently normal lambs and these show no greater incidence of blindness at a later age than the lambs from unaffected ewes.

The possible association with avitaminosis A cannot be excluded particularly as flocks with blindness tended to be those grazing the poorer moors. If vitamin A deficiency *per se* were involved the greatest number of cases would perhaps be expected in spring when the nutritional status is lowest and not in the autumn. The high incidence found in sheep over three years of age is also unexpected (Table V). Little supporting evidence for a hypovitaminosis A was obtained from the biochemical estimations on blood and liver from affected sheep. These results could however be misleading as most of the sheep had shown symptoms for some time before the examinations were made and any lowering of vitamin A could have been corrected.

The information from the field investigations suggests a possible association between the amount of available bracken, the grazing of bracken and the incidence of blindness. This suggestion is supported by the apparently seasonal incidence of the condition.

Garrett-Jones (1960, 1961) in South Wales and Fidler (1963) in Wharfedale, have both made observations on the grazing of bracken by sheep. Garrett-Jones suggested that any persistent grazing of bracken must be considered remarkable and he found no evidence that the sheep suffered any ill-effect. There is no doubt that many flocks on the Yorkshire moors eat a considerable amount of bracken and more is being grazed each year as this type of vegetation increases. It is not known why certain areas, often in the centre of beds of bracken, are grazed heavily by sheep but it has been observed by Braid (1934) that sheep will graze bracken wilting as the

result of a fungus infection. Confirmation of the possible rôle of fungi or of bracken in the aetiology of bright blindness must await further field and laboratory investigations.

Acknowledgments.—It is a pleasure to acknowledge the help of all those who contributed to this investigation. The work was made possible by the ready co-operation of flock owners in Yorkshire who provided information and sheep for examination. The farmers' veterinary surgeons kindly allowed free access to material from these flocks. Mr. R. Parker was responsible for the collection and care of the sheep, all the technical staff of the Leeds V.I.C. were concerned in their examination and Mr. D. Watson, The Moredun Institute, the preparation of the photomicrographs.

Miss G. Lewis kindly arranged for the biochemical examinations to be performed at Weybridge.

We are grateful to Dr. J. McC. Howell of Liverpool University and Dr. J. K. A. Beverley for useful discussions on this problem and for the examinations for toxoplasma antibody.

The early investigational work was assisted by the help of the N.A.A.S., Harrogate. Several of the veterinary officers at Leeds obtained flock histories with the consent of Mr. H. B. Allan, Miss J. Williamson, Leeds, prepared the maps and Mr. T. Evans visited several farms to study the distribution of bracken.

Finally may we thank Mr. W. B. V. Sinclair and Mr. L. E. Hughes for their ready help and advice.

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28.

The Transmission of a Specific Encephalopathy of Mink to the Goat

Sir,—Recently a specific encephalopathy of mink has been described in Wisconsin (Burger & Hartsough, 1965) and Idaho (Hadlow, 1964) and has excited considerable interest because of its similarities with scrapie. The disease has an incubation period of seven months or more and a course lasting six to eight weeks. It is characterised by behavioural changes, ataxia and wasting. Mortality is 100 per cent. The lesions are microscopic, confined to the CNS and consist of severe vacuolation of the grey matter and diffuse gliosis. In the cerebral cortex, vacuolation gives rise to a spongy appearance of the tissues, whereas in the larger subcortical neurones single or multiple intracytoplasmic vacuoles are observed. Hitherto the disease has only been transmitted among mink.

In 1964 one of us (I.Z.) obtained, through the courtesy of Dr. Burger, brain material from affected mink and inoculated 0.03 ml. of a 10^{-1}

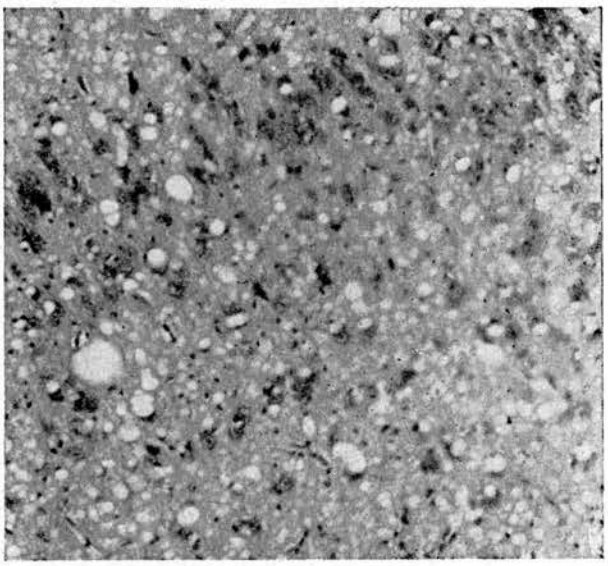

FIG. 1.—Status spongiosus in temporal cortex of goat (H. & E. ×120).

suspension intracerebrally into each of 37 weanling mice and 1 ml. of similar material intracerebrally into each of three young goats. The mice were kept for 16 months and showed no evidence of clinical disease during this time. Histological examination of their brains failed to reveal any significant lesions. Twenty months after inoculation, however, one goat developed a high stepping gait with the left forelimb, circling to the right, apparent blindness of the right eye and ptosis of the left eyelid. The clinical signs progressed slowly and the goat was killed four months later. The gross pathology was negligible; the brain was small and firm, and the left occipital cortex appeared abnormally thin.

Histologically there was diffuse intense status spongiosus (Fig. 1) of the archipallium and neopallium, the lateral geniculate and paracentral nucei of the thalamus, the hypothalamus, fornix and paraterminal body, the anterior colliculi of the corpora quadrigemina and the olives. Less severe vacuolation of the ground substance was evident in the lateral caudal, lateral cuneate, spinal trigeminal and hypoglossal nuclei, and in the grey matter of the spinal cord. Neuronal vacuolation was present

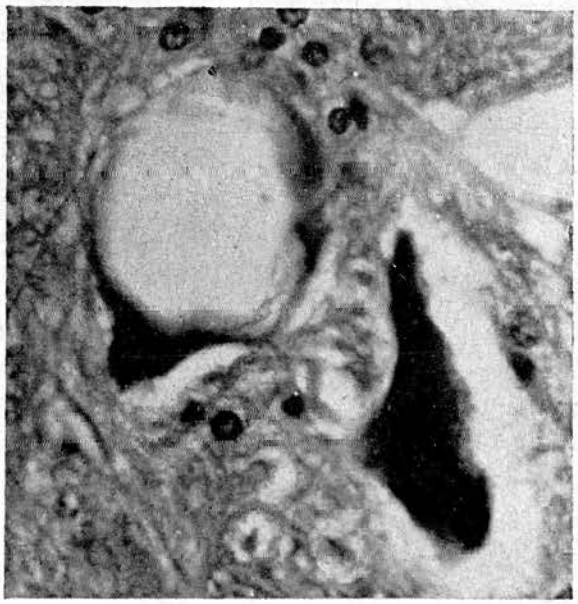

FIG. 2.—Vacuolated neuron in reticular formation of goat (H. & E. ×480).

in the larger cells of the raphé, reticular formation and dorsal motor vagus nuclei (Fig. 2) and chromatolysis was observed amongst cells of the red nucleus. The cerebellum was largely spared, only small numbers of "punched out" holes being seen in the granular layer in paraffin sections.

Qualitatively the lesions are identifiable with those of both scrapie and mink encephalopathy. The quantitative distribution of the lesions is in accord with published descriptions of mink encephalopathy, but in our opinion, it is unlike any form of scrapie hitherto described in the goat. Scrapie in goats typically produces neuronal vacuolation at subcortical levels, the amygdaloid nucleus and subcallosal gyrus (Pattison, Gordon & Millson, 1959; Zlotnik, 1961) but after serial passage in mice, status spongiosus may be observed also in restricted areas of the cerebral cortex and hippocampus of experimentally infected goats (Zlotnik & Rennie, 1965). For these reasons we are inclined to believe that the disease in this goat represents transmission of encephalopathy of mink, rather than some chance contact infection with scrapie. Further characterisation of the mink disease in goats may clarify its relationship to scrapie.

At the time of writing (28 month after inoculation) one of the two remaining goats is showing mild bilateral ptosis and hyperaesthesia while the other remains clinically normal.

May 17th, 1967.

Yours faithfully,
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29.

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Familial Convulsions and Ataxia in Angus Calves

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SUMMARY.—The clinical and pathological manifestations of a hitherto unreported neurological disease in Aberdeen Angus calves are described. The condition is characterised by recurrent convulsions in young calves and a residual ataxia in older animals which is referable to a selective degeneration of Purkinjé cells. The disease appears to have a dominant mode of inheritance, and its spread and control have been discussed.

Introduction

THIS paper records the existence of a neurological disease in the Aberdeen Angus breed, the dissemination of which is consistent with a dominant mode of inheritance. The disease has been located so far in one leading herd and the use of bulls from this herd has resulted in several peripheral and associated outbreaks. Affected calves show single or multiple seizures and a residual ataxia. The lesions are microscopic and consist of degeneration of the Purkinjé cells of the cerebellar cortex.

History

Three affected calves were observed in a leading herd in 1961 and another three or four had previously been disposed of—one, an affected heifer, becoming supreme champion at a fat stock show. All were sired by an apparently normal bull and their mothers were the offspring of several bulls. The transmitting bull also left 14 or so normal calves. A bull mated contemporaneously to a corresponding group of females left no defects and the breeder was carefully avoiding inbreeding.

In a second outbreak, investigated in 1967, a young bull bought from the first herd produced eight affected and nine normal calves on mating to unrelated

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A.A. heifers as well as one or two cases out of six crossbred females. Again a contemporary bull left no defects.

This young bull was then transferred to Orkney where he left affected offspring in six separate herds—the actual numbers of affected and normals being two and sixteen; six and nine; two and five; two and eight; two and one; and three and fifteen respectively. On these farms the bull was mated mainly to crossbred cattle and was the only common factor linking the outbreaks.

In a third outbreak, a bull, whose mother came from the first herd, was used along with several other bulls on a large herd of Hereford females, and six or seven defective calves appeared. Blood typing of three affected animals indicated that this bull could have been the parent, but blood was unavailable from the other bulls to exclude their possible parentage.

In a fourth outbreak, another clinically normal bull obtained directly from the original herd, has also left two or three affected and several normal calves.

A clinically affected bull aged one year old has also been observed.

Clinical Signs

The clinical signs are neurological in character and become apparent during the first few hours of life, or less commonly when the calves are two to three months old. The disease takes the form of single or multiple tetaniform seizures of variable intensity, lasting from three to 12 hours or more. These decline into a residual ataxia which persists for a few weeks or several months, and may be interrupted by further seizures.

There is a tendency for symptoms to abate after the calves are turned out to grass in the spring and complete or incomplete clinical recovery is common. Relapses may occur after considerable periods of time, especially when the animals are brought in for wintering or moved to fresh premises.

The seizures are sudden in onset and are of two forms. In the mild form movements become laborious and exaggerated due to a general increase in muscular tone. The tail is held out stiffly, the limbs are not easily bent and the calves walk with a straddled gait. The head is held high and may show a fine tremor, but there is no trismus. The eyes are prominent but sight is not affected and there is no papilloedema. There is hyperaesthesia, but temperature, respiration and appetite are not affected at this stage.

In the severe form, which occurs especially in the earlier phases of the disease, convulsions may develop. The calf goes down and struggles; opisthotonos develops with limbs and tail held in rigid extension. Sometimes, in the absence of opisthotonos, exaggerated paddling movements of the forelimbs are seen. Spasm of the masseter muscles is absent, but temperature and respiratory rate may be elevated.

There would appear to be a "panic" element in the convulsive attacks, since if the animal can be assisted to rise and steadied against a wall there may be immediate improvement. The attacks are frequently precipitated by excitement, such as driving by dogs, or loading into lorries. The fits do not respond to calcium

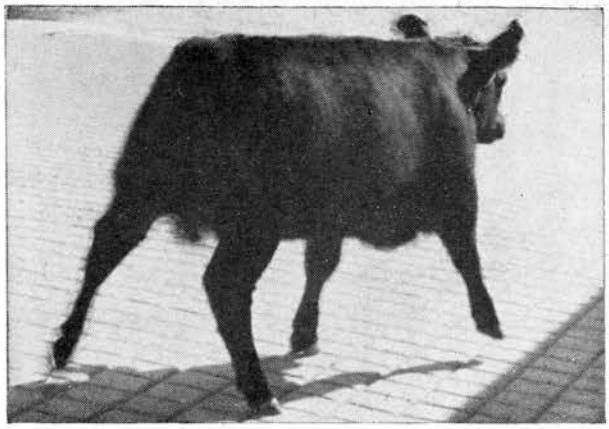

FIG. 1.—Residual ataxia, yearling A.A. heifer. Note the "goose-stepping" movements of the forelegs and the wide stance of the hindlegs. (Retouched print from 16-mm. cine film.)

borogluconate, magnesium sulphate or high potency vitamin injections, but they can be controlled with chloral hydrate or pentobarbitone sodium. When a series of convulsive seizures occurred over a short period, the animal was usually slaughtered.

The residual ataxia is characterised by spasticity. The walk has a "goose stepping" quality especially with the forelegs (Fig. 1), and there is difficulty in placing the hindquarters. In some cases animals showed marked disinclination to walk down hill. After periods of activity the trunk appears to sag between the shoulders and there may be slow rhythmic lateral movements of the head.

Biochemical examination of blood showed normal levels with respect to Ca^{++}, Mg^{++}., $^{--}PO_4$, Cu^{++}, Pb^{++}, vitamin A, sugar, SGOT and SGPT but K^{+} was slightly raised in five to six cases examined. (Mean 25, Range 20·5 to 29·5, Normal 17 to 22 mg. per 100 ml.). Complement fixation test for toxoplasmosis and the haemagglutination inhibition test for louping-ill were negative in all cases. Cerebrospinal fluid was bacteriologically sterile and showed no microscopic abnormalities.

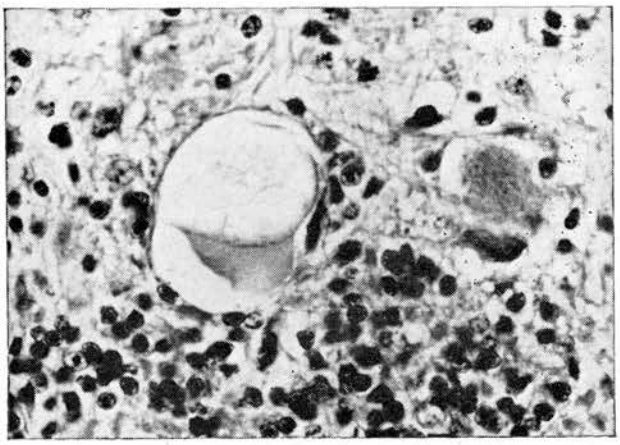

FIG. 2.—Swollen vacuolated Purkinjé cell. Cerebellar cortex. (H. & E. ×480.)

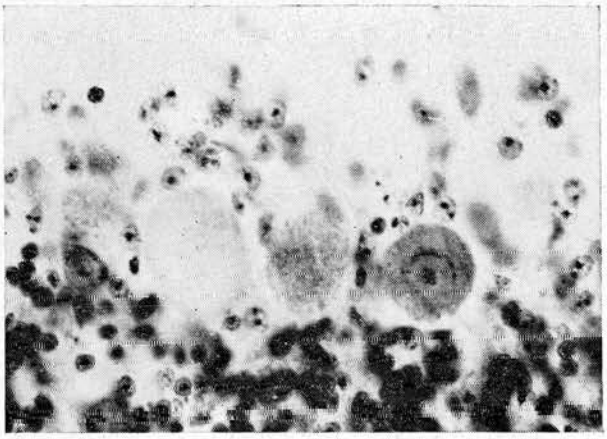

FIG. 3.—Various stages of chromatolysis. Cerebellar cortex. (Luxol fast Blue ×480).

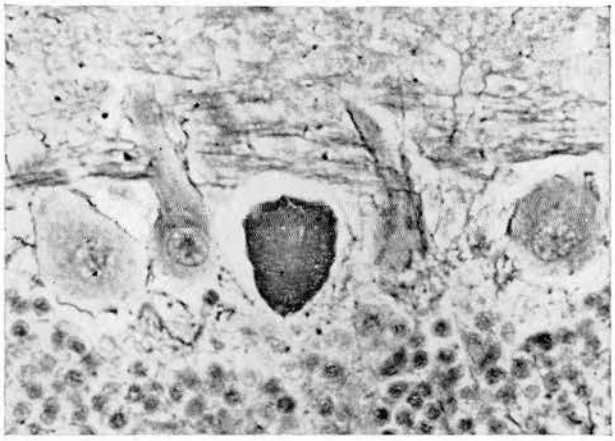

FIG. 4.—Various stages of neurofibrillar degeneration. Cerebellar cortex. (Holmes ×480.)

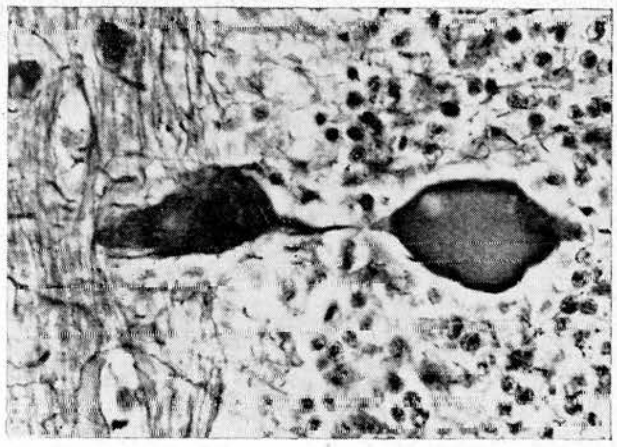

FIG. 5.—Axonal torpedo on Purkinjé cell axon. Cerebellar cortex. (Holmes ×480.)

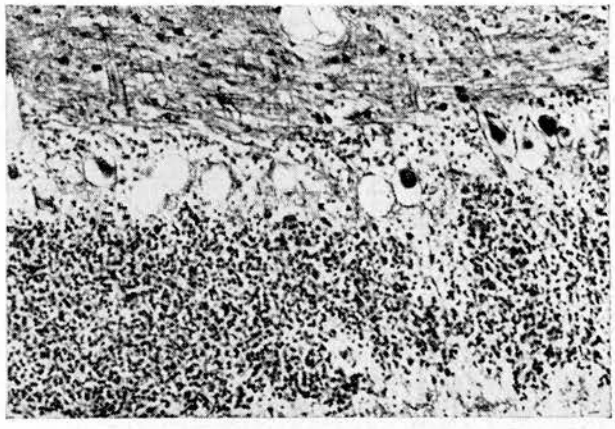

FIG. 6.—Loss of Purkinjé cells and formation of "empty baskets." (Holmes ×120.)

