DISSEMINATED SCLEROSIS IN MAN AND EXPERIMENTATION WITH SHEEP.

JOHN M. SUTHERLAND, M.D.,*
and D. R. WILSON, D.Sc., M.R.C.V.S.,
from the Western Infirmary, Glasgow.
and the Animal Diseases Research Association, Moredun Institute, Edinburgh.

Recent literature has emphasized a possible relationship between disseminated sclerosis and agricultural or rural exposure (Campbell *et al.*, 1947 ; Lhermitte, 1947 ; Shield, 1947 ; Dean, 1949). Such a concept is not a new one, and articles to this effect have been published for well over a quarter of a century (Morawitz, 1904 ; Steiner, 1918 ; Dreyfus, 1921 ; Adams, 1923, 1927 ; Wilson, 1927). This aspect is also referred to in the textbooks of Wilson (1940) and of Boyd (1944).

There is at present no uniformity of opinion as to the etiology of disseminated sclerosis. We believe, as does McAlpine (1946), that there is increasing evidence in support of the infective nature of the disease, particularly as many aspects of the disease process may be explained on a secondary allergic basis (Ferraro, 1944). It follows, however, that should the progress of disseminated sclerosis depend to at least some extent on altered myelin acting as antibody, the etiology of this clinical syndrome may be a diverse one. Thus, any process, whether infective, toxic, vascular or metabolic, which has the property of altering myelin may be followed by a clinical syndrome of disseminated sclerosis. On the other hand, the view that disseminated sclerosis as judged by its histology is a distinct pathological entity has not been contradicted. Further, Steiner (1941) presented strong evidence in support of the hypothesis that disseminated sclerosis is an ' etiologically uniform infectious disease.'

For the purpose of the present investigation the hypothesis was formulated that infection with the causative agent of disseminated sclerosis is influenced by rural or agricultural exposure.

This view, however, gained no support from a study of the occupational incidence of the disease. Table 1 records the occupations in 173 male cases of disseminated sclerosis, and it would appear from this that occupation has no etiological significance. Such an opinion is shared by Bramwell (1917), Brain (1930 & 1947), Adie (1932), Wilson (1940), McAlpine (1946), and Walshe (1947).

* Now at Raigmore Hospital, Inverness.

TABLE 1.
Occupational incidence in 173 male cases.

Occupation	No. of Cases	%
Agricultural occupations	18	10.4
Metal Workers	19	11.0
Textile Workers	3	1.7
Makers of Foods, Drinks and Tobacco	2	1.2
Workers in Wood and Furniture	8	4.6
Painters and Decorators	4	2.3
Shipwrights	2	1.2
Transport Workers	4	2.3
Commercial, Finance and Insurance Occupations	18	10.4
Public Administration and Defence	6	3.4
Professional Occupations	15	8.7
Personal Service	2	1.2
Clerks and Draughtsmen; Typists	11	6.3
Other and Undefined Workers	12	6.9
Not Stated	49	28.4
Total	173	100.0

TABLE 2.
Country associations in 389 cases.

	Males	Females	Total No.	Total %
Lived in Country	55	63	118	30.3
Worked in Country	3	1	4	1.0
Been in Country	38	35	73	18.8
Not been in Country	6	6	12	3.1
Association with Country not stated	71	111	182	46.8
Total	173	216	389	100.0

In contrast to the occupational incidence we have been impressed by the fact that a history of exposure to rural conditions is an almost constant finding in cases of disseminated sclerosis seen by us. From Table 2 it will be observed that, in a series of 389 cases, many patients have lived in the country, and further, that the majority have at one time or another been in the country. A note of caution must be struck in connection with the statistical evaluation of occupational incidence and exposure to rural conditions. For example, we have found that the occupation of a patient has not always been that which he is following at the time of seeking treatment for disseminated sclerosis. Again, a short holiday in the country, perhaps several years previously, may be readily forgotten by a patient. It is therefore suggested that occupation is significant only in so far as it brings a person into contact with conditions obtaining in country districts. Exposure to rural conditions rather than agricultural employment or rural residence is necessary for exposure to the causative agent of the disease. Such a hypothesis, however, demands an explanation

