Preliminary Communications

Transmission Experiments with Multiple Sclerosis: An Interim Report

Brit. med. J., 1966, 2, 564–565

The association between multiple sclerosis and rural or agricultural pursuits suggested by some early epidemiologists (summarized by Sutherland and Wilson, 1951) has not been borne out by most subsequent workers. While it received a powerful impetus from the report by Campbell, Daniel, Porter, Russell, Smith, and Innes (1947) that four out of seven workers studying swayback at Cambridge had developed the disease, an addendum by Campbell (1963) has much diminished the force of the association. In 1951 Sutherland and Wilson published an account of some attempts they had made to infect sheep by injection of blood and spinal fluid from early cases of multiple sclerosis. Their results were indefinite, and they concluded that their work had "afforded no evidence in support of the suggested relationship between disseminated sclerosis and either swayback or scrapie in sheep." Their experiments embraced the period November 1948 to May 1950—that is, some 17 months.

Meanwhile, however, studies initiated by Sigurdsson at the Keldur Institute, Reykjavik, and extending over the years 1948–56 had suggested that certain virus diseases, notably visna (Sigurdsson and Pálsson, 1958), had an exceptionally long latent or incubation period. In view of this a reassessment of multiple sclerosis becomes important. Since such experiments necessarily extend over a period of years, it is hoped the present interim report may stimulate independent studies as suitable material comes to hand.

Material and Methods

At the end of September 1959 there became available in Newcastle the brain of a patient (X.) in her early forties who had died from an acute attack of multiple sclerosis which had virtually transected her medulla. Certain unusual features of this case (together with the clinical history) were the subject of a report by Field, Miller, and Russell (1962). Material from the vicinity of a fresh lesion in the frontal lobe was obtained within four hours of death and inoculated into sheep, goats, and Moredun strain of white mice.

In April 1962, through the kind cooperation of Mr. John Hankinson, a fragment of parietal cortex, together with subjacent white matter, was obtained from a patient (Y.) upon whom a stereotactic operation was being performed for the relief of severe unilateral tremor occurring in the course of multiple sclerosis. The material did not appear to be part of a lesion. It was made up in sterile saline (1:10, w/v) and 0.05 ml. of the opalescent supernate (after centrifugation at 3,000 r.p.m. (2,000 *g*) for five minutes) inoculated intracerebrally into six cages of Moredun mice, six in each cage.

X. Material

A full account of the results obtained with X. material has been presented by Pálsson, Pattison, and Field (1966), and need only be summarized here. In Reykjavik four out of four sheep inoculated with X. brain developed a neurological disturbance which was diagnosed by histological examination as scrapie, while all four inoculated at the same time with control brain (of the same age) remained well. The average incubation period in this experiment was 18.5 months. When material from the brain of the first two sheep of the series was passaged intracerebrally all five animals developed a scrapie-like condition, and this time the incubation period was reduced to 10.8 months.

The X. material was stored at −20° C. and repetition of the original experiment with a new sample in February 1962 gave only two out of five sheep positive—again with a long incubation period. A third group of sheep inoculated in March 1963 produced only one out of five positive.

Neither at Compton nor in Newcastle has a positive result been obtained with sheep, though in both cases scrapie-susceptible animals were used—Herdwicks at Compton and Cheviots at Newcastle—nor has inoculation of mice with X. material followed by two blind passages resulted in clear disease.

Y. Material

Of the 36 mice injected with Y. material none showed clinical disease at the end of eight months, and all were killed and examined histologically by haematoxylin and eosin straining of paraffin sections. (The more delicate Cajal impregnation of astrocytes was not adopted as a routine until later.) All were negative. Brains were stored in 10% formalin. At about this time it became known that scrapie agent was highly resistant to formalin (Pattison, 1965). A brain which had been in formalin for 10 months was washed in running water for 48 hours and a 1:10 suspension prepared as before. Three cages of six mice (4–5 weeks old) each were inoculated with 0.05 ml. of a sterile 1:10 suspension intracerebrally and were clinically normal when killed for examination at 13, 16, and 17 months. In each cage one mouse was unexpectedly found to have the marked astroglial proliferation and vacuolation of nervous parenchyma associated with scrapie in this species (Chandler, 1961; Pattison and Smith, 1963). Portions of brain from two of these mice were further passaged (0.05 ml. of 1:10 suspension) intracerebrally, and in one case 15 out of 15 mice came down with scrapie in five to six months; in the other all of 11 developed the disease in eight to nine months. A further passage in this case reduced the incubation of the disease to five months.

Discussion

The emergence of scrapie in sheep as the result of any experimental procedure must always be interpreted with the utmost caution. The disease is widespread both in this country and in Iceland (where it is called "rida"), and there is an ever-present possibility that the condition has been lighted up by injection of foreign material. Reasons for accepting the Icelandic results as genuine have, however, been given by Pálsson *et al.* (1966) and may be summarized thus:

(*a*) The flock from which the sheep were drawn is well known at first hand to Dr. Pálsson and has supplied animals for experiment over many years without rida having occurred.