Pathology

Post-mortem examination of eight cases, varying from eight to 17 months of age, revealed no gross lesions in viscera, musculature or skeletal structures. Lesions were confined to the C.N.S. and consisted of a very selective cerebellar cortical degeneration. Apart from two cases with small focal depressions in the superior semilunar lobule, the changes were microscopic and affected Purkinjé cells almost exclusively.

The earliest change recognised was acute swelling of the cell bodies accompanied by vacuolation (Fig. 2), chromatolysis, loss of neurofibrils and the formation of axonal torpedoes (Figs. 3, 4 and 5). The ultimate fate of degenerate Purkinje cells was lysis and the formation of empty baskets (Fig. 6). Many affected Purkinjé cells were displaced into the granular or molecular layers (Fig. 7) but heterotopic cells of normal cytological appearances were not observed. The changes were widespread, without localisation to particular regions of the cerebellum and affected all or a proportion of the Purkinjé cells in any folium (Fig. 8).

Neuronophagia was absent and cellular reaction—proliferation of Bergmanns glia especially around the dendritic tree of degenerating Purkinjé cells (Fig. 9)—was observed in only two cases. Basket cells, granule cells, Golgi type II cells and glomeruli appeared normal and silver preparations did not reveal any loss of mossy or climbing fibres. The *corpus medullare* showed slight pallor of myelin and small quantities of sudanophilic lipid were present in the perivascular spaces. In a single case a few dentate nucleus cells were chromatolytic but otherwise no nuclei projecting to, or receiving fibres from the cerebellar cortex showed any morphological change.

Some cells of the third (pyramidal cell) layer of the cerebral cortex of two cases showed severe shrinkage and hyperchromasia, but otherwise no lesions were detected elsewhere in the nervous system.

Discussion

Differential Diagnosis

The tetaniform convulsions must be differentiated from several commonly occurring diseases of cattle, and some difficulty may be encountered with the clinical diagnosis in a single case. The muscular spasms and opisthotonos resemble tetanus but there is no trismus and the course of the disease is different. There is no lowering of serum magnesium levels and no response to treatment with magnesium salts as in hypomagnesaemia. Blindness and grinding of teeth

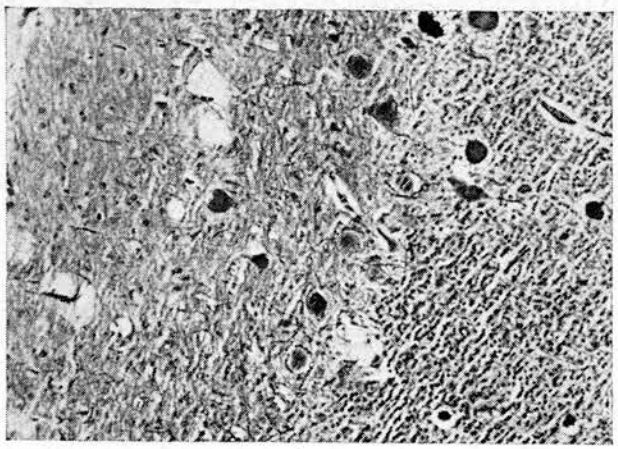

FIG. 7.—Degenerating Purkinjé cells in heterotopic situations. Neuronal material is present in the molecular layer and deep within the granular layer. (Holmes ×120.)

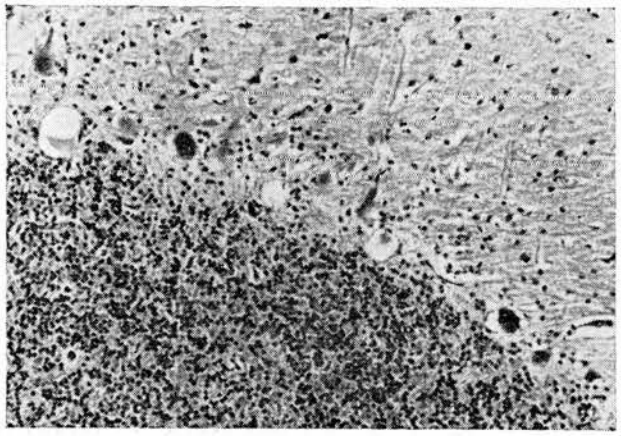

FIG. 8.—Part of a cerebellar folium showing varying degrees of Purkinjé cell degeneration. (H. & E. ×120.)

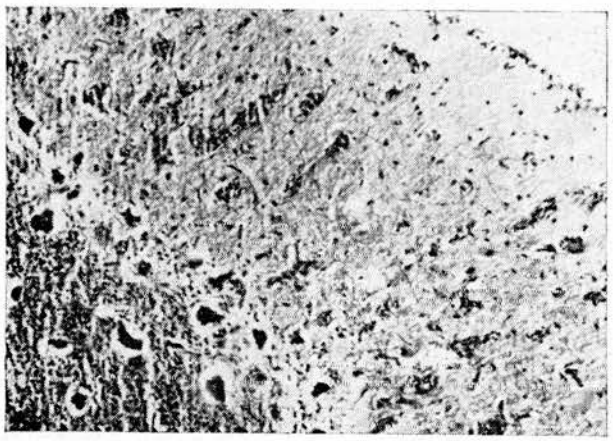

FIG. 9.—Proliferation of Bergmanns glia in molecular layer of cerebellar cortex. (Holmes ×120.)

such as may be present in lead poisoning and polioencephalomalacia (cerebrocortical necrosis), are absent. Furthermore, blood lead levels are low and there is no response to vitamin B1 therapy as is usually the case in polioencephalomalacia.

Meningoencephalitis would be expected to progress more rapidly and also to stimulate a febrile reaction and a leucocytosis in the C.S.F. The cerebral pseudolipidosis of Aberdeen Angus cattle (Whittem & Walker, 1957) should be distinguished clinically from the present condition by its progressive nature, failure to grow and intention tremor, and pathologically by the presence of eosinophilic plaques and widespread neuronal degenerations.

The convulsive attacks resemble those described by Hill (1956) in Hereford and Hereford × Jersey calves, but those animals recovered spontaneously without relapses or residual ataxia. In the condition described here the residual ataxia may require differentiation from spastic paresis (Love & Weaver, 1963) and muscular dystrophy. Remission of signs and other more widespread evidence of cerebellar disease are not features of spastic paresis. A history of spasms should eliminate muscular dystrophy.

Pathology

Prima facie, Purkinjé cell degeneration could be, the fundamental lesion of the disease and heterotopia result from displacement of degenerating cells. Alternatively heterotopia may be a manifestation of a more extensive malformation of the cerebellar cortex, of which Purkinjé cell degeneration is one result. The complete absence of normal Purkinjé cells in heterotopic situations, however, suggests that the lesion is solely a Purkinjé cell degeneration and not a more generalised defect.

Other explanations, however, are also possible. Many degenerating Purkinjé cells in the oldest animals (17 months) showed changes which can only be interpreted as of recent origin and thus the observed cerebellar lesions may only appear after the clinical symptoms have developed. Selective Purkinjé cell degeneration is found frequently in the cerebellum of children dying from conditions associated with severe convulsions (Zimmerman, 1938; Dow & Moruzzi, 1958) and the commonly held view is that Purkinjé cell degeneration results from anoxia produced by the tonic phase of the convulsive state. Dow and Moruzzi, moreover, have suggested that Purkinjé cells are uniquely susceptible to a variety of exogenous toxins and to alterations in metabolism such as hypoglycaemia, hyperthermia and anoxia. Thus the cerebellum may not represent the primary site of action of the factor responsible for the development of this disease, the Purkinjé cell degeneration being the result, rather than the cause, of the acute convulsive phase. Purkinjé cell degeneration does, however, provide a physiologically satisfactory explanation of the residual ataxia. In view of this it is very unfortunate that we have been unable to obtain cerebellar material from very young calves with only the acute convulsive form of the disease.

Comparative Aspects

The literature on calves does not appear to contain any reference to cerebellar disease of the type reported here. The congenital bovine cerebellar ataxias hitherto

described fall broadly into two types: those where varying degrees of hypoplasia are combined with degeneration (Robin, 1911; Innes, Russell & Wilsdon, 1940; Anderson & Davis, 1950; Johnston, McGavin & Simmons, 1962; Finnie & Leaver, 1965; Howell & Ritchie, 1966) and those with neuronal aplasia and hypomyelinogenesis (Saunders, Sweet, Martin, Fox & Fincher, 1952; Hulland, 1957; Johnston, Fourt, Ross & Bailey, 1958; Young, 1962). Most of these conditions are described as genetic and generally considered to be recessive.

Daft lamb disease (Innes, Rowlands & Parry, 1949; van Bogaert & Innes, 1950) however, resembles the present condition in that the cerebellum is often grossly normal and there is loss of Purkinjé and granule cells. Daft lamb disease is inherited as a recessive.

Congenital granuloprival cerebellar hypoplasia in kittens also has some pathological features in common with the present disease. It, however, is the result of intrauterine infection with a picodna virus (Kilham & Margolis, 1966).

Genetics

Four clinically normal bulls, used on separate farms, left affected cases on mating to females of several breeds, while bulls used contemporaneously left only normal offspring. One bull left affected offspring out of cross cows on seven different farms. Cows producing affected offspring had never previously or subsequently (to date) had abnormal calves. The bull, therefore, directly transmitted the condition.

This direct transmissibility, the absence of signs of infection, and the apparently hereditary nature of other cerebellar ataxias, indicate a genetic basis for the disease, though clear cut genetic ratios are absent. On the other hand, a viral aetiology has recently been shown for feline cerebellar ataxia, and a sire-transmitted virus could provide an alternative explanation.

Direct transmission by the bull implies a dominant mode of inheritance. The Orkney outbreak, and the data from the first herd, suggest transmitting bulls leave around a quarter of their offspring affected. The disease is, therefore, probably due to a dominant with incomplete (around 20 to 30 per cent.) penetrance.

Although direct descent over several generations has not been demonstrated (pedigrees from the first herd are unavailable), the time relationships suggest that the more recent transmitting bulls are probably descended from the original bull in the first herd.

Spread and Control

Possibly the disease arose as a fresh mutation in the first herd and is, as yet, restricted to a few herds. So far other outbreaks have not been located. Most of these herds are now selecting against transmitting bulls and their offspring, and the current localised outbreak may die out.

The disease could, however, cause considerable trouble in a small breed like the Aberdeen Angus. While a straight dominant is easily controlled (every carrier can be recognised), as penetrance declines, more and more clinically normal animals transmit the condition and 20 to 30 per cent. penetrance permits the existence of many carriers. Breeders could also be tempted to use recovered cases.

Moreover, as the first outbreak shows, pedigree herds using a valuable transmitting bull may be reluctant to dispose of all the progeny and even breeders willing to cull a transmitting bull may, to cut their losses, pass it or its progeny on to other owners. Affected calves can be reared and fattened and heavily involved breeders may be disinclined to suffer extensive losses through culling.

Possibly, therefore, other leading herds are involved. The disease could, indeed, be fairly widespread already in the Aberdeen Angus, either being hushed up (the first breeder discouraged further enquiry as soon as he realised the condition was genetic) or being confused with other conditions. While around one in four offspring are affected, the disease could even be transmitted by A.I. in small herds without relationship to the bull being suspected.

Once the disease is recognised as a clinical entity, however, and it is realised that the bull transmits the condition, breeders will select against involved herds and these will be forced to eliminate transmitting bulls and their progeny. Penetrance is sufficiently high to make detection of transmitting bulls fairly easy.

The disease fortunately appears in early life. Some human dominant diseases, like Huntingdon's chorea, only appear when reproduction is over and hence can persist through many generations—more than 1,000 cases are currently known in direct descent from two brothers who arrived in America in 1630. Once the present disease is recognised it should not exhibit a similar spread, because it appears early in life and direct selection against it can be rigorously applied.

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Addendum

Since this paper was submitted, the opportunity has arisen through the courtesy of Mr. J. Doyle, M.R.C.V.S., to examine the brain of a two- to three-month-old calf affected with this disorder. Vacuolation and chromatolysis of Purkinjé cells was present, but localised to the tips of the cerebellar folia. It is suggested that these findings support the concept that the neuropathological changes are probably the result rather than the cause of the acute convulsive attacks.

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NEUROPATHOLOGICAL OBSERVATIONS IN GRASS SICKNESS OF HORSES

By

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INTRODUCTION

The various clinical forms of grass sickness are well known to clinicians in regions where the disease is endemic. The morbid anatomy however, is insufficiently characteristic to provide precise confirmation of the diagnosis. The recognition by Obel (1955) of neuronal degenerations in the vertebral and prevertebral ganglia and alimentary mural plexuses of the autonomic nervous system was a most important advance, though even the specificity of these changes has since been questioned (Brownlee, 1959, 1965).

The purpose of this communication is to draw attention to the occurrence of histological changes in the central nervous system of a small sample of horses with clinical grass sickness.

MATERIALS AND METHODS

During the period April to September, 1968, 6 horses with clinical grass sickness were obtained for pathological examination from 4 veterinary surgeons with long experience of the disease. Table 1 defines this material in terms of the classification recognised by clinicians and those gross post-mortem findings which have been considered most characteristic of grass sickness (Pool, 1928; Begg, 1936; Holman, Gordon and Pattison, 1944). In the two horses which died, post-mortem examination was carried out within 5 hours of death. The remainder were autopsied immediately or within 2 hours of a lethal intravenous dose of thiopentone sodium or chloral hydrate.

As soon after death as possible, each horse was bled out by removing the head at the atlanto-occipital articulation and the left forelimb at the shoulder. The severity of the lesions in the nervous system was scored on an arbitrary scale + to + + + +, the latter indicating the maximum lesion density observed.

The tissues, which were placed in 10 per cent. formalin for subsequent histological examination, were brain; spinal cord from C_1-T_2 and L_1 to the filum terminale; the corresponding dorsal root ganglia; the cranial and caudal cervical, stellate, coeliaco-mesenteric and caudal mesenteric ganglia; thoracic sympathetic ganglia; thymus; cervical and mesenteric lymph nodes; upper and lower oesophagus; cardia, fundus and pylorus of the stomach; duodenum; jejunum, and ileum; 4 regions of the colon; rectum; liver; kidney; adrenals; pancreas; pituitary and eyes.

Blocks of each tissue were post-fixed in saturated mercuric chloride, dehydrated in an alcohol—benzene series and embedded in paraffin. The remaining tissue was stored in formalin for frozen sections where necessary. The C.N.S. blocks were taken from transverse slices of cerebrum at the levels of the prefrontal gyrus and the occipital lobe to include the hippocampus; the corpus striatum and basal ganglia; the thalamic and hypothalamic nuclei; the medial and later geniculate bodies and the subcommissural organ; the superior and inferior colliculi of the corpora quadrigemina; the cerebellum through the fissura prima and the middle cerebellar peduncle, and

serially through the medulla. Blocks of cord were taken initially from about C_2, C_5, T_1, L_2 and S_2 spinal segments.

Section of all blocks were cut at 6 μ and stained with haematoxylin and eosin. C.N.S. sections were also cut at 12 μ and stained with Luxol fast blue. Special neuro-histological methods—Holmes' silver method for neurofibrils, Cajal's gold chloride for astrocytes, OTAN for myelin and products of myelin degeneration, Smith Quigley for myelin and Sudan IV for neutral fat—were applied to appropriate tissues as required.

RESULTS

The findings in the six clinical cases of grass sickness are summarised in Tables 1 and 2. Four geldings and 2 mares, all saddle horses between 3 and 9 years old,

TABLE 1

CLINICAL AND MORBID ANATOMICAL SUMMARY OF CASES

Case No.	1[1]	2[2]	3[3]	4[4]	5	6[5]
Breed	Thorough-bred	Hunter	Dartmoor pony	Pony	Highland pony	Thorough-bred
Sex	F	M	F	M	M	M
Age (years)	3	5	5	9	3	3
Duration of illness (days)	2	1	1	10	14	7
Clinical classification	Acute	Acute	Acute	Chronic	Chronic	Chronic
Natural death	+	−	−	−	+	−
Destroyed Chloral/ Pentobarb. Na	−	C	P	P	—	P
Delay before P.M. (hours)	2	2	Nil	Nil	4–5	Nil
Oesophageal ulceration	+	−	−	−	−	+
Sanguinous cervical lymphadenopathy	++	+	−	−	−	−
Stomach distended	++++	+	−	+	+	−
Small intestine distended	−	+	++	+	+	−
Large intestine distended	++	−	−	++	+	−
L.I. contents blackened and hard	++++	−	−	++	−	+
Submucosal oedema	−	+	+	−	−	−
Splenomegaly	+++	+	−	+	+	++
Adrenal cortical haemorrhages	+++	+	−	−	−	−

F = Mare M = Castrated male

[1] Bruising and abrasion of intestinal wall near ileocaecal valve. Peritoneal fibrin tags from seepage of stomach contents through gastric wall. Infiltration of hepatic portal tracts with lymphocytes and polymorphs.

[2] Intestinal mucosa heavily infiltrated with eosinophils.

[3] Massive infestation of small intestine with tapeworms of genus Anoplocephala. Necrotising enteritis with sloughing of mucosa at ileocaecal valve and intense local inflammatory oedema. Neuroaxonal dystrophy in cuneate nucleus. Microscopic inflammatory foci in liver; larval migratory tracts.

[4] Liver and lungs show heavy hydatid infestation. Light, focal, polymorph infiltrations of portal tracts.

[5] Patchy jejunitis, masses of lymphocytes and eosiniphils infiltrating mucosa and breaching muscularis mucosa.

In some places Meissner's plexus was involved in this process and destroyed: elsewhere it appeared normal. Adrenals very soft and yellow.

were involved. Three were classed as acute and three as chronic cases. One acute case and one chronic case died and the remainder were destroyed on humane grounds. The most characteristic morbid anatomical features of grass sickness, namely gastric distention, distention of the large intestine with blackened firm

ingesta and splenomegaly, were present to a greater or lesser degree in 5 out of the 6 cases. Case 3, however, is exceptional in that the morbid anatomy was dominated by a parasitic enteritis due to very large numbers of tapeworms of the genus Anoplocephala.