of the fact that more people do not develop disseminated sclerosis, since the vast majority of the population have at some time been exposed to conditions obtaining in country districts. We suggest that this may be explained in one or more of the following ways. Only certain districts may harbour the causative agent. It is well known that disseminated sclerosis has a clearly defined geographical incidence (Bing, 1932; Sallstrom, 1942). It is further believed that disseminated sclerosis has, in a given country, a district incidence. Thus, Bing found the incidence in North Switzerland to be four times greater than that in South Switzerland. Similarly, the high incidence of the disease in the region of the Great Lakes of North America is well known. In England, Wilson (1927) in a statistical study found that the death rate was much higher in some counties compared with others. We are of the opinion that in Scotland disseminated sclerosis has a definite district incidence. In some areas the disease is common; in others it is seldom if ever encountered. It has not yet been found possible to undertake an accurate investigation on this subject, but in several areas known to the writers the disease is particularly common. Andrewes (1948) has pointed out that no mere contact of virus and host is enough to produce overt infection. He considers that many virus diseases may normally be subclinical or latent. With regard to disseminated sclerosis we believe that infection with the causative agent may be compatible with perfect health and a normal life. In other instances, subsequent to the initial manifestation, the disease may remain asymptomatic for an average life-time. The development of disseminated sclerosis may thus depend on personal susceptibility, predisposing factors, or exposure to an overwhelming infection.

Burnet (1945) has suggested that most of the virus diseases of man are of relatively recent origin and that they are originally contracted from some animal. Although we would hesitate at this stage to implicate a virus as the causative agent of disseminated sclerosis, it would appear reasonable to suppose that this disease is caused by a closely allied micro-organism. We therefore proceeded on the assumption as a working hypothesis, that an animal or animals might prove to be the natural reservoir of an agent responsible for the disease. Because of the suggested relationship between disseminated sclerosis and scrapie, a chronic disease of sheep caused by a virus infection of the central nervous system, or between that disease and swayback, also a disease of the nervous system but commonly accepted as a conditioned copper deficiency in the animal, sheep were chosen for the purposes of the investigation.

It was therefore decided to determine whether materials obtained from suitable cases of disseminated sclerosis, when used as inocula, had any pathogenic effects in sheep and if so, whether the morbid changes were such as to justify the assumption of a relationship between disseminated sclerosis and either of the diseases mentioned.

MATERIAL AND METHODS.

The cases of disseminated sclerosis chosen. Because of the belief that in established cases of disseminated sclerosis immunity reactions may have destroyed large numbers of the infecting micro-organisms it was decided to utilize early cases of the disease. It was considered that only in such cases would the causal agent be present in significant numbers. Three cases selected were of recent onset and were diagnozed on clinical and serological grounds as suffering from disseminated sclerosis. Blood obtained by venepuncture and cerebro-spinal fluid by lumber puncture from each patient was withdrawn aseptically into sterile containers which were immediately sealed by heat. In the case of blood an anticoagulant, ' Liquemin ' (Heparin), was employed in the proportion of 0.2 c.c. to every 5 c.c. of blood withdrawn. The samples, which consisted of approximately 15 c.c. of blood and 15 c.c. of cerebro-spinal fluid from each patient, were divided into two equal parts thus making a total of 12 samples. Three samples of blood and three of cerebro-spinal fluid were placed in a container at 0°C. and the other six in a container at 37°C. This precaution was taken because of the uncertainty as to the optimum temperature at which the potential infective agent in the blood and cerebro-spinal fluid should be stored pending inoculation into the test sheep. The interval between withdrawal of the fluids and inoculation of the animals was approximately three hours.

Test sheep. These were approximately seven months old and appeared in normal health. Cerebro-spinal fluid which previously had been withdrawn by cysternal puncture from six animals had revealed no abnormal features and the colloidal gold reaction in each instance had been normal. On 24th November, 1948, while under pentothal anaesthesia six animals were inoculated intracerebrally and five of them, in addition, received the inoculum by injection into the sciatic nerve. Two animals were retained as controls. The eight sheep in the experiment, details of which are shown in Table 3, were thereafter strictly segregated from other animals. Inoculated animals showed no immediate ill effects apart from a transient lameness due to trauma of muscle which occurred while exposing the sciatic nerve.

TABLE 3.

Inoculation of sheep.

Sheep No.	Inoculation (24/11/48)	Subsequent Course	Pathological Findings
33	C.S.F. 1 c.c. intracerebrally C.S.F. 0.2 c.c. into sciatic nerve	Survived to end of Experiment (May 1950)	————
34	ditto.	Deterioration in general condition—death (18/11/49)	Pneumonia
36	ditto .	Survived to end of experiment (May 1950)	————
40	Blood 1 c.c. intracerebrally Blood 0.2 c.c. sciatic nerve	Deterioration in general condition—destroyed (3/12/49)	Pneumonia
42	ditto.	Survived to end of Experiment (May 1950)	————
45	Blood 1 c.c. intracerebrally	Accidental death (7/4/49)	Subacute meningo-encephalitis
49	Control	Survived to end of Experiment (May 1950)	————
56	Control	Survived to end of Experiment (May 1950)	————

RESULTS.