(*b*) A 100% scrapie "take" would be a highly improbable occurrence even if known scrapie material had been injected.

(*c*) Absence of disease in control animals.

(*d*) Shortening of the incubation period with passage—a very characteristic occurrence when the "species barrier" is crossed in scrapie experiments (Pattison, 1966). Moreover, the early signs of neurological disturbance noted by Pálsson in his experiments were not those which occur in Icelandic rida, and this initial variability

in signs has also been emphasized by Pattison (1966) when attempts are made to establish scrapie in a new species.

In the interpretation of the Y. mouse experiments difficulties of a different order emerge. No one who has kept mice for prolonged periods can be entirely confident that animals do not find their way into the wrong box on rare occasions, despite the most rigid discipline in the animal house. However, at the time of the first experiments no scrapie material was being handled in the laboratory, and later, when it was imported, separate animal houses were used for scrapie and non-scrapie work. Scrapie was long believed not to be infectious from cage to cage, but recently there have been reports of the disease affecting normal mice kept in the same room as infected ones (Dickinson, MacKay, and Zlotnik, 1964; Morris, Gajdusek, and Gibbs, 1965). If these are true cases of cross-infection they must be very rare indeed. Nevertheless, the possibility of their occurrence makes it important that attempts to pass multiple sclerosis into mice should be carried out in laboratories not working with scrapie material at all.

Again in the case of the Y. mice, the long incubation period (shortening on passage) coupled with absence of symptoms is exactly what would be expected if a species barrier was being crossed. In view of this, these results, highly unexpected though they are, may well be genuine, and it becomes of importance that independent experiments should be set up as suitable material becomes available.

Sheep of different breeds are known to differ remarkably in susceptibility to scrapie (Gordon, 1959). Genetic make-up has indeed been regarded by some workers as all-important for the development of the condition (Parry, 1962). Icelandic sheep are a special case in that they are related to German goats, and it might well be that unusual genetic factors make them specially suitable for experiments of this type. Dairy goats can in general be "infected" much more consistently than sheep.

Dick, McAlister, McKeown, and Campbell (1965) in Northern Ireland, like Pálsson *et al* (1966) in this country, have been unable to produce disease in sheep by inoculation of multiple sclerotic brain material. On the other hand, Campbell, Norman, and Sandry (1963) reported positive results in experiments carried out by Pálsson with brain from a case of "subacute encephalitis . . . associated with necrotizing myelitis," and believed not to be multiple sclerosis. However, it must be borne in mind that necrosis sometimes does occur with multiple sclerosis (Adams and Kubik, 1952) and that some cases of neuromyelitis optica (Devic's disease) show classical multiple sclerosis plaques in the brain.

Clinically the progressive relentless course of scrapie terminating fatally differs from the common type of multiple sclerosis. Pathologically, too, they show little similarity at first sight, though both present a remarkable overgrowth of astroglial cells (Müller, 1904; Anton and Wohlwill, 1912; Chandler, 1961; Pattison, 1965), a feature apparently shared with kuru. The possible implications of this have been briefly discussed elsewhere (Field, 1966).

Even if the results in Icelandic sheep and in the Moredun strain of white mice are accepted as genuine and not a "laboratory error," many problems would yet remain in the absence of any real understanding of the scrapie process. On rare occasions, for example, inoculation of normal brain has produced scrapie (Pattison, 1966). To what extent this capacity may depend upon the age of the injected brain or non-specific pathological processes within it remains to be determined. The recent suggestion that kuru may be a slow virus infection (*Brit. med. J.*, 1966a) and that a virus may be responsible for multifocal leuco-encephalopathy (*Brit. med. J.*, 1966b) should encourage further transmission experimentation with multiple sclerosis. Recent work, if verified, suggests that the scrapie agent "may be able to increase in quantity without itself containing nucleic acid" (Alper, Haig, and Clarke, 1966). In other words, the scrapie agent might be a non-nucleic acid containing "replicon"—a highly interesting and far-reaching possibility. Neither in scrapie nor in multiple sclerosis has a virus been observed in the electron microscope, and it might well be that some new class of particle—perhaps a replicating polysaccharide—might be involved in certain slow or latent "virus" infections. At present the most important need is for further patient experiment with multiple sclerosis material.

SUMMARY

By inoculation of biopsy material from a case of multiple sclerosis into white mice followed by blind passage, a few cases of scrapie (with shortening incubation period) have emerged. The difficulties in interpreting these results are considered.

I would like to thank Miss Greta Joyce, A.I.M.L.T., for the large number of histological preparations she has made for this work.

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