Table 2 shows that degenerative changes in the vertebral and prevertebral

TABLE 2

DISTRIBUTION AND SEVERITY OF CHROMATOLYSIS

	Case No.					
	1	*2*	*3*	*4*	*5*	*6*
Oculomotor complex	++	−	−	++	++++	+
Lat. vestibular nucleus	−	−	−	+	++	+
Hypoglossal nucleus	−	−	−	−	++++	+
Dorsal motor vagus nucleus	−	−	−	−	++	−
Facial nucleus	−	−	−	++	++++	++
Nucleus VII b (Palmer, 1958)	−	−	−	+	−	+
Spinal cord (Ventral horn)	−	−	−	+	+++	−
Dorsal root ganglia	−	−	−	−	−	−
Sympathetic ganglia						
cranial cervical	++	NE	−	++	NE	+++
caudal cervical	++	NE	−	++	+++	+++
stellate	++	NE	−	++	++++	+++
cran. mesenteric	++	NE	−	++	+++	+++
caudal mesenteric	+++	+	−	++	+++	+++
thoracic chain	+++	+	−	++	+++	++++
Myenteric plexus (Auerbach)	++	+++	−	+*	+*	++
Submucous plexus (Meissner)	+	*	−	*	+*	+

N.E. = Not examined.
* = Cells rarely observed, many presumed to have been lost.
\+ = Occasional chromatolytic cell observed.
++ = Moderately extensive chromatolysis.
+++ = Extensive chromatolysis.
++++ = Very extensive chromatolysis.

ganglia and the gastro-intestinal mural plexuses of the autonomic nervous system occurred in all cases except case 3. The earliest change observed was central chromatolysis followed by margination and pyknosis of the nucleus and the formation of numerous, tiny, cytoplasmic vacuoles (Fig. 1). Neuronophagia sometimes accompanied the later stages of degeneration, especially in the cells of the mural plexuses (Fig. 2). In the longest standing chronic cases (4 and 5) it was sometimes difficult to find cells of both the submucous and myenteric plexuses and it is presumed that many had completely disintegrated.

In one acute and all 3 chronic cases neuronal lesions were also found in the C.N.S. The lesions varied in extent, but appeared to be selective for medium sized nerve cells of particular cell groups. They occurred in the oculomotor, facial, lateral vestibular, hypoglossal and dorsal motor vagus nuclei, in decreasing order of frequency. In the spinal cord they were confined to a few cells at the ventral or lateral margins of the ventral horns. As in the autonomic nervous system, these neuronal lesions in the C.N.S. were degenerative in character, commencing with central chromatolysis, margination and pyknosis of the nucleus and granular disintegration of neurofibrils (Figs. 3 and 4). Vacuolation of the cytoplasm and neuronophagia were not observed. Affected cells became transformed into homogeneous, pale, eosinophilic masses which gradually merged with the ground

substance and disappeared to leave holes in the tissue (Figs. 5 and 6). No abnormal accumulations of lipid or products of myelin degeneration were found. Lipofuscin granules were irregularly observed in both affected and apparently normal nuclei.

In one case (4) a different type of change involving the cerebellar Purkinjé cells was also seen. This was an acute vacuolation and fragmentation of a few neurons at the tips of the folia and was probably an artefact of handling or fixation.

DISCUSSION

Six cases of clinical grass sickness have been examined pathologically. Five of them showed the characteristic morbid anatomy of the disease and those neuronal degenerations in the vertebral and prevertebral ganglia and the alimentary mural plexuses which, hitherto, have been associated with grass sickness (Obel, 1955). In the remaining case such pathological support for the clinical diagnosis was lacking and it is tempting to conclude that this was not, in fact, a case of grass sickness, but an acute colic arising from parasitic enteritis with severe inflammatory oedema of the ileocaecal valve.

It is of considerable interest that 4 of the 5 cases for which pathological support was obtained also showed chromatolysis in certain nuclei of the C.N.S. The extent of this change appeared to be in direct proportion to the duration of clinical illness and it might be argued that this lesion is not a primary feature of the disease, but results from other non-specific causes. Support for this view can be claimed from the thorough work of Obel (1955) in whose series of 9 acute and 5 chronic cases C.N.S. changes were recognised only in the hypoglossal nucleus of one animal. On the other hand the distribution of the lesion was highly selective. With the exception of the lateral vestibular nucleus, all the C.N.S. regions involved govern final motor pathways and might relate to the parasympathetic division of the autonomic nervous system. Furthermore, it seems unlikely that a secondary, non-specific toxic process would cause severe selective chromatolysis in the brain stem and cord, and yet spare the cerebellar cortex which is notably susceptible to toxic-anoxic processes (Dow and Moruzzi, 1958).

For these reasons it is tentatively suggested that the C.N.S. changes described here may be a specific part of the pathology of grass sickness though their extent may depend upon the duration of clinical illness.

SUMMARY

Pathological examinations were made on 6 horses with clinical grass sickness. Support for the diagnosis was obtained in 5 cases, in 4 of which chromatolysis and necrosis of neurons in specific regions of the central nervous system were also found. The local severity of these lesions appeared to be directly proportional to the duration of clinical illness. As the distribution of the lesions indicates a possible relationship to the parasympathetic division of the autonomic nervous system it is tentatively suggested that these changes in the central nervous system may be of direct significance in the pathogenesis of grass sickness.

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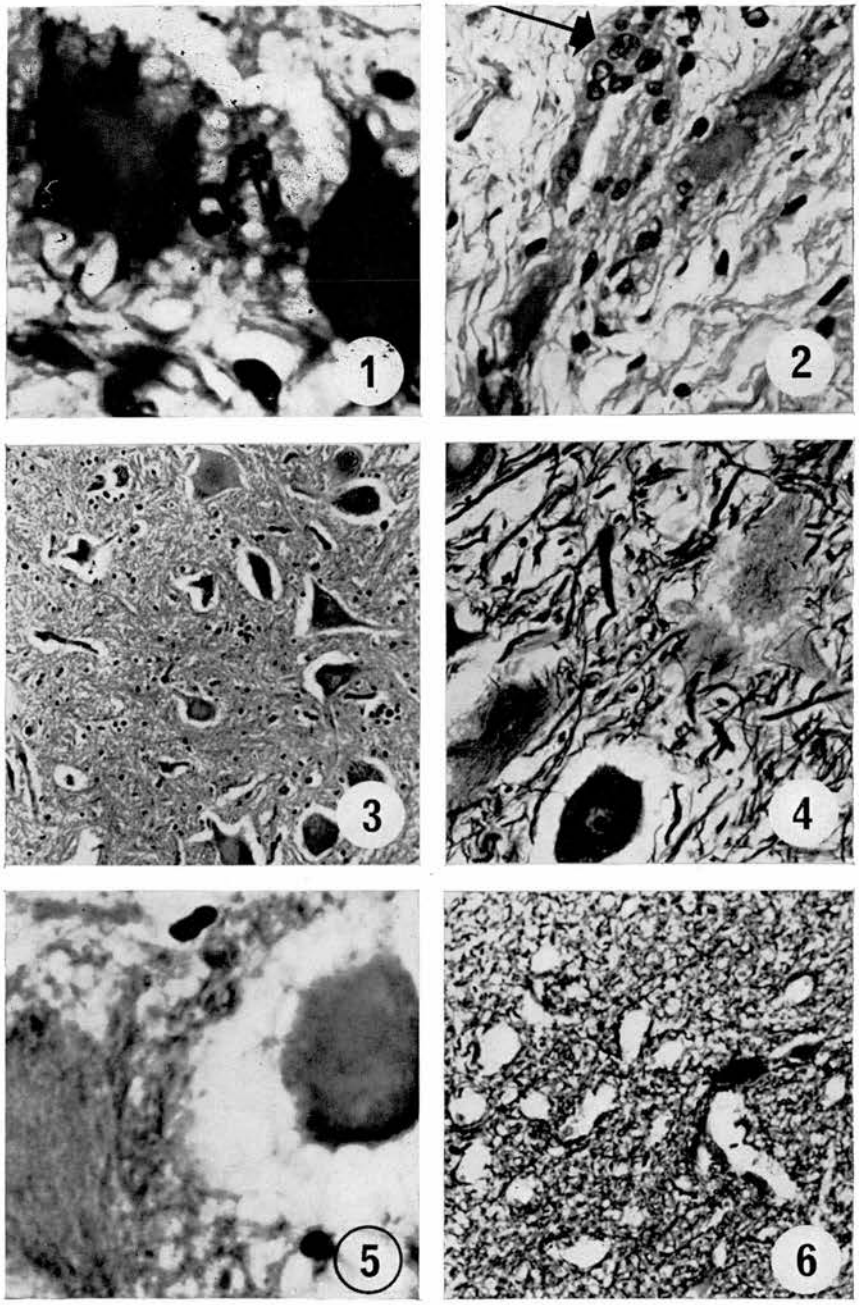

LEGENDS

Fig. 1. Auerbach's plexus. Chromatolysis of nerve cells with margination of the nucleus and fine vacuolation of cytoplasm. H & E × 1120.

Fig. 2. Auerbach's plexus. Chromatolysis of nerve cells and neuronophagia (arrow). H & E, × 515.

Fig. 3. Facial nucleus. Stages of chromatolysis. H & E, × 110.

Fig. 4. Oculomotor nucleus. Chromatolysis with granular disintegration of neurofibrils. Holmes, × 380.

Fig. 5. Facial nucleus. Chromatolysis and lysis of neurons. H & E, × 945.

Fig. 6. Oculomotor nucleus. Vacuolation due to fall-out of effete neurons. Holmes, × 110.

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31.

SPONGY CHANGES IN THE BRAINS OF SHEEP POISONED BY EXCESS DIETARY COPPER

BY

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Spongy Changes in the Brains of Sheep Poisoned by Excess Dietary Copper

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SUMMARY. The brains of 6 sheep that died as a result of long-term feeding of a diet containing 80 p.p.m. of copper were examined histologically. There were marked lesions of spongy transformation, particularly of the white matter in the midbrain, pons and cerebellum. The telencephalon was unaffected.

WILSON'S DISEASE (HEPATOLENTICULAR DEGENERATION) of man is characterised by severe pathological changes in the brain, especially in the basal ganglia, and in the liver. This has been associated with the presence of excess copper in many tissues (Cumings, 1959). It is the only naturally occurring neuropathological condition of man or animals in which copper poisoning has been implicated.

Marked cirrhosis of the liver is invariably seen in sheep dying from chronic copper poisoning (Pearson, 1956). We wish to report the finding of pathological changes in the central nervous systems (C.N.S.) of such animals.

MATERIALS AND METHODS

A group of lambs being raised at the Institute were fed from 6 to 12 weeks of age on a commercially prepared lamb weaner ration. Seventeen of these came to *post-mortem* examinations, having been seen alive the previous night and found dead in the morning. The macroscopic appearance was of generalised icterus with swollen orange-red livers and dark kidneys. Histologically there was severe liver cirrhosis and necrosis of kidney tubules. This pathology, together with liver copper values from two cases of 3225 and 4325 p.p.m. d/w, led to a diagnosis of chronic copper poisoning. The diet was examined and found to contain 80 p.p.m. d/w of copper.

Treatment was instituted after an early tentative diagnosis and all were dosed orally for 5 days of each week with 20 ml. of a mixture containing 100 mg. of ammonium molybdate and 1 g. of sodium sulphate (Ross, 1966). The brains of 6 of the 17 that

TABLE I
THE DISTRIBUTION OF SPONGY TRANSFORMATION* IN THE CENTRAL NERVOUS SYSTEM

		Sheep number						
		6H30	5H66	5H97	6H35	6H60	6H73	6H15
Cerebrum		—	—	—	—	—	—	—
Internal capsule		—	NE	NE	+	+	+	+
Midbrain		—	+++	++++	+++	++	++	+++
Cerebellum	R.H.S.	—	+++	+++	+++	++	++	+++
Corpus Medullare	L.H.S.	—	++	+++	+++	++	++	+++
Pons	R.H.S.	—	++	+++	+++	+	++	+++
	L.H.S.	—	++	+++	+++	+	+	+++
Medulla		—	+	++	+++	—	++	+++
Spinal cord		NE	NE	++	NE	—	NE	NE

* The most severe lesion seen was classified as ++++. A +++ lesion is illustrated in Fig. 1, ++ in Fig. 2.
NE Not examined.

died and one (6H30) that was clinically normal when killed, were taken for histology. These 7 sheep had been treated with the molybdate-sulphate preparation for at least 9 days and were sampled over a one month interval, in the order given in Table I.

After fixation in 10% formol saline the brains were sliced coronally and blocks taken as described by Barlow *et al.* (1960). Paraffin sections (6μ) were stained with haematoxylin and eosin (H&E) and luxol fast blue (LFB). Selected sections were stained by the following techniques: Sudan black, periodic acid-Schiff, Holmes' silver stain, gallocyanin, methyl green pyronin and Lendrum's modification of Mallory's stain. Frozen sections were stained by the OTAN, Sudan IV, Smith Quigley and Cajal's gold chloride impregnation methods.

RESULTS

There was marked spongy transformation (Figs. 1 to 4), predominantly in the white matter, in the brains of all sheep that died. The distribution of this lesion is summarised in Table I.

The telencephalon was not affected and the most rostral lesion was slight status spongiosus of the internal capsule. The superior cerebellar peduncles were particularly severely damaged. The white matter of the cerebellar folia was rarely affected, and then only centrally.

The use of the various special stains did not result in further elucidation of the nature of the lesion. No free lipid was detected. Damage to neurons was slight (Fig. 3), though a few cells appeared vacuolated (Fig. 4).

There was a diffuse inflammatory reaction with small perivascular cuffs in the caudate nucleus of one sheep, 6H73.

DISCUSSION

It is unlikely that the time lag between death and *post-mortem* examination was responsible for the lesion described. We examine the brains of many sheep that die in the field and have not previously seen pathology of this type. The possible effect of the molybdate-sulphate treatments cannot be discounted.

The spongy changes observed by us are very similar to the acute pathology described in laboratory animals inoculated directly into the C.N.S. with copper. Intraventricular injection of a copper albumin complex into the brains of cats produced severe damage, the earliest signs of which were swelling and sponginess of the white matter (Vogel & Evans, 1961). This acute change was characterised by hydropic swelling of myelin sheaths and was rapidly followed by necrosis of all parenchymal structures. Wisniewski *et al.* (1965) injected small doses of copper into the brains of rabbits, which caused marked sponginess of white matter and necrosis. In sheep dying from chronic copper poisoning there is a massive release of copper from the liver into the blood shortly before death (McKosker, 1968). The presence of a great excess of direct reacting copper in the plasma might result in increased levels in the cerebrospinal fluid, which could mimic the effect of direct inoculation of copper into the C.N.S.

It is important that the present report be confirmed, in both experimental and field situations.

ACKNOWLEDGMENTS

The history of the incident was provided by Mr. M. G. Christie. The copper estimations were done by Dr. N. F. Suttle. The photographs were taken by Mr. D. G. Watson.

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FIG. 1. Spongy changes in the midbrain of sheep 6H15. All the white matter tracts are affected, yet the central grey matter around the aqueduct is quite normal. H & E × 15.
M = Medial lemniscus.
D = Decussation of the superior cerebellar peduncles.
F = Medial longitudinal fasciculus.

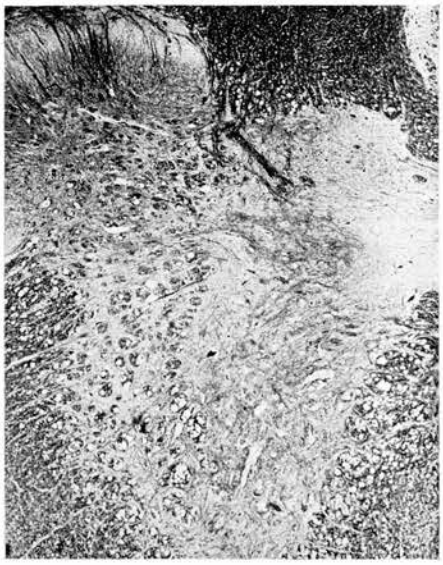

FIG. 2. Spongy transformation in the dorsal and ventral fasciculi proprii of the cervical spinal cord of sheep 5H97. LFB × 32.

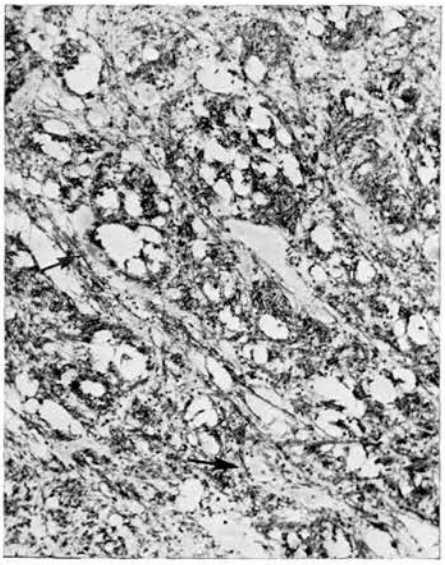

FIG. 3. Sponginess in the corpus medullare of the cerebellar body of sheep 6H15. The arrows point to normal neurons LFB × 225.

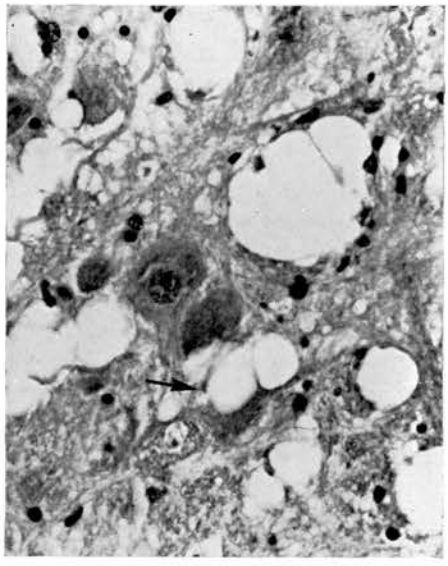

FIG. 4. Vacuolated cell (arrow) and status spongiosus in the reticular formation of the medulla of sheep 6H15. H & E × 825.

32.

TRANSMISSION EXPERIMENTS WITH A SCRAPIE-LIKE ENCEPHALOPATHY OF MINK

By

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INTRODUCTION

North American mink encephalopathy (Hadlow, 1964; Burger and Hartsough, 1965) has a long incubation period, a clinical course of about 8 weeks and a pathology closely resembling that of scrapie in sheep (Zlotnik, 1958), goats (Pattison, Gordon and Millson, 1959; Hadlow, 1961) and mice (Chandler, 1961, 1962; Zlotnik and Rennie, 1962). The disease is readily transmissible to mink by intracerebral inoculation of affected brain tissue and Zlotnik and Barlow (1967) have reported the probable transmission of mink encephalopathy to the goat. They failed, however, to produce the disease in Swiss white mice within 16 months of inoculation.