This experiment embraced the period November, 1948, to May, 1950. During this time three of the six inoculated sheep died ; both controls survived.

Sheep No. 45 died on 7th April, 1949, *i.e.*, four and one half months after inoculation. The cause of death was suffocation following pentothal anaesthesia which had been administered to permit follow-up cysternal puncture being performed. The manner of death was entirely accidental, and there was no clinical evidence of disease of the animal's nervous system.

Histological examination, however, revealed the presence of a meningo-encephalitis of subacute type. The lesions which were characterized by marked perivascular cuffing with lymphocytes and a few polymorphonuclear cells, predominated in the cerebrum, but were also present in the cerebellum, medulla and cord. Gram-stained specimens disclosed no pyogenic micro-organisms and no spirochaetes were seen on appropriate examination. Sections stained by Weigert's method failed to reveal any evidence of demyelination. The histological picture obtained is demonstrated in the photomicrographs.

In July, 1949, it was apparent that the condition of sheep Nos. 34, 36 and 40 had deteriorated. No cause for this was found and blood and biochemical investigations revealed no abnormal features. The worm burden in each animal was determined. In only one instance (sheep No. 40) was this such as to be possibly injurious to health.

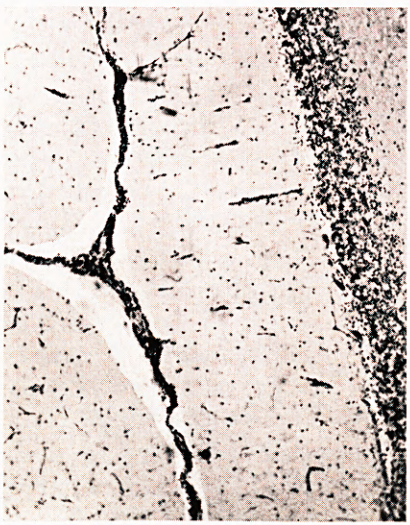

Fig. 1. Sheep No. 45. Cerebellum showing cellular infiltration of the pia mater and reaction in the molecular cell layer of grey matter. H. & E. (× 100).

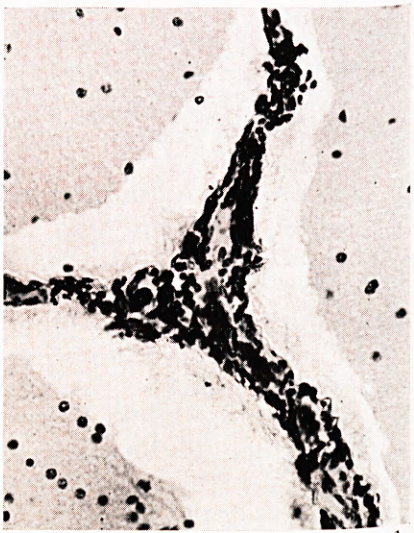

Fig. 2. Area of cerebellum present in Fig. 1, showing the infiltrating cells (mainly lymphocytes) present in the pia mater. H. & E. (× 250).

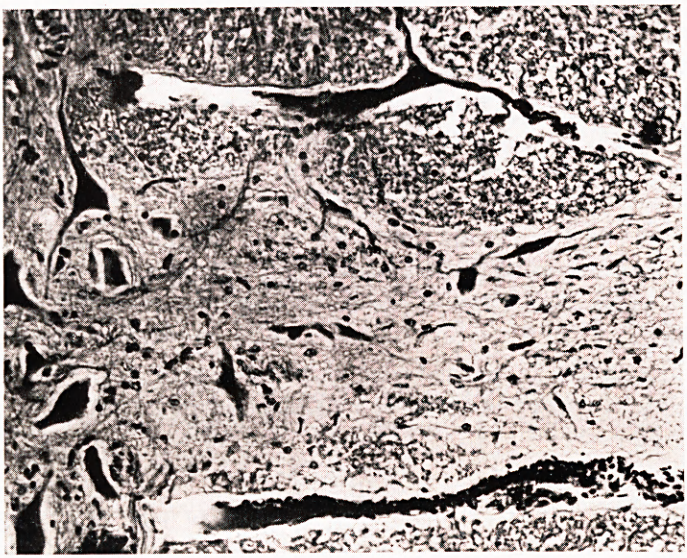

Fig. 3. Medulla, showing congested blood vessel with infiltrating cells (mainly lymphocytes) in region of central median fissure. H. & E. (× 250).

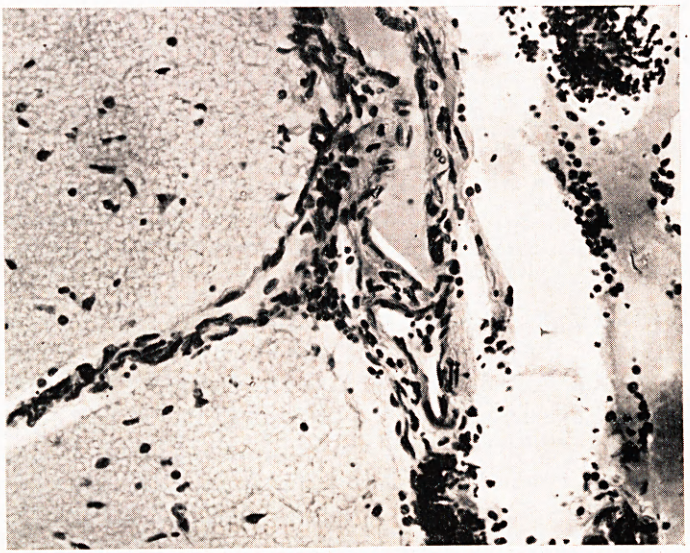

Fig. 4. Cerebrum, showing infiltrating lymphocytes and polymorphonuclear leucocytes in pia mater of cerebral sulcus. In neighbouring cortex the same types of infiltrating cells are present together with proliferating microglial elements. H. & E. (× 250).

Sheep No. 36 subsequently improved but sheep No. 34 died on 18th November, 1949, from a terminal pneumonia and on 3rd December, 1949, sheep No. 40 was destroyed at the onset of what would have proved to have been a terminal respiratory tract infection. In neither instance was there any evidence of paresis or of other involvement of the central nervous system, with the exception that the cough reflex appeared to be diminished. Histological examination of the brain and spinal cord of both animals revealed no abnormal features.

Cerebro-spinal fluids obtained from each animal on two occasions during the course of the experiment proved to be normal. In particular Lange's colloidal gold test revealed no abnormal precipitation of the gold sol.

Both control and the surviving three inoculated sheep appeared in normal health when the experiment was terminated in May, 1950.

DISCUSSION.

Histological examination of the central nervous system of one inoculated sheep revealed the presence of a diffuse meningo-encephalitis of subacute type. The histological picture, however, apart from being suggestive of the existence of a subacute infective process, did not appear similar to that of any known specific disease of the nervous system in sheep. Further, as it did not resemble that of disseminated sclerosis in the human subject, the experiment has afforded no evidence in support of a suggested relationship between disseminated sclerosis in man and either swayback or scrapie in sheep.

The cause of this inflammatory process is obscure. There was no evidence of pyogenic or spirochaetal infection. Facilities did not permit further investigation into a possible virus etiology. It is therefore not possible to say whether or not this disease process was occasioned by the causative agent of disseminated sclerosis, or whether some contaminant was responsible.

Despite many attempts to transmit disseminated sclerosis to animals (Steiner, 1919 ; Adams *et. al.*, 1924) ; both Brain (1930) and Schumacher (1950), in critical reviews, consider that the disease, as it exists in the human subject, has never been experimentally reproduced in animals. It is, of course, open to speculation whether animal tissues ever react in the same way as human tissues do to a specific infective agent. Further, although experimental support is inconclusive, the theory that disseminated sclerosis may result from a toxic-infective process has never been disproved, and the studies of Dawson (1916) suggest that the histology of the disease is compatible with such a process. Finally, the recent work of Margulis *et al.* (1946) furnishes support for the theory that a virus may be the causative agent of disseminated sclerosis.

The occupation of the individual plays no significant rôle in the etiology of disseminated sclerosis. It is possible, however, that exposure to rural conditions in certain localities may be of importance in bringing an individual into contact with the causative agent of the disease. Following on this, the demarcation of localities in which disseminated sclerosis is common and the study of conditions obtaining in such areas are of obvious importance.

SUMMARY.

The results of inoculating six sheep with blood and cerebrospinal fluid from three cases of disseminated sclerosis are described.

One animal inoculated in this way developed a meningo-encephalitis of subacute type.

This work has afforded no evidence in support of the suggested relationship between disseminated sclerosis and either swayback or scrapie in sheep.

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