The present paper reports the results of further transmission experiments with this agent and underlines the similarity of this disease to scrapie.

MATERIALS AND METHODS

Inocula

Mink brains. A pool of brains from affected mink was obtained from Dr. Burger and stored at −20°C. The supernatant of a 10 per cent. suspension of brain was inoculated intracerebrally (0·03 ml.) into random bred mice of the Porton and Moredun Swiss white strains.

Goat brain. A 10 per cent. saline suspension supernate of goat brain No. 1 (Zlotnik and Barlow, 1967) was similarly prepared and inoculated intracerebrally into goats (1·0 ml.), and Porton and Moredun Swiss white mice (0·03 ml.).

Mouse brain. An inoculum was similarly prepared from a pool of the Porton mice mentioned above and inoculated intracerebrally in 0·03 ml. doses into mice of the strains Porton and Moredun Swiss white, C57 Black and C57 Brown.

Animals

The goats were of mixed breeding, predominantly Anglo-Nubian and Toggenburg and were inoculated at 2 to 3 months of age. The mice were inoculated as 19 to 21 day old weaners. The random bred mice consisted of groups of 10 to 40 (Table 1) drawn from the pooled females of between 35 and 40 litters. The groups of inbred mice were composed of the female offspring of 2 to 3 litters.

Sterile utensils and disposable syringes were used for all manipulations and all brains were examined histologically.

RESULTS

The results including those of Zlotnik and Barlow (1967) are summarised in Table 1.

TABLE 1

TRANSMISSION OF MINK ENCEPHALOPATHY TO GOATS AND MICE

Times given in brackets indicate duration of experiments and sign denotes whether or not lesions were found.

Mink Brain Inoculum

Goats. As reported previously, clinical neurological signs (hyperaesthesia, ptosis and ataxia) appeared in one goat (No. 1) at 20 months and another (No. 2) at 27 months after inoculation with mink brain supernate. When killed at 25 and 28 months respectively both showed neuronal vacuolation and status spongiosus which was generalised and severe in the first animal, but restricted to the striae of the midbrain in the second. The third goat (No. 3) remained clinically normal, but when slaughtered 43 months after inoculation mild status spongiosus of the cerebral cortex and amygdaloid nuclei was found.

Mice. As in the experiment of Zlotnik and Barlow (1967) no clinical disease or neuropathological changes were evident in Swiss white mice 15 months after inoculation with mink brain supernate. In Porton mice, however, clinical cases were found 8 months after inoculation. Histologically these showed severe neuronal vacuolation of the brain stem, cerebral cortex, amygdaloid nuclei and hippocampus. The incidence in Porton mice was 50 per cent. in 14 months.

Goat Brain Inoculum

Goats. Three weanling goats (Nos. 4, 5 and 6) inoculated with material from goat No. 1, showed intermittent, very mild hyperaesthesia, scratching and bilateral ptosis about 9 months after inoculation. They were clinically normal when slaughtered 15 months after inoculation, but on histological examination all showed vacuolation of nerve cells in the brain stem and amygdaloid nuclei and one also had widespread severe status spongiosus in the hippocampus and cerebral cortex.

Mice. Porton and Moredun Swiss white mice inoculated with the same material remained well and were histologically normal when killed 15 months after inoculation.

Porton Mouse Brain Inoculum

This was prepared from the mice which developed a scrapie-like condition after inoculation with mink brain supernate. In Porton mice clinical signs appeared at about 13 weeks after inoculation with this material and the first cases were confirmed histologically at 18 weeks. By the 20th post-inoculation week all the Porton mice were clinically affected and were found to have widespread scrapie-like lesions of the cerebrum and brain stem. The C57 Black and C57 Brown mice all developed clinical signs at about 20 weeks and severe brain stem and cortical lesions were found without exception in those surviving at that time. In the Moredun Swiss white mice, however, the earliest confirmed case occurred 23 weeks after inoculation and a further 9 weeks elapsed before the whole group was affected. The lesions were indistinguishable from those in the other groups of mice.

The inoculum was not tested in goats.

DISCUSSION

These experiments with mink encephalopathy reveal patterns of transmission and lesion distribution which must be considered in relation to those of scrapie. It is clear from the results that the earlier report (Zlotnik and Barlow, 1967) did

represent transmission of the mink disease to goats and not some chance infection with scrapie. The disease in mink produces cortical and sub-cortical status spongiosus and vacuolation in brain stem neurons (Burger and Hartsough, 1965), closely resembling the lesions encountered in the present experiments. These lesions are similar to those produced by the scrapie agent of sheep origin after serial passage in mice and hamsters (Zlotnik, 1965).

From these considerations mink encephalopathy might be regarded as a mink-passaged form of scrapie akin to scrapie passaged in other rodents, e.g. mice and hamsters. The differential susceptibility of the various mouse strains used is therefore of interest. Had the Swiss mice been allowed to live beyond 16 months, they might eventually have developed lesions, but this would have further emphasised the difference in incubation period between mice of the Porton strain and other breeds. The incubation period of scrapie in mice appears to be genetically controlled (Dickinson, Meikle and Fraser, 1968). If similar genetic factors operate in mink encephalopathy it is surprising that such differences in incubation period can be overcome so dramatically by a single passage in Porton mice and that the virulence of the agent for these mice can be so reduced by a single goat passage. In the context of current genetic theories on scrapie (Dickinson and Meikle, 1969) these findings can be explained by postulating that the original "agent" contained a mixture of strains, different elements of which were favoured by the various genetic endowments of different host species. However the "all or none" effect within the different groups of random bred mice argues against this. The alternative hypothesis of agent modification remains open.

The behaviour of this agent in goats is unusual. Only one of the first 3 goats showed marked clinical and pathological changes; all 3 of the goats to which its brain was passed showed mild, intermittent and rather indeterminate clinical signs, but all had severe and widespread histological changes when killed at 15 months. These findings demonstrate lack of correlation between clinical signs and pathological changes in goats.

The present experiments have not resolved the question whether mink encephalopathy is a variant of scrapie with a possible mixture of agents or a distinct entity. The results suggest that the causal agents are similar, but that the agent of mink encephalopathy has a greater degree lability which might be exploited in the search for the nature of both agents.

SUMMARY

North American encephalopathy of mink has been transmitted to mice and goats. The histopathology of the disease in these species resembles that of mouse-passaged scrapie of sheep origin. The characteristics of the agent as at present revealed by passage are unlike those of scrapie.

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A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE SUBCOMMISSURAL ORGAN OF YOUNG AND OLD SHEEP*

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Summary. Cytologic alterations with age are evident in the subcommissural organ of sheep, but evidence of a secretory function has not been found. In young lambs the cytoplasm has numerous, irregular, interconnecting, endoplasmic sacs with electron dense material. Lamellar cytoplasmic lipid bodies (lipid surrounded by membranous whorls) are scattered throughout the deep layer.

With age, the cytoplasm contains electron-lucent, flocculent material and lamellar cytoplasmic lipid bodies are less apparent. In adolescent and adult sheep tightly-coiled membranous whorls (fingerprints) enclosing cytoplasmic constituents are noted. Gliosis is progressive. These changes probably indicate involution.

Histochemical studies demonstrate distinct Golgi patterns in superficial and deep layers and a rich vascular network in the deep layer. Acid phosphatase activity is present and defines lysosomes, a larger cholesterol ester-containing body (Type II) and macrophages. Type II bodies are less frequent with age.

Bioassays and chemical analyses should be interpreted with reference to the age of the experimental animal.

The subcommissural organ (SCO) is a plate of modified ependyma attached to the ventral surface of the posterior commissure. It forms the lining of the roof of the third ventricle near its junction with the cerebral aqueduct. The cells of the SCO contain numerous granules which stain with periodic acid-Schiff (P.A.S.) and Gomori's chrome alum-hematoxylin. Morphologic appearances suggest apical secretion into the ventricle (Dendy and Nicholls, 1910; Wislocki and Leduc, 1953; Kivalo et al., 1961; Vigh et al., 1961; Lin and Duncan, 1961; Laatsch, 1964), though endocrine functions have also been proposed (Gilbert, 1960, 1963; Taylor and Farrell, 1962; Palkovits and Lukas, 1963; Brown and Afifi, 1964). In some species, including man, it has been suggested that the SCO may involute with advancing age (Friede, 1961; Stanka et al., 1964; Rakic, 1965). The present study is concerned with the ultrastructural and histochemical changes which occur in the SCO of sheep of various ages between 2 weeks and approximately 7 years.

Materials and Methods

The SCOs of 28 sheep were studied. With the exception of 3 ram lambs (15, 29 and 74 days old) and 4 castrated males approximately 8 months of age, all were from female sheep. Eight lambs of Rambouillet or Suffolk breeds, which were also part of a study of

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axonal reaction to sciatic nerve section, were examined 15—81 days after birth. Eleven subcommissural organs from adolescent lambs 5—8 months of age and 9 from ewes approximately 7 years old were obtained from a local abattoir and were of undetermined breeds. The sheep were killed by decapitation, which in the case of the young lambs was performed under pentobarbital sodium anesthesia. Within 5 min of death each brain was removed and the SCO isolated for further processing. For electron microscopy small pieces were fixed overnight in phosphate-buffered 4% glutaraldehyde. After washing in phosphate buffer the pieces were further subdivided and post-fixed for 1 hour in phosphate-buffered 1% osmium tetroxide. In a few instances (7 year old ewes) electron microscopy of sites of acid phosphatase activity was performed. In these cases the primary fixation was in a sodium cacodylate-buffered 3% glutaraldehyde for 2 hours. Frozen sections were cut at 40 μ, incubated in Gomori's β-glycerophosphate-lead nitrate acid phosphatase medium in acetate buffer at pH 5.0 (Holt, 1961) and post-fixed in 1% buffered osmium tetroxide. Thereafter, all tissue for electron microscopy was dehydrated in alcohol and embedded in Epon 812. For orientation, sections 1—2 μ thick were stained with alkaline toluidine blue or cresyl violet. Thin sections were stained with lead citrate, uranyl acetate or double-stained.

For oxidative enzyme histochemistry (slaughterhouse material only) the block of fresh brain was received on aluminum foil and quickly frozen on dry ice. When hard, it was tightly wrapped in foil and Parafilm and returned to the dry ice container until required for processing. Sections were cut at 10 μ in a cryostat and mounted on clean dry slides or coverslips and stored briefly in dry Coplin jars at 4° C. Adjacent sections were used for each of the following enzymes and 6 replicas were incubated for succinic dehydrogenase (Nachlas et al., 1957), cytochrome oxidase by Burstones method using 1-hydroxy-2 naphthoic acid as a coupler (Pearse, 1961) and monoamine oxidase (Glenner et al., 1957). In 2 cases the presence of steroid 3 β-ol dehydrogenase was tested by the method of Levy et al. (1959) using both fresh sections and cold acetone-extracted sections with pig adrenal gland as positive control. Negative control sections were incubated in the medium without dehydroepiandrosterone to distinguish non-specific formazan production.

For phosphatase and lipid histochemistry, blocks were fixed in cold formol-calcium for 36—48 hours, washed in cold distilled water and stored in the refrigerator in isotonic sucrose-acacia solution. Frozen sections were cut at 10 μ into cold distilled water for use in the following techniques: acid phosphatase (Holt, 1961), thiamine pyrophosphatase (Novikoff and Goldfischer, 1961), alkaline phosphatase (Pearse, 1961), Sudan IV and a method for unsaturated fatty acids (Barlow, unpublished) based on Adams (1959), osmium tetroxide-α-naphthylamine development of the Marchi technique. Perchloric acid-naphthoquinone (Adams, 1961), Okamoto's iodine-sulphuric acid (Pearse, 1961), Schultz's modification of the Liebermann-Burchardt reaction (Pearse, 1961) and the digitonin reaction (Feigin, 1956) were used to determine the presence of cholesterol and cholesterol esters. At each stage frozen sections were stained with hematoxylin and eosin to maintain morphologic control.

Bouin-fixed paraffin sections were treated with Sudan black B and with P.A.S. with and without diastase digestion.

The extractability of lipid was tested by treatment with 1 N NaOH at room temperature and 37° C for 1 hour, or with 96% alcohol, absolute acetone, or equal parts of absolute alcohol and ether for 15 min. The sections were mounted in water and examined by polarized and ultraviolet light. Similarly treated sections were examined by the method for unsaturated fatty acids.

Observations

Light Microscopy

The SCO of the sheep is a clearly demarcated thickening of the lining of the roof of the 3rd ventricle close to the origin of the cerebral aqueduct. It lies beneath the posterior commissure and lines a diverticulum curving dorsally into the pineal stalk. In the adult it presents 2 distinct layers, a superficial ependymal portion composed of pseudo-stratified columnar epithelium and a deep hypendymal portion (Stanka et al., 1964) consisting of an irregular arrangement of cells in a

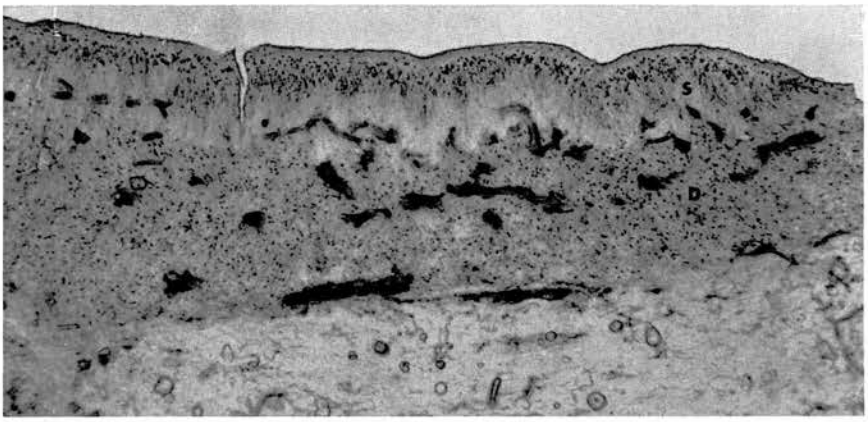


Fig. 1. Subcommissural organ. The ventricle is above and the posterior commissure is below. In the superficial layer (*S*) of the subcommissural organ the Golgi apparatus has a polar distribution which is not evident in the deep layer (*D*). Blood vessels are prominent in the deep layer. Thiamine pyrophosphatase. ×200

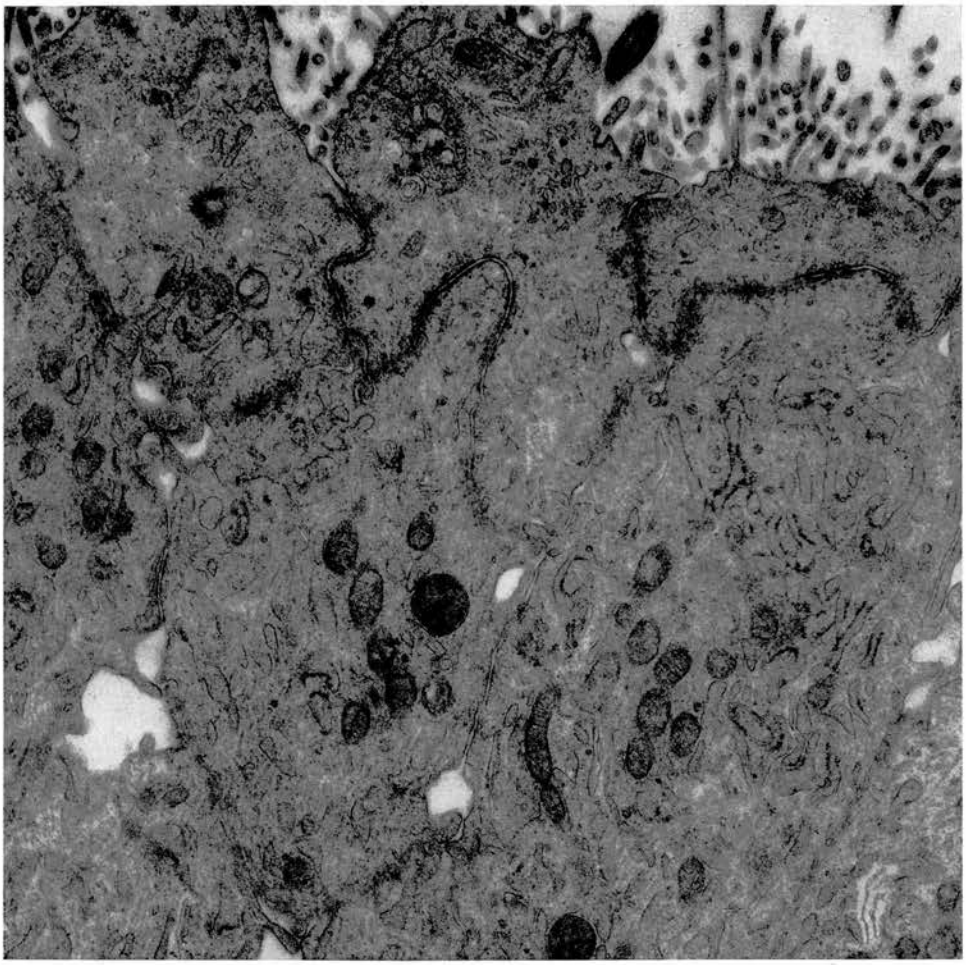


Fig. 2. The superficial cells have terminal bars, abundant microvilli and few cilia. There are numerous irregular sacs of the smooth and rough endoplasmic reticulum with electron-dense material. 38 day lamb. Lead citrate. ×12,000

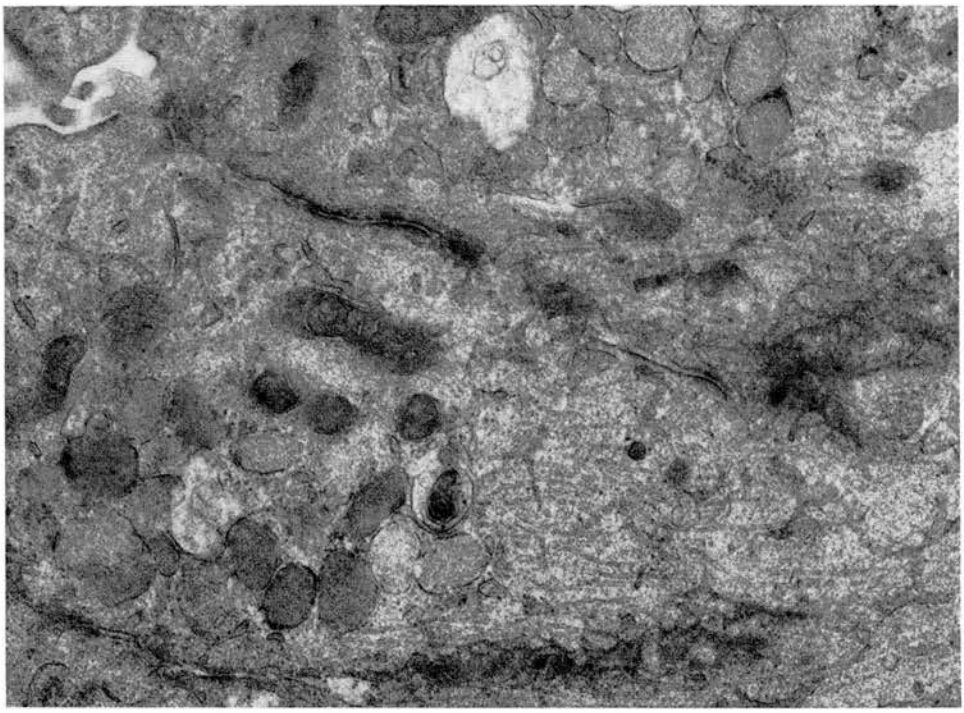


Fig. 3. Superficial cells with numerous tubules and groups of membrane-bound, spherical dense bodies. Adult sheep. Lead citrate. ×20,000

dense glial matrix. The hypendyma and the junction between the ependyma and hypendyma are richly supplied by blood vessels (Fig. 1). Myelinated nerve fibers from the posterior commissure extend a variable distance into the hypendyma.

In lambs up to 81 days of age the distinction between the two layers is not as clear as in older lambs and adults. The hypendyma is more cellular and the glial matrix less evident. In young lambs the organ extends a greater distance into the pineal stalk than in adolescent and adult sheep.

Electron Microscopy

The arbitrary terms superficial and deep are preferred to ependyma and hypendyma in the description of the ultrastructure because of the difficulty of exact localization within thin sections. The deep layer includes the hypendyma and the portions of the basal cell processes of the ependyma which abut on the perivascular space; while superficial refers to the remaining portions of the ependyma.

In young lambs 15—30 days of age the superficial portion of the SCO consisted of long slender cell processes which, in contrast to the adjacent unmodified ependyma lining the ventricle, presented relatively few cilia (Fig. 2). Reissner's fiber (Horsley, 1908; Dendy and Nicholls, 1910) was not observed, but the methods of tissue preparation were unfavorable for its preservation. Microvilli occurred along the ventricular border and terminal bars were present between

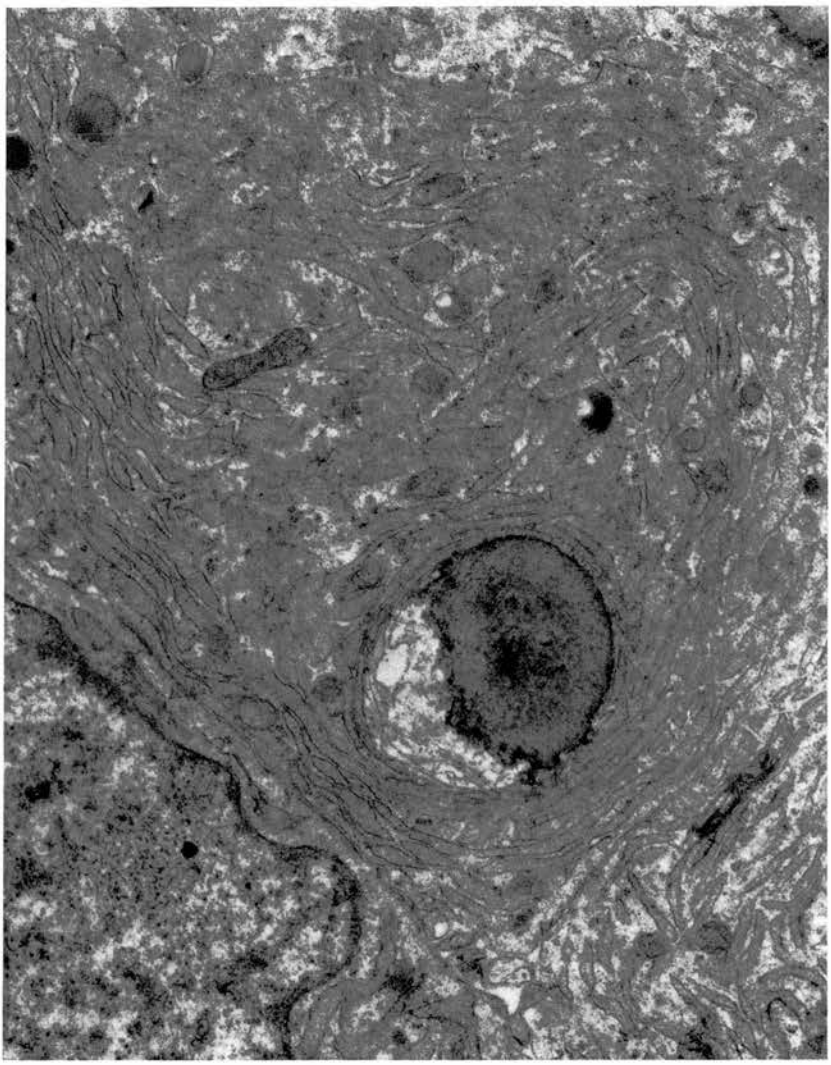
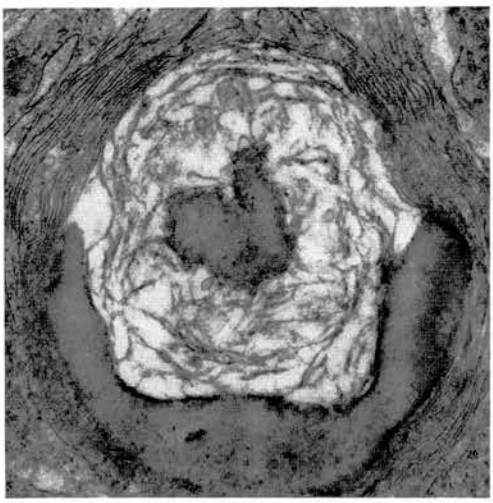


Fig. 4. Deep layer. This cell has an extensive system of interconnecting endoplasmic sacs with electron-dense material and a lamellar cytoplasmic lipid body. 15 day lamb. Lead citrate. ×17,000

adjacent cells. Slightly deeper in the superficial layer terminal bars were less evident; the cells were separated by a space containing slender cytoplasmic processes.

The cytoplasm of superficial cells in young lambs was filled with an elaborate system of endoplasmic sacs containing homogeneous, moderately dense material. This material was noted in both rough and smooth sacs of endoplasmic reticulum but more frequently in the latter. Occasionally, the contents of the sacs were electron-lucent and flocculent. With increasing age, the only significant change in the superficial layer was the occurrence of cytoplasmic tubules (Fig. 3) and pigment granules.



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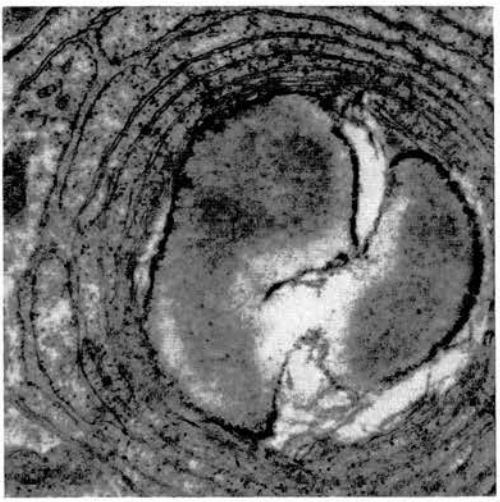

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Fig. 5. Lamellar cytoplasmic lipid body. In this instance the lipid core is surrounded by closely approximated membranes. 15 day lamb. Lead citrate. ×17,000

Fig. 6. Lamellar cytoplasmic lipid body. The smooth and rough endoplasmic reticulum surrounding the lipid core contains electron-dense material. 38 day lamb. Lead citrate. ×17,000

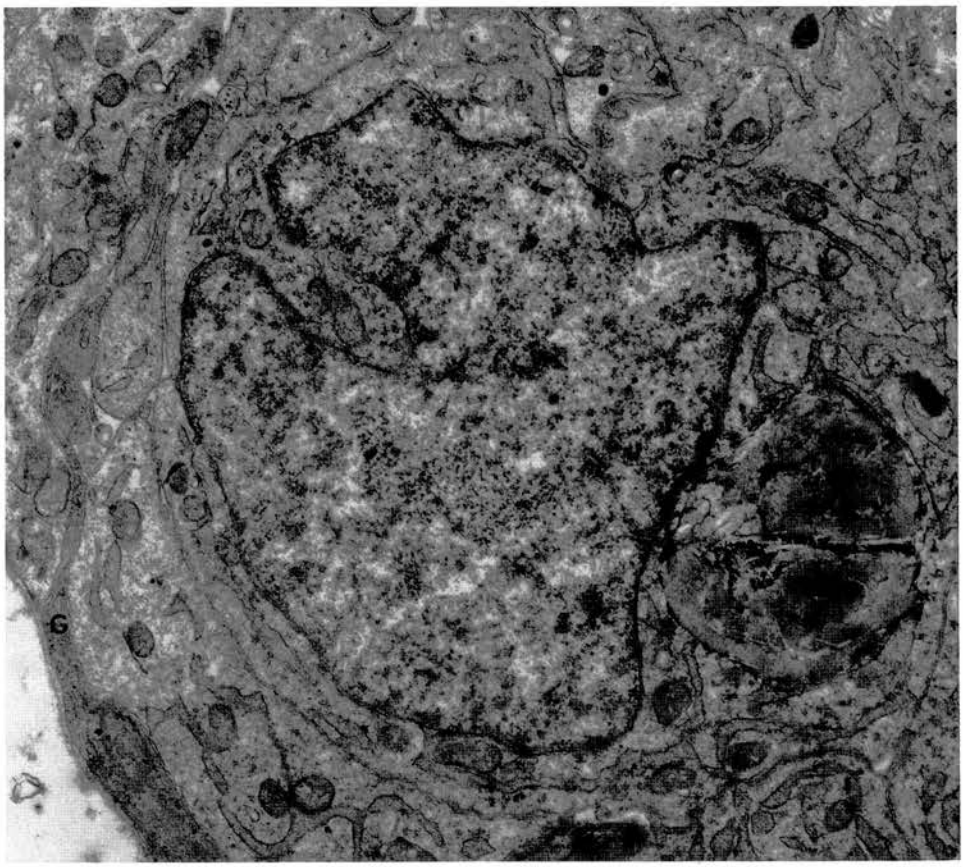


Fig. 7. There is a glial process (*G*) between a deep cell and its basement membrane. A lipid body is adjacent to the nucleus. The endoplasmic reticulum is continuous with the nuclear membrane and contains electron-dense material. 15 day lamb. Lead citrate. ×17,000

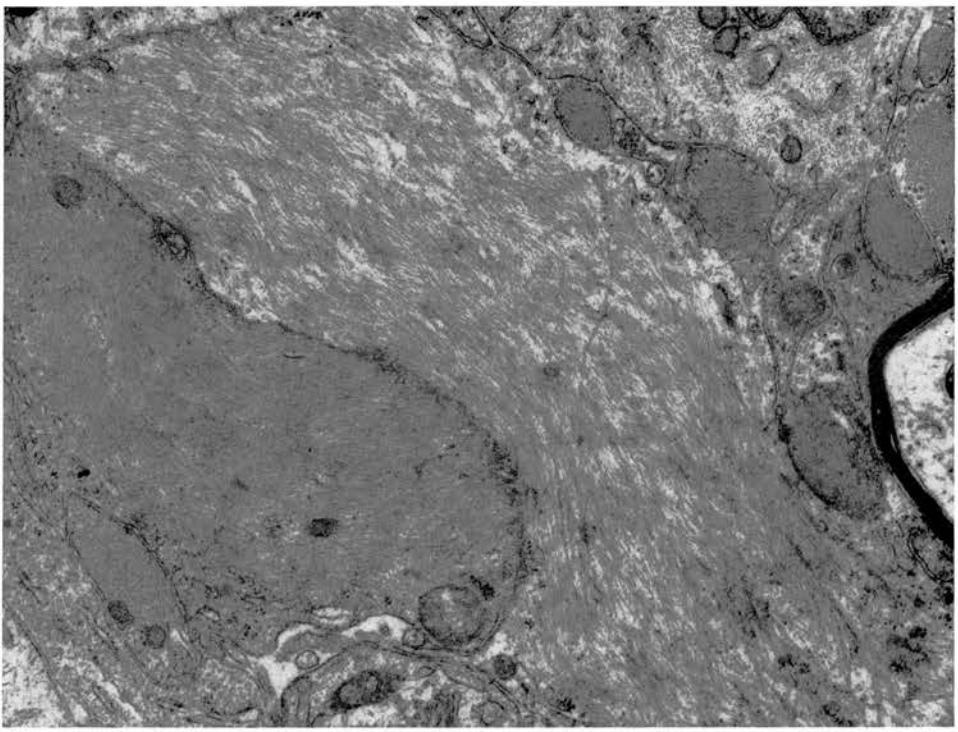


Fig. 8. Deep layer with glial processes containing many fibrils. A myelinated nerve is present. Adult sheep. Lead citrate. ×14,000

The deep layer was as thick as the superficial but lacked the patterned arrangement of cells. In 15—30 day old lambs the deep cells contained an extensive system of interconnecting endoplasmic sacs (Fig. 4). A prominent feature of the deep layer, observed infrequently in the superficial layer, was the presence of lamellar cytoplasmic lipid bodies (Fig. 5). These bodies varied, but virtually all contained a center represented by dense lipid and filamentous forms. Elements of the endoplasmic reticulum surrounded this core. In some instances the concentrically arranged endoplasmic reticulum contained dense material similar to that observed in the endoplasmic sacs of the superficial layer (Fig. 6).

Slender glial processes with fibrils and glycogen granules coursed through the deep layer. In addition, a glial process often extended along the perivascular space between the parenchymal cell and its basement membrane (Fig. 7). Collagen fibers and fibroblasts were present in the perivascular space. Desmosomes occurred between endothelial cells of the capillaries; fenestrations and pores were not observed. Histiocytes with lipid were sometimes seen in the perivascular space.

In adolescent and adult sheep there was a progressive increase of glial fibers and processes in the deep layer (Fig. 8). The network of endoplasmic sacs containing moderately dense material became progressively less evident, but cells containing electron-lucent, flocculent material were encountered with greater frequency (Fig. 9).

In adolescent sheep, instead of a lipid core, membranous whorls enclosed cytoplasmic constituents which resembled the fingerprints described in other

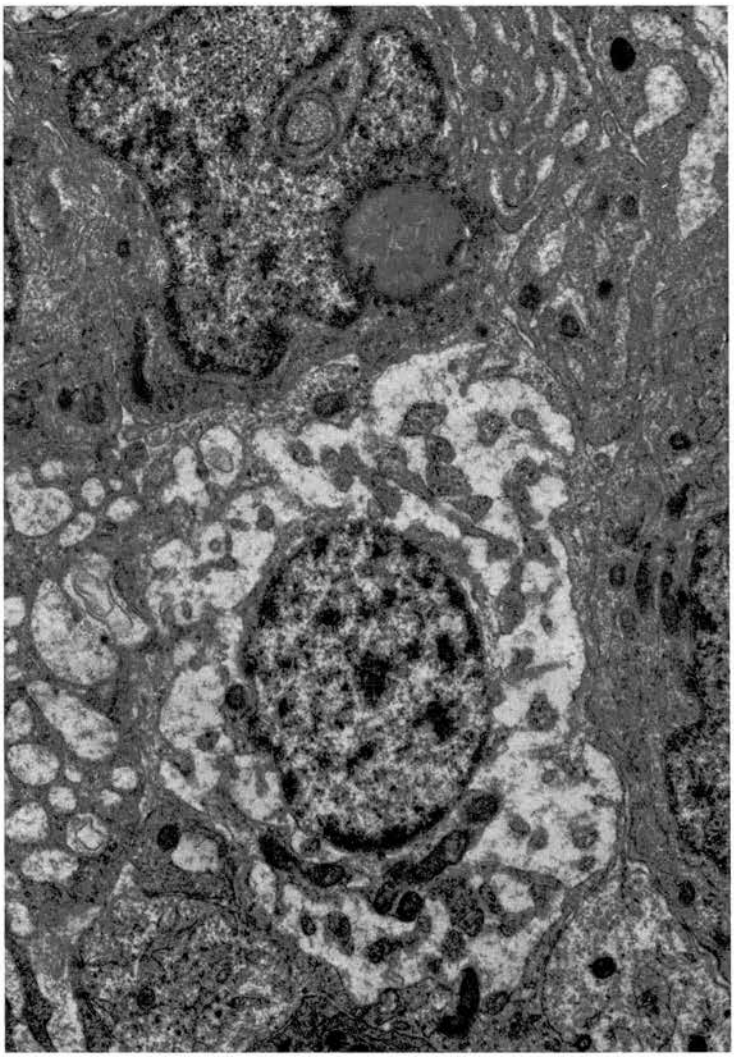


Fig. 9. Cells of the deep layer with dilated endoplasmic reticulum containing dispersed, finely granular material. Adolescent sheep. Lead citrate. Uranyl acetate. ×9,000

organs (Emmelot and Benedetti, 1960) (Figs. 10, 11). In adult sheep both lamellar cytoplasmic lipid bodies and fingerprints were observed less frequently.

Histochemistry

Alkaline phosphatase and thiamine pyrophosphatase delineated the blood vessels of the SCO. Thiamine pyrophosphatase also demonstrated the Golgi apparatus (Fig. 1). This structure was observed in distinctly different forms in the superficial and deep layers. Beneath the ventricular surface of the superficial layer the Golgi apparatus consisted of vesicular configurations connected by straight fine threads arranged parallel to the long axis of the cell. In the deep layer the Golgi network lacked this patterned arrangement and was closely applied to the nucleus. These patterns remained unchanged with age.

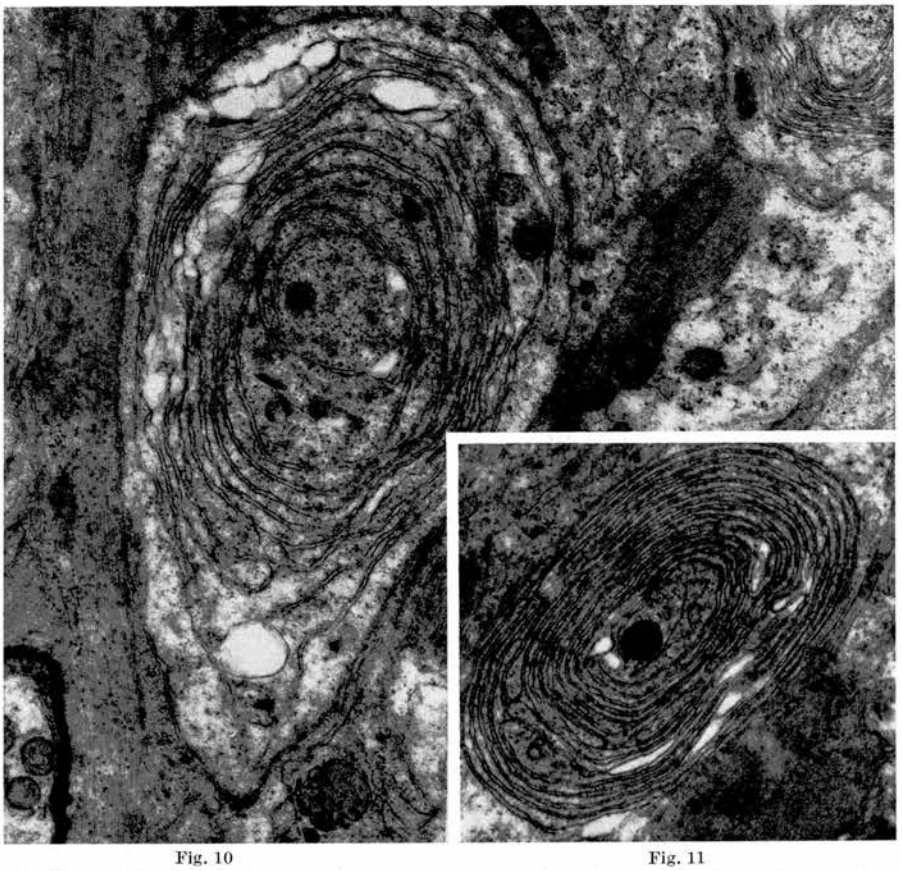


Fig. 10 Fig. 11

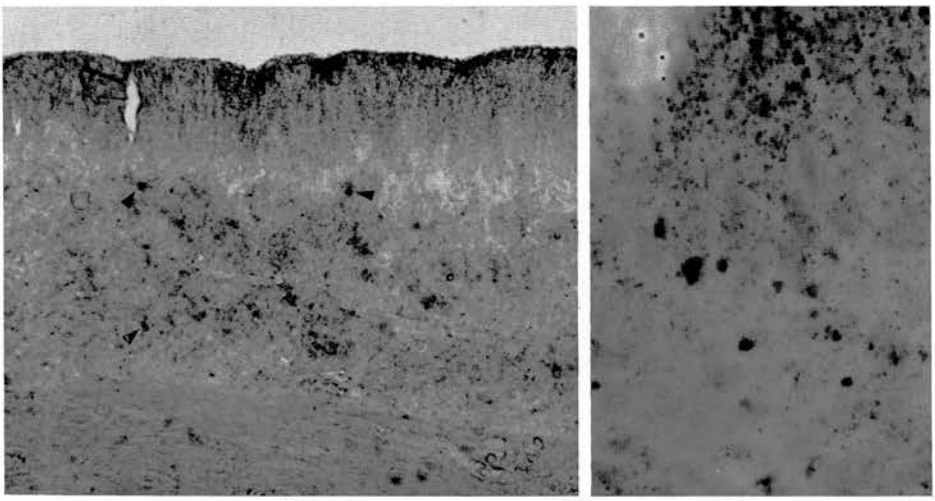
Fig. 10. Fingerprints. In cells of the deep layer membranous whorls enclose portions of cytoplasm. Adolescent sheep. Lead citrate. ×11,000

Fig. 11. The membranous whorls forming the fingerprint are more compact than those in Fig. 10. Adult sheep. Lead citrate. ×10,000

Acid phosphatase revealed structures of 3 sizes (Fig. 12). The smallest were lysosomes. They appeared throughout the SCO, were most numerous in the apical half of the superficial cells and were observed with equal frequency in all ages of sheep.

The second type of acid phosphatase positive body (Type II) was larger (3—6 μ diameter) than the usual lysosome (Fig. 12). They were found in relation to the nucleus or near the base of superficial cells and also in the cells of the deep layer. They were particularly numerous in lambs up to 38 days of age. In older lambs, Type II bodies were fewer and were recognized with certainty only in the deepest regions, especially towards the lateral margins of the SCO. No difference between adolescent and adult sheep in the number of Type II bodies was observed.

The third type of body with acid phosphatase activity was the largest and was located near capillaries. It was most frequent in lambs less than 38 days of age. Bodies of this size and distribution stained intensely with Sudan IV.



A B

Fig. 12. Subcommissural organ. (A) The ventricle is above and the posterior commissure is below. Lysosomes are numerous in the superficial layer. Large Type II bodies (arrow) are present in the deep layer. Acid phosphatase. ×200. (B) Higher magnification to emphasize the difference in size between large Type II body in the deep layer and the smaller lysosomes above. ×450

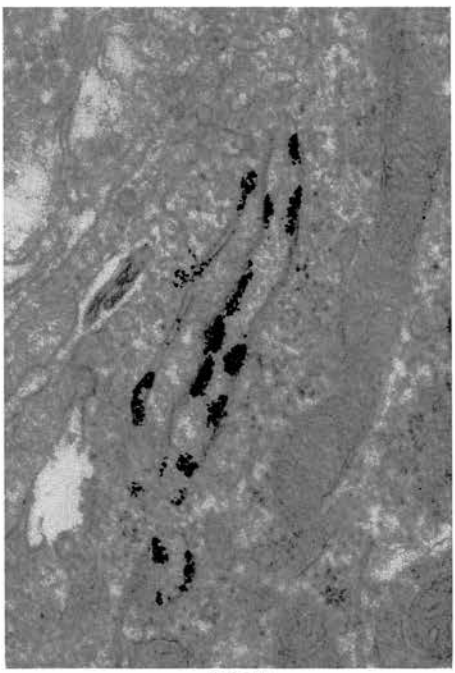


Fig. 13

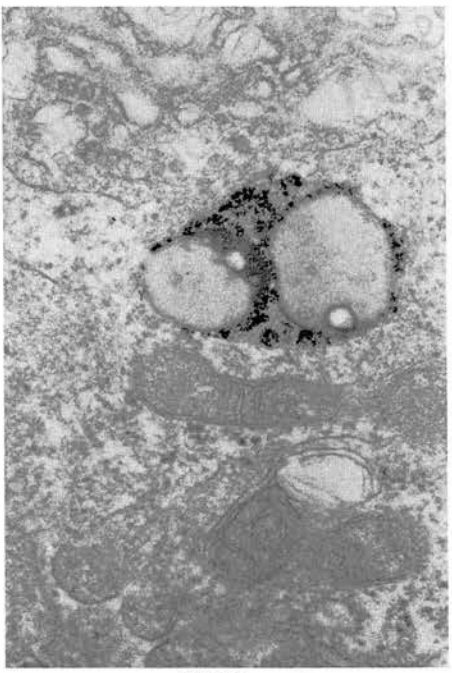


Fig. 14

Fig. 13. Endoplasmic reticulum with acid phosphatase reaction product. Adult sheep. Acid phosphatase. Uranyl acetate. ×47,000

Fig. 14. Residual body with acid phosphatase reaction product about lipofuscin granules. Adult sheep. Acid phosphatase. Uranyl acetate. ×40,000

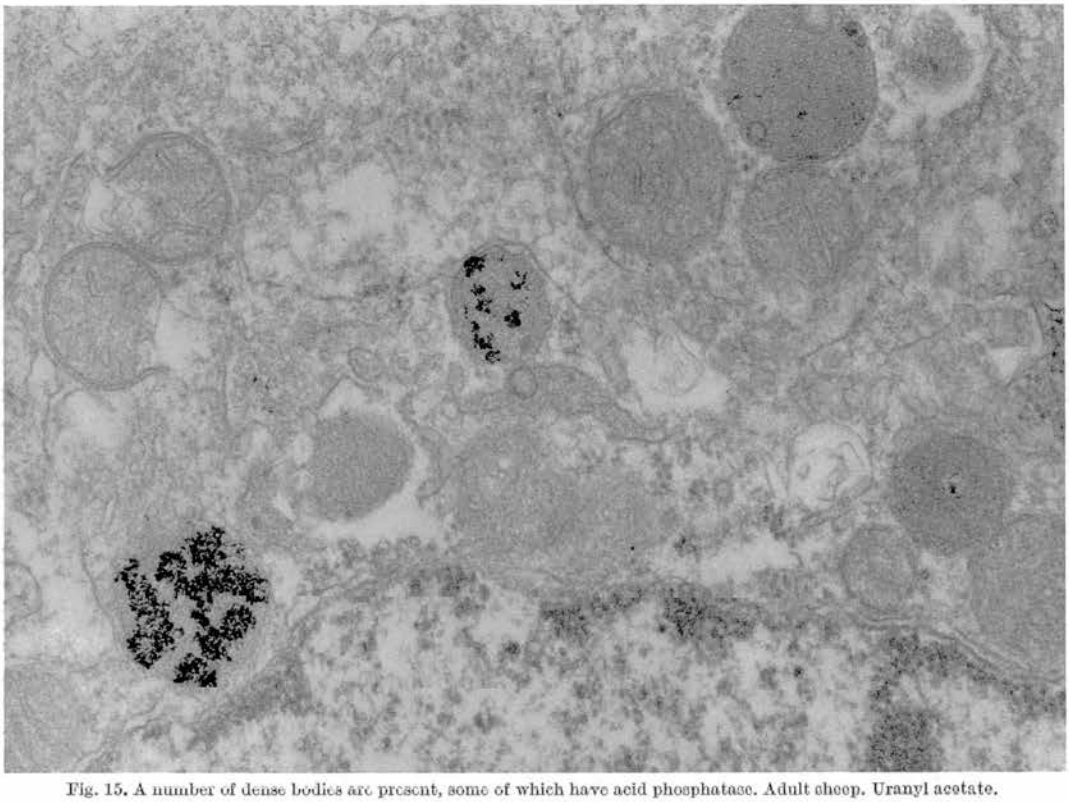


Fig. 15. A number of dense bodies are present, some of which have acid phosphatase. Adult sheep. Uranyl acetate. ×47,000

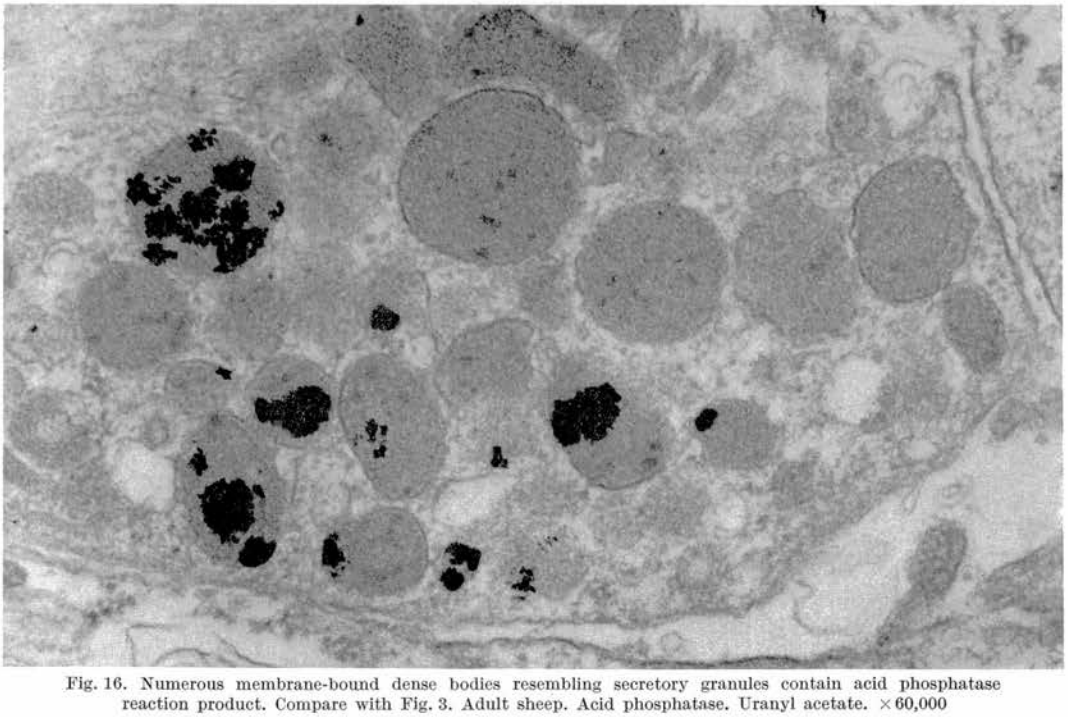


Fig. 16. Numerous membrane-bound dense bodies resembling secretory granules contain acid phosphatase reaction product. Compare with Fig. 3. Adult sheep. Acid phosphatase. Uranyl acetate. ×60,000

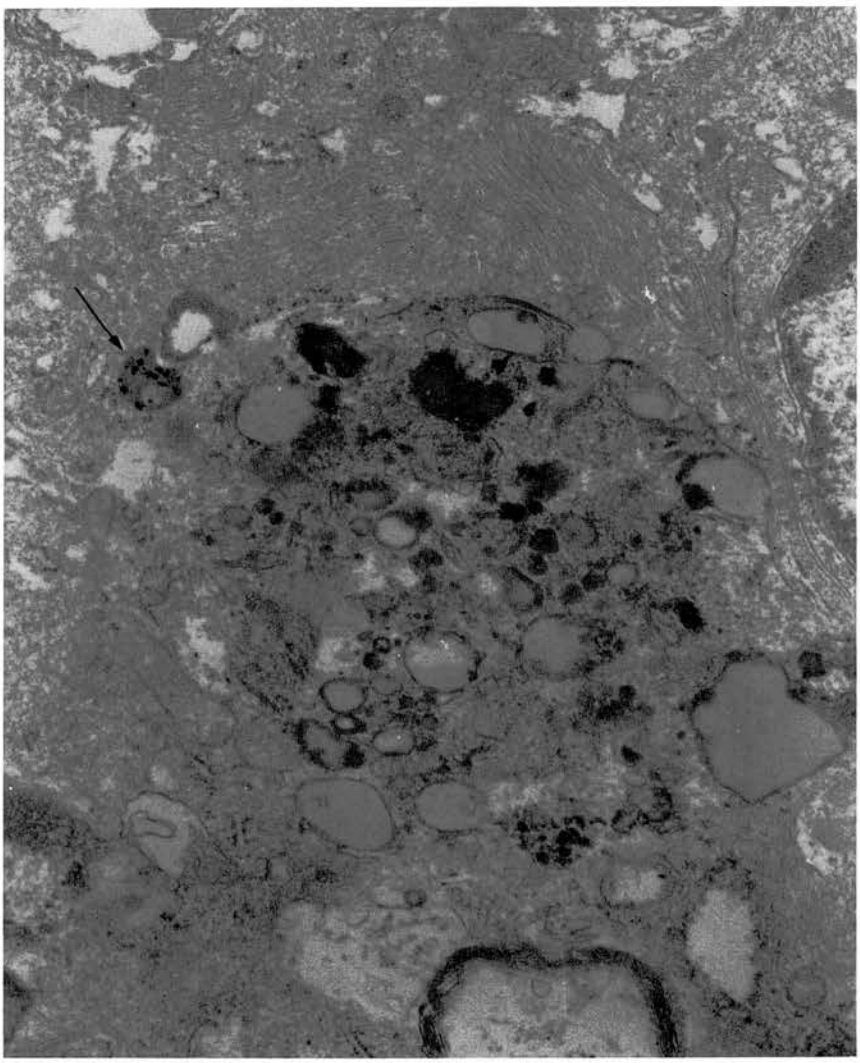


Fig. 17. Type II body with lipids of varying density and peripheral acid phosphatase reaction product (arrow). Adult sheep. Acid phosphatase. Uranyl acetate. ×25,000

Acid phosphatase preparations examined in the electron microscope revealed localization within sacs of the endoplasmic reticulum (Fig. 13). There was inconstant staining of dense bodies and lipofuscin granules (Figs. 14, 15). In addition, occasional collections of dense bodies were encountered with heavy staining (Fig. 16). The Type II body was identified by its size and location. It consisted of a circumscribed structure containing aggregates of lipid and membranes. There was activity within the body and in adjacent dense bodies (Fig. 17).

The lipid of the Type II body was investigated by a number of methods and the results are summarized in the Table. It is assumed on the basis of size and situation that the same structures were demonstrated with each method. The

results of the tests indicate that the SCO contains lipoidal particles, probably esterified cholesterol. In young lambs these particles also contained neutral fat.

Table. *Lipid reactions* of type II bodies*

Method	Result	Remarks
1 Sudan black B	Black	Indicates lipid or carotenoid
2 Sudan IV	Tan/scarlet	More scarlet (neutral fat) in young lambs than adolescent and adults
3 $OsO_4/KClO_3$/phenothiazine (BARLOW)	Black/brown	Unsaturated fatty acids and ? phospholipids
4 Okamoto's I_2/H_2SO_4 (PEARSE, 1961)	Blue-green	Cholesterol or cholesterol esters
5 PAN (ADAMS, 1961)	Blue	
6 Schultz (PEARSE, 1961)	Green (Smaller numbers than in 4 and 5)	
7 Digitonin reaction (FEIGIN, 1956)	Red	Cholesterol esters
8 Digitonin reaction after alcohol/ether extraction	No color. No evidence of birefringence	Indicates absence of cholesterol
9 UV light	Persistent green fluorescence	Eliminates carotenoids
10 Polarized light	Some particles anisotropic but results irregular	
11 1N NaOH for 1 hr at rm. temp.	Withstands treatment	Tested by methods 3 and 9
12 1N NaOH for 1 hr at 37°C	Diminished	Tested by methods 3 and 9
13 96% ethanol for 15 min	Diminished	Tested by methods 3 and 9
14 Absolute acetone for 15 min	Eliminated	Tested by methods 3 and 9
15 Ether/ethanol for 15 min	Eliminated	Tested by methods 3 and 9

* After CAIN (1950).

In view of the lipid content and the postulated endocrine role of the SCO, the reaction for steroid 3β-ol dehydrogenase was performed in 2 cases. The results were negative.

A difference between the SCO and contiguous ependyma was demonstrated in oxidative enzyme preparations. In the ependyma succinic dehydrogenase, cytochrome oxidase and monoamine oxidase were localized near the ciliated border. In the SCO, however, monoamine oxidase activity was virtually absent. Respiratory enzyme activity was evident throughout the superficial and deep layers. In locations similar to those in which Type II bodies had been found, respiratory enzyme activity was more intense. The precipitates frequently took the form of open or closed circles or granules 3—6 μ in diameter.

Intracytoplasmic P.A.S.-positive material, unaffected by diastase digestion, was present at all levels of the SCO of lambs aged 8 months. The distribution

and morphology of this material were unlike those of the Type II bodies. They were not investigated in sheep of other age groups.

Discussion

The large endoplasmic sacs with electron dense material seen in this study presumably correspond to the P.A.S. and chrome alum-hematoxylin-positive secretory granules described by Wislocki and Leduc (1953). This electron dense material is considered a secretion for purposes of description and discussion. Wislocki and Leduc (1953) concluded that the P.A.S.-positive material was secreted from the apex of the cell to form Reissner's fiber. This suggestion was supported by the electron microscopic studies of Lin and Duncan (1961) and Laatsch (1964). In the guinea pig and the rat the membrane-enclosed secretory material became smaller and more regular in size towards the luminal surface, and vesicles apparently discharging their contents into the aqueduct were occasionally seen (Lin and Duncan, 1961). Laatsch (1964) noted that the secretory droplets increased in density towards the apical surface. In the sheep we obtained no morphologic evidence to indicate a discharge of secretion into the CSF, although P.A.S.-positive droplets were noted within the cells of the SCO and in amorphous material lying in the ventricle in paraffin sections. Alternatively Murakami and Tanizaki (1963) have suggested that the small vesicles beneath the ventricular surface of the rat SCO may be involved in micropinocytosis.

Evidence for basal secretion into the abundant perivascular space of the deep layer has not been demonstrated convincingly (Vigh et al., 1961; Kivalo et al., 1961; Murakami and Tanizaki, 1963). In the extracellular space of the rat SCO, Naumann (1963) has described sheets of striated material with a periodicity of 1070 Å. Similar material with a repeating pattern of approximately 940 Å was noted by Stanka et al. (1964), who suggested that the structured bodies represent mucopolysaccharide secretion. In lambs and sheep, collagen fibers are present in the extracellular space, but structures with a greater repeating pattern were not observed. Furthermore, the secretory material in the deeper portions of the SCO was contained in large intracellular endoplasmic sacs and was never demonstrated within the perivascular space. In the perivascular space of the rabbit SCO Schmidt and D'Agostino (1966) noted cytoplasmic processes containing granular and vesicular material similar to those described in the pineal gland. Thus, this study provides no evidence for a secretory role, apical or basal, in the SCO of the sheep over a wide range of ages.

In the SCO of the toad, numerous whorled membranes with attached ribosomes are conspicuous (Murakami and Tanizaki, 1963). These authors suggest that the concentric lamellae may participate in the synthesis of secretory substances into the perivascular space, but admit that their functional significance is not clear. Similar membranous whorls were seen in our young lambs often surrounding lipid, forming the lamellar cytoplasmic lipid bodies. Abundant agranular endoplasmic reticulum enclosing lipid is commonly observed in steroid secreting cells (Carr and Carr, 1962; Enders and Lyons, 1964; Christensen, 1965). Our results, however, provide no direct evidence of a steroid hormone in the SCO of young lambs despite the presence of cholesterol esters.

In adolescent and adult sheep involution of the deep layer of the SCO is suggested by progressive gliosis and a decrease of endoplasmic secretory material. In this layer the "fingerprint" type of membranous whorl was frequently encountered (EMMELOT and BENEDETTI, 1960). It has been noted in other organs, principally liver under experimental conditions (FAWCETT and WILSON, 1953; ADAMS and PRINCE, 1959; EMMELOT and BENEDETTI, 1960; HERDSON et al., 1964), but also in normal and autolyzed kidney (LATTA et al., 1965). In liver cells STEINER and BAGLIO (1963) have described tightly coiled membranes enclosing cytoplasmic constituents and consider them to be manifestations of non-specific injury. Alternatively, FAWCETT and ITO (1955) observed that the hydration of testicular cells resulted in the formation of membranous whorls. In adolescent and adult sheep the cytoplasm of the cells of the SCO contained electron-lucent, flocculent material which might indicate an increased water content and favor the formation of fingerprints.

The mode of formation of the fingerprint is unknown. Its resemblance to the lamellar cytoplasmic lipid body suggests that it may be derived from the latter by loss of secretory material from the endoplasmic reticulum, apposition of the membranes and a progressive reduction of the lipid. This suggestion does not explain satisfactorily the presence of non-lipoidal cytoplasmic elements in the center of some fingerprints. The initial development of the concentric membranes might be a active process in response to the lipid, or a passive one due to displacement and approximation of the endoplasmic reticulum. The functional significance of such concentric membranes is not known.

In light microscopic preparations from adolescent and adult sheep focal concentrations of respiratory enzyme activity were present, their size and distribution suggesting a possible localization within the membranous whorls. Mitochondria, however, were infrequent within the fingerprints.

Our light microscopic observations indicate that the Type II body is lipoidal and contains esterified cholesterol. In electron microscopic histochemistry of adult sheep this body was found to consist of discrete lysosomal particles disposed about lipid aggregates resembling lipofuscin granules. Unfortunately, the ultrastructure of the Type II body of young lambs was not determined. Aggregates of spherical membrane-bound bodies in the superficial layer were observed to have heavy acid phosphatase activity. They may represent secretory granules or lysosomes.

This study of the SCO of the sheep has revealed cytological differences with age, which can be interpreted as involutionary. No evidence of a secretory role has been found. Bioassays and chemical analyses should be interpreted with reference to the age of the experimental animal.

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The Foetal Sheep: Morphogenesis of the Nervous System and Histochemical Aspects of Myelination

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ABSTRACT The development of the central nervous system of the sheep foetus with particular reference to myelin forming cells and myelination has been studied by morphological and histochemical methods. The development of the spinal cord resembles that described for other species and broadly similar patterns of development have been found for the other major regions. Differentiation of definitive cell types is marked by the appearance of cytochrome oxidase activity in their cytoplasm. In gray matter strong cytochrome oxidase activity persists into post-natal life whereas in white matter activity declines following commencement of myelination.

Lipids having the histochemical reactions of myelin first appear in a particular region as the capillary circulation of that region develops. It is suggested that phospholipid elements of perivascular lipid may be rapidly incorporated into the developing myelin sheath whilst galactolipids from the same location are more slowly utilized. The implications of the findings are discussed.

Myelin formation and degeneration have received much attention due to their importance in human neurological disease. Myelination of the spinal cord of the chick embryo has been studied intensively, (Cajal, '09; Bensted, Dobbing, Morgan, Reid and Payling Wright, '57; El-Eishi, '67) and among domestic mammals, the kitten (Tilney and Casamajor, '24; Windle, Fish and O'Donnell, '34) pig (Ziolo, '65) calf (Kostyra, '58) and lamb (Romanes, '47) have been examined, but usually with very limited objectives.

Due to its size and the concurrence of myelination with a rapid growth phase of nervous tissue (Barcroft, '46; Romanes, '47) the foetal sheep is a very suitable animal in which to carry out a variety of investigational procedures simultaneously in a number of regions. Furthermore, there are at least two diseases of newborn lambs, swayback and Border disease, in which disturbance of myelin is a feature (Barlow, Purves, Butler and Macintyre, '60; Barlow and Dickinson, '65).

As it is probable that the interfascicular oligodendroglia are the cells most concerned with myelination (Bunge, Bunge and Pappas, '62) the development and metabolic activity of these cells together with the origin and utilization of myelin lipids would appear to be central issues in the problem of the formation of myelin. The work reported here attempts to define and correlate the changes occurring in different regions of the central nervous system (C.N.S.) of the foetal lamb with particular reference to these factors.

MATERIALS AND METHODS

Over a period of five years 78 foetuses of mixed breeding and known gestational age were obtained by Caesarian section at intervals between 40 and 160 days (16 days post partum) from conception. Initially at least four foetuses at each 20 day interval from 40–160 and 90–130 days were used (46 in all), and these were later supplemented by a further 32 foetuses at random ages from 47–104 days after conception. Conception was assumed to have occurred on the day of service to which there was no return. Within each age group slight variations in the physical dimensions and degree of development were observed but as in the general population these were of the same order as variations between siblings of the same sex, no attempt was made to analyze the data with respect to breed, sex, and litter size. Approximately half the number of foetuses at each gestational age were fixed by

perfusion via the umbilical vein with Baker's formol-calcium. The remainder were dissected immediately, blocks taken for the demonstration of cytochrome oxidase by Burstones method as used previously (Barlow, '63) and the remaining central nervous tissue fixed by immersion in Baker's fixative. Blocks were taken from as standard sites as possible; transverse slices of cerebrum and diencephalon at the level of the posterior commissure to include portions of hippocampus; through the midbrain at the summits of the anterior colliculi; the cerebellum and medulla at the level of the fissura prima and the brachium pontis, and both transverse and longitudinal slices of the spinal cord from cervical and lumbo-sacral enlargements. Blocks of fixed tissue were post-chromed in Mueller's fluid and double-embedded or used for frozen sections. Histological procedures included H. & E., Luxol fast Blue and Smith-Quigley for myelin and Holmes method for neurofibrils. Polarized light, Sudan black B, Sudan IV and OTAN (Adams, '59) were used for the study of lipids. Frozen sections were also stained with cresyl violet.

RESULTS

During closure of the neural groove the ectoderm proliferates to form a thick mantle layer around the newly formed neural canal. Those cells closest to the lumen elongate about a radial axis and consolidate to form the ependyma whilst outside the mantle layer, there forms a fine fibrillar network virtually devoid of cell nuclei and known as the marginal layer.

At 40 days in the sheep foetus histogenesis is incomplete in regions other than the medulla, midbrain and cervical spinal cord, and thus the last stages of this process overlap the earlier phases of morphogenesis. The sequence of events is described in six stages.

Stage I Neuroblast differentiation and migration

Phase (a). The cells of the mantle layer divide repeatedly to form a thick layer of small dark-staining nuclei around the neural canal. A number of these cells develop larger more vesicular nuclei. These are the neuroblasts and they migrate towards their final positions in the nervous system. In the brain stem and spinal cord, these final positions remain associated with the mantle layer, but in the cerebrum the neuroblasts migrate into the marginal layer which increases greatly in size behind them. Cerebral neuroblasts thus become effectively separated from a predominantly spongioblastic mantle layer by a widening band of mesenchymal "marginal" layer. In the cerebellum which has arisen as cellular masses on the rhombic lips of myelencephalon, the major portion of the mantle layer is peripherally situated at 40 days gestation and the "marginal" layer develops as a fibrillary network between it and the neuroblasts of the dentate and cerebellar roof nuclei. This enclosed portion of marginal mesenchyme is continuous with the peripherally located marginal layer of the medulla through the developing cerebellar peduncles. The more marginal mantle layer of the cerebellum gradually develops a narrow molecular layer between the granular layer proper and the predominantly spongiobalstic subpial mantle or external granular layer.

Phase (b). The continued proliferation of neuroblasts in the cerebral and cerebellar cortices is accompanied by folding of the surface which becomes increasingly complex as gestation proceeds. Cortical growth is also accommodated by expansion of the enclosed "marginal" layer i.e., the primitive white matter in which fine axons can be demonstrated in increasing numbers.

Stage II Neuronal differentiation

Towards the end of histogenesis the neuroblast nuclei enlarge and this is followed by a marked increase in the volume of their cytoplasm in the marginal regions of which Nissl granules appear. In some cells this enlargement is such as to leave a clear perinuclear halo giving the cell the appearance of chromatolysis (fig. 1). As the neurones differentiate in this manner cytochrome oxidase activity becomes evident in their cytoplasm for the first time.

Stage III Spongioblast migration and differentiation

Phase (a). Coincident with the development of the gray matter streams of

spongioblasts are shed from the mantle layer into the marginal layer or primitive white matter where their small darkly-staining nuclei continue to divide.

Phase (b). Shortly two types of cells can be distinguished; one remains small, dark and pleiomorphic, whilst the other is larger vesicular and contains one or more nucleoli (c.f. Types A and B — Spatz, '18). The latter are believed to be astrocytes. Traces of cytochrome oxidase can be demonstrated in the perikarya of these cells.

The smaller nuclei continue to proliferate (fig. 2) and become arranged in rows (fig. 3). Their perikarya then also develop cytochrome oxidase activity the granular reaction product of which serves to delineate the axons (figs. 4–6). These small cells are thus recognized as the interfascicular oligodendroglia which Bunge, Bunge and Pappas ('62) suggested were the cells responsible for the formation of myelin.

Stage IV Vascular proliferation and lipid importation

Phase (a). During the preceding stages capillaries are budded off from the primary vascular trunks of the various regions. The rate of growth of these buds appears to be uniform but regions in proximity to two or more trunks become thoroughly vascularized more quickly than those supplied by a single major vessel. Thus, those portions of the ventral funiculi receiving capillary buds from both the sulcal and vasocoronal branches of the ventral spinal artery become throughly vascularized in advance of the more lateral and dorsal funiculi which are supplied by the arterial vasocorona alone. Similarly those portions of the primitive cerebral cortex adjacent to branches of the major cerebral arteries vascularize more quickly than more remote regions. In the spinal cord, the establishment of a complete capillary bed is associated with a more advanced state of cell division and maturation in the ventral funiculi than elsewhere. It was not possible to determine whether this is a causal relationship which exists elsewhere in the nervous system, but if so, the local cortical cell proliferations essential to the formation of gyri and cerebellar folia may be a result of the early establishment of a rich capillary circulation.

Phase (b). Following closely upon the penetration of nervous tissue by blood vessels, lipid appears in the perivascular spaces, beneath the pia mater and also amongst adjacent glial cells (figs. 9, 10). Close to the blood vessels the lipid forms large globules (8–15 μ diameter) (fig. 10) but as distance from the vessel increases the droplets become smaller (1–2 μ diameter). They stain strongly with Sudan Black B, weakly with Sudan IV, give a predominantly red reaction with OTAN and contain birefringent elements (fig. 11). They also stain with cresyl violet but not with P.A.S. These features suggest that they contain phospholipids, and probably also cholesterol. By the methods employed it was not possible to determine whether the larger particles all have an intracellular location, though the smaller droplets appeared to be contained in astrocytes.

Stage V Myelination

The actual process of myelination is heralded by the development of maximum cytochrome oxidase activity in the interfascicular oligodendroglia which have outlined the course of axons since stage III (b) (fig. 6). Tiny lipid granules with the same staining characteristics as those in the perivascular glia appear within their cytoplasm. Thus in its earliest recognizable phase of development the myelin sheath appears extremely delicate, finely granular and locally discontinuous. With continued incorporation of lipid droplets the granularity of the myelin sheath is lost and the sheath broadens. The tissue becomes more compact as other fibers develop myelin sheaths, though within a given region there is considerable variation in thickness of individual sheaths.

Stage VI Maturation of myelin

As myelination proceeds there occurs a gradual reduction in cytochrome oxidase activity associated with the interfascicular oligodendroglia (figs. 7, 8). The enzyme reaction product first becomes localized to the immediate perikaryon as tiny granules and then ceases to be demonstrable. The quantity of lipid observed in the perivascular spaces is also reduced progressively, and its nature changes. It stains more strongly with Sudan IV, brown/black or

TABLE 1

Age of onset of various stages of development (in days from conception)

Developmental stages		Regions of C.N.S.							
		Cerebrum	Cerebellum	Hippocampus	Diencephalon	Mesencephalon (Tectum)	Mesencephalon (Tegmentum)	Medulla	Spinal cord
Neuroblast differentiation	I (a)	40	47	40	60	60	?	?	?
Neuroblast migration	I (b)	80	70	60	60	60	?	?	?
Neuronal differentiation	II	80	80 (60) [1]	40	60	100	40 [2]	40	40
Spongioblast migration	III (a)	60	70	40	40	80	40	40	60
Spongioblast differentiation	III (b)	70	80	47	40	80	40	40	60
Vascular proliferation	IV (a)	70	70	47	60	70	60	40	47
Lipid importation	IV (b)	70	70	47	60	70	60	60	47 [3]
Myelination	V	100	80	80	80	110	60	60	60
Maturation of myelin	VI	140	110	?	120	120	120	140	90

[1] (60) Fastigial and dentate nuclei only.
[2] Indicates the process was well established at age examined.
[3] Trace in a single specimen.

black with OTAN. Bi-refringent elements are less frequently encountered in these lipid droplets. These qualities suggest that the material is predominantly neutral fat. This residual lipid, however, also stains metachromatically with cresyl violet.

The gestational age at which each stage of development was first observed in each of the 6 tissue blocks is given in table 1. The method of approach to the problem does not lend itself to an uninterrupted assessment of the development of specific neural system though certain facts may be deduced. The first sign of morphogenesis in the C.N.S. is the differentiation of multipolar neurones close to the neuroaxis of the brain stem and cervical cord whither the process extends to neurons situated laterally, caudally and cranially in this order. The development of the myelin sheaths (and the processes leading up to it) follow the same general pattern, efferents from these brain stem nuclei arising first followed by afferents from the cord, cerebellum and cerebrum respectively. Tables 2 and 3 indicate this development in more detail.

The amount of cytochrome oxidase activity, the accumulation of subpial and perivascular lipid and the density of myelin in the regions studied were scored on a scale from a trace (1 +) to a maximum (3 +) at each gestational age and the results are presented in table 4. Whereas there is no relationship between the scores for the various parameters examined, the method is sufficient to show that the rate of change in the nervous system as a whole which respect to these parameters is greatest between 70–110 days gestation.

DISCUSSION

These results indicate that differentiation of both neurones and glia is accompanied by a change in the metabolic activity of the definitive cell types which is manifested by the appearance of cytochrome oxidase activity in the cytoplasm. In nerve cells there is a progressive increase in intensity of the enzyme reaction product during foetal life, whilst in the white matter activity rises to a maximum coinciding with the first appearance of myelin and then declines during the matu-

TABLE 2

Gestational age at which myelin was demonstrated in specific regions

Regions	Days of gestation													Romanes ('47)
	40	47	60	70	78	80	90	100	110	120	130	140	160	
Medial longitudinal fasciculus			+	+	+	+	+	+	+	+	+	+	+	(63)
Trigeminal nerve (central parts)			+	+	+	+	+	+	+	+	+	+	+	(63)
Uncinate fasciculus			+	+	+	+	+	+	+	+	+	+	+	(66)
Vestibulospinal tract			+	+	+	+	+	+	+	+	+	+	+	(63)
Internal capsule					+	+	+	+	+	+	+	+	+	(96)
Fibrae vestibulocerebellares					+	+	+	+	+	+	+	+	+	
Facial nerve (central part)					+	+	+	+	+	+	+	+	+	(63)
Trochlear nerve (central part)					+	+	+	+	+	+	+	+	+	(63)
Abducent nerve (central part)					+	+	+	+	+	+	+	+	+	(63)
Dorsal tegmental decussation					+	+	+	+	+	+	+	+	+	
Ventral columns spinal cord				+ [1]	+	+	+	+	+	+	+	+	+	(63)
Dorsal columns spinal cord					+	+	+	+	+	+	+	+	+	(78)
Vestibular nerves (central part)						+	+	+	+	+	+	+	+	(78)
Posterior commissure						+	+	+	+	+	+	+	+	(78 tr)
Rubrospinal tract						+	+	+	+	+	+	+	+	(78)
Brachium pontis						+	+	+	+	+	+	+	+	(96)
Restiform body							+	+	+	+	+	+	+	
Corpus medullare of cerebellum							+	+	+	+	+	+	+	(96)
Ventral white commissure of spinal cord							+	+	+	+	+	+	+	
Centrum semiovale								+	+	+	+	+	+	(111)
Subfoliar digits of paraflocculus									+	+	+	+	+	
Subgyral digits of cerebral white matter										+	+	+	+	
Tectal strata										+	+	+	+	(111)
Arcuate fibers												+	+	
Precuneal gyrus												+	+	
Pyriform area												+	+	(129)

[1] Cervical enlargement only.

TABLE 3

Gestational age at which neuronal differentiation was recognized

Nucleus	Days of gestation												
	40	47	60	70	78	80	90	100	110	120	130	140	160
Ventral horn cells	+	+	+	+	+	+	+	+	+	+	+	+	+
Oculomotor nucleus	+	+	+	+	+	+	+	+	+	+	+	+	+
Red nucleus	+	+	+	+	+	+	+	+	+	+	+	+	+
Trigeminal nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+
Vestibular nuclei		+	+	+	+	+	+	+	+	+	+	+	+
Dentate nucleus			+	+	+	+	+	+	+	+	+	+	+
Cerebellar roof nuclei			+	+	+	+	+	+	+	+	+	+	+
Substantia nigra			+	+	+	+	+	+	+	+	+	+	+
Pontine nuclei			+	+	+	+	+	+	+	+	+	+	+
Substantia gelatinosa Rolandi					+	+	+	+	+	+	+	+	+
Purkinje cells (vermis)						+	+	+	+	+	+	+	+
Cerebral cortex (V and VI)								+	+	+	+	+	+
Cerebral cortex (I–IV)									+	+	+	+	+

ration process until it is no longer demonstrated by Burstones method.

A similar premyelination rise in oligodendroglial enzyme activity has also been reported for three other oxidative enzymes concerned with carbohydrate metabolism, succinic dehydrogenase and di- and tri phosphopyridine nucleotide diaphorases in myelinating rat tissue cultures (Yonezawa, Bornstein, Peterson and Murray, '62) and

TABLE 4

Duration and scale of histochemical events in morphogenesis

		40	47	60	70	78	80	90	100	110	120	130	140	160
Cytochrome Oxidase in Gray matter	Cerebrum	(1)	(1)	(1)	(1)	(1)	1	2	3	3	3	3	3	3
	Cerebellum			1	1	1	2	2	3	3	3	3	3	3
	Brain stem and cord	1	1	2	2	2	3	3	3	3	3	3	3	3
Cytochrome Oxidase in White matter	Cerebrum						1	2	3	2	1	1	1	1
	Cerebellum				1	1	2	3	3	1	1	1	1	
	Brain stem and cord	1	1	2	2	3	3	2	1	1	1	1	1	1
Subpial Lipid	Cerebrum				1	2	3	2	1	1				
	Cerebellum				1	3	3	2	1					
	Brain stem and cord		1	3	3	1	1	1						
Perivascular Lipid	Cerebrum				1	3	3	3	3	2	1	1	1	1
	Cerebellum				1	3	3	3	1	1	1			
	Brain stem and cord		1	3	3	3	2	1	1	1				
Myelin	Cerebrum								1	2	2	2	3	3
	Cerebellum						1	2	2	3	3	3	3	3
	Brain stem and cord			1	1	1	2	2	2	2	3	3	3	3

(1), Cytochrome oxidase activity in Hippocampus only.
1, Traces present.
2, Intermediate amounts or intensities of reaction.
3, Maximum amount or intensity of reaction.

for DPN-diaphorase in human white matter (Friede, '61). As cytochrome oxidase is a mitochondrial enzyme the decline in enzyme activity may be due to the gradual elimination or redistribution of these organelles during the spiral wrapping process of sheath formation. Alternatively as Blunt, Wendell-Smith, Paisley and Baldwin ('67) have suggested a high activity of glyceryl phosphate dehydrogenase (an enzyme which can also have cytochrome C as an acceptor) may indicate the cell's concern with phospholipid membrane production and turnover, or its dependence on lipoproteins as an energy source. In oligodendroglia such metabolic activities may be of a temporary nature. Friede ('62) has demonstrated the adaptability of astrocytes with respect to activity of several enzymes during certain pathological processes and it may be that oligodendroglia in the changing conditions of myelination are similarly adaptable.

Cell division and differentiation are accompanied by vascular proliferation and since areas of the spinal cord and cerebral cortex which are supplied by a number of major vessels appear to develop respectively more rapidly and to a greater degree than similar tissue supplied by a single vessel, it is suggested that the early establishment of a rich capillary network may promote localized regional development. In the brain stem and medulla of the rat a rapid increase in vascularity occurs between the tenth and twenty-first days of life (Craigie, '24) and coincides with the development of myelin in these regions.

The occurrence of very numerous phospholipid-rich droplets in the perivascular spaces and neuropil adjacent to the vascular pia mater prior to the appearance of any myelin corroborates the views of Tilney and Casamajor ('24) and Ziolo ('65) that "the material of the myelin sheaths is distributed by the blood," and renders the hypothesis that such accumulations represent attempts to remove the products of lipid hypersynthesis from the nervous system, an unlikely alternative. By histochemical methods and polarized light, tiny droplets of this material have been visualized in situations distant from the perivascular spaces and appear to become incorporated unchanged into the developing myelin sheath. The mechanisms of transportation from the vessel and incorporation into the sheath have not been revealed. The persistence of reducing lipid in the perivascular spaces of well-myelinated areas may indicate that this material is principally a vehicle for transporting phospholipids to the brain. The metachromatic qualities of this residual lipid suggest that it may also be rich in galactolipids (sulphatides) to be more slowly incorporated into the myelin sheath as the structural proteins are formed.

Adams and Davison ('59) have also found lipid droplets in nervous tissue from chick embryos and children prior to myelination. In its earliest stage of formation this lipid was hydrophobic, staining black orgray with OTAN, the normal red-staining reaction of myelin being delayed. They concluded that the first lipid was esterified cholesterol which was later de-esterified, free cholesterol being deposited in the sheath or alternatively, was diluted by the deposition of larger amounts of other hydrophilic lipids as the sheath matured. It would appear that the foetal lamb may differ from the child and chick embryo in that hydrophilic lipids also, are present prior to myelination.

The granularities and discontinuities of the very young myelin sheaths are of great interest. They could indicate simple incorporation of the smallest lipid droplets into the sheath, or may correspond to the dark and light bands developing in the lateral loops of several myelin forming cells along the length of the axon (Hirano and Dembitzer, '67).

Davison and Dobbing ('65) have proposed that since myelin is a metabolically stable material, adverse external circumstances may affect the biosynthesis of myelin constituents before their incorporation into the lamellae of the sheath, and thus myelination is a vulnerable period in brain development. Dickerson, Dobbing and McCance ('67) have shown that, in the pig, undernutrition during development may permanently affect the myelin sheaths. Reference to table 4 indicates that in addition to lipid importation and myelinogenesis *per se*, the period 70–110 days gestation in the sheep foetus is the time when the rate of change of cytochrome oxidase

activity (and associated phenomena such as vascularization and cellular differentiation) is greatest for the C.N.S. as a whole. Thus adverse circumstances other than those directly influencing myelin-lipid synthesis, but operating during this period are also likely to produce widespread, deleterious effects.

Reference to table 2 shows that the age at which myelin was first demonstrated was similar for the most part to that in Romanes ('47) study where comparable regions were examined. Allowing for age distribution of the foetuses and possible distinctions in the sites of sampling the only notable differences between these results and those of Romanes are or the uncinate fasciculus in which myelin was found six days earlier, cranial nerves IV, VI and VII, ventral columns and pyriform area in which myelin was found respectively, 15, 7 and 11 days later than in Romanes work. However, as the age of Romanes two youngest foetuses was estimated from the crown-rump lengths and weights some of these differences may be more apparent than real.

In the spinal cord of the foetal sheep the overall pattern of myelination of the ventral columns is similar to that described for the kitten (Windle et al., '34) the calf (Kostyra, '58) and the piglet (Ziolo, '65) and beautifully illustrated for the chicken by Bensted et al. ('57). The process commences close to the ventral sulcal angle and spreads in a dorso-lateral direction. As has been suggested already this may be a reflection of the pattern of development of the capillary blood supply.

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PLATE 1

EXPLANATION OF FIGURES

1 Nerve cell of the oculomotor nucleus of an 80 day foetus showing eccentric nucleus and margination of chromatin. The changes resemble chromatolysis in some respect. (Neutral red. × 1,200).

2 Undifferentiated glial cell nuclei in spinal cord. 40 day foetus. (H. & E. × 750).

3 Differentiation of astrocytes and oligodendroglia. The latter are beginning to form up as chains of nuclei along the course of the nerve fibers. Internal capsule 60 day foetus. (H. & E. × 250).

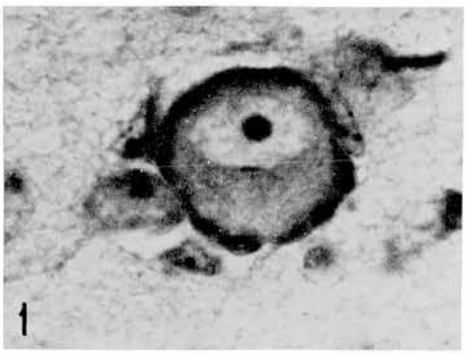
4 L.S. ventral funiculus of spinal cord of 60 day foetus showing cytochrome oxidase activity in white matter. (Burstone. × 440).

5 L.S. ventral funiculus of spinal cord of 80 day foetus showing cytochrome oxidase activity in white matter. (Burstone. × 440).

6 L.S. ventral funiculus of spinal cord of 90 day foetus showing cytochrome oxidase activity in white matter. (Burstone. × 440).

7 L.S. ventral funiculus of spinal cord of 100 day foetus showing cytochrome oxidase activity in white matter. (Burstone. × 440).

8 L.S. ventral funiculus of spinal cord of 140 day foetus showing traces of cytochrome oxidase activity in a few fibers (lower half of picture). The upper parts of the picture show the intense activity in gray matter. (Burstone. × 440).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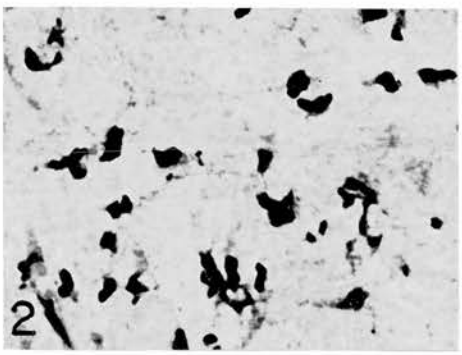
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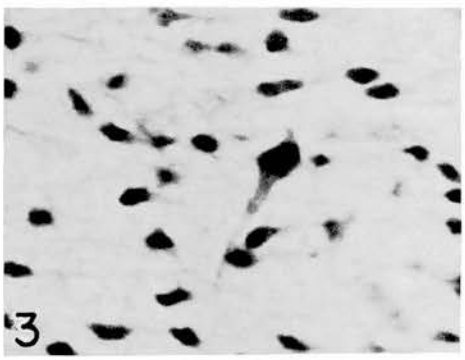
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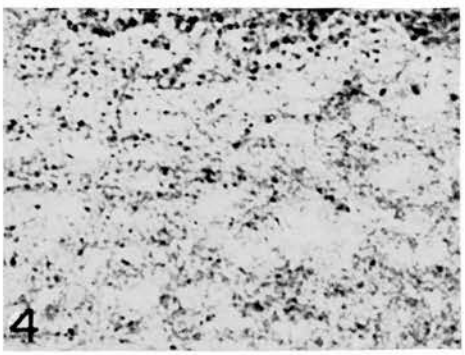
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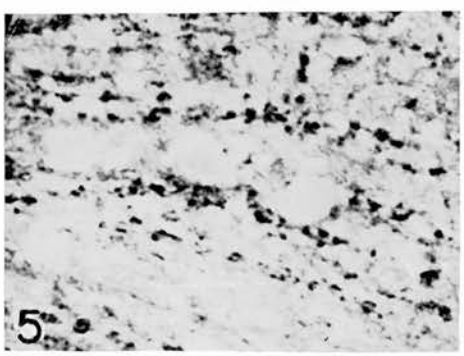
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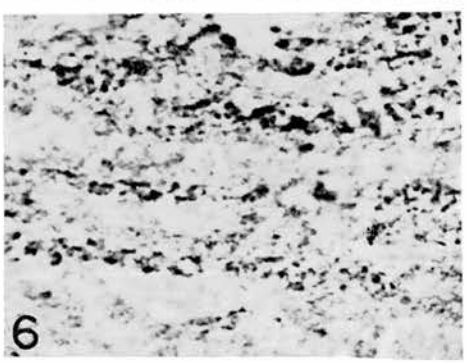
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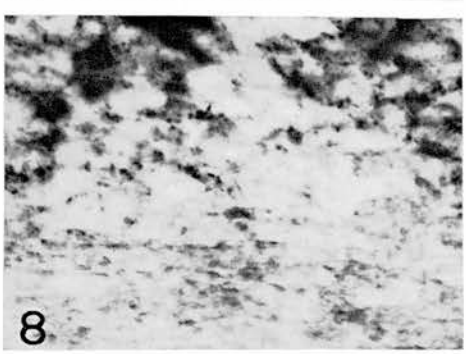
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PLATE 2

EXPLANATION OF FIGURES

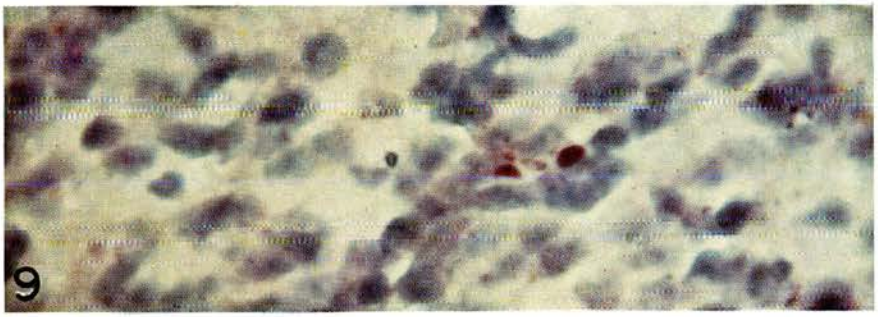
9 Cerebral white matter of a 47 day foetus. Sudanophilic lipid droplets are associated with a small capillary. (Sudan IV. × 750).

10 Dorsal spinal nerve roots of a 97 day foetus showing myelinated fibers adjacent to perivascular lipid droplets. (Sudan IV. × 480).

11 The same section as figure 10 but photographed with polarized light.

12 Early myelination in spinal cord of an 82 day foetus. The myelin sheaths are finely beaded with phospholipid material. Tiny droplets of similarly staining material are evident in the perikarya of the oligodendroglial cells and lying apparently free in the tissue. (OTAN. × 480).

13 A single myelinated fiber of the vestibulocerebellar tract of a 78 day foetus. Arrows indicate phospholipid droplets lying outwith the myelin sheath. (OTAN. × 750).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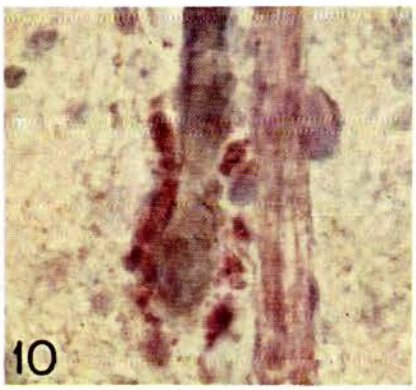
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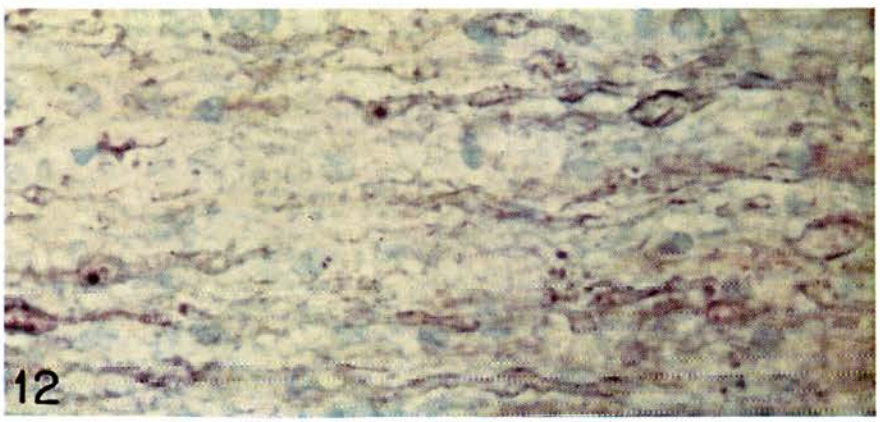
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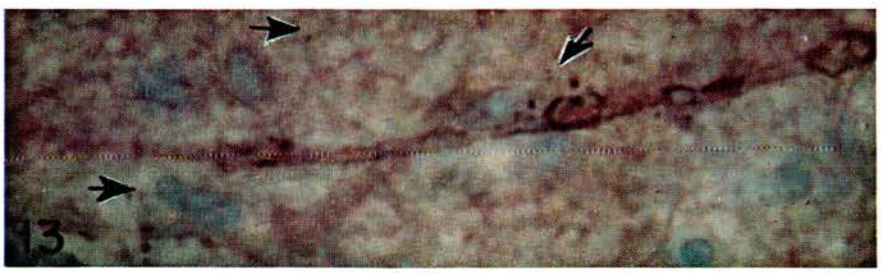
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