

## Multiple sclerosis and scrapie

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Field, Miller, and Russell (1962) reported that sheep in Iceland injected intracerebrally by Dr. Palsson with a suspension of the brain of an acute case (SELL) of multiple sclerosis developed a disease histologically indistinguishable from scrapie. In the following year, Campbell, Norman, and Sandry (1963) noted that Dr. Palsson had again elicited a scrapie-like disease in sheep injected intracerebrally with a suspension of pathological human brain material. On this occasion the inoculum was a suspension of brain and spinal cord from a fatal case of subacute encephalitis of unknown aetiology. Histological examination of the central nervous system of two of the four inoculated sheep showed changes which have been regarded as characteristic of scrapie by Zlotnik and Stamp (1962), but the ponto-cerebellar and other abnormalities observed in scrapie sheep in England by Beck, Daniel, and Parry (1964) were not seen.

In 1964 Palsson, Pattison, and Field (unpublished data) reported that all of the original sheep, four in number, inoculated with SELL brain in Iceland developed neurological illnesses and showed histological signs of scrapie. Furthermore they recorded that the experiment had been repeated in Iceland in that two of five sheep inoculated with the same brain material developed signs of scrapie, and that sheep-to-sheep passage experiments had been successfully done.

The clinical pictures of multiple sclerosis and scrapie are very different as are also the lesions in the central nervous system. Indeed the only common features in the two conditions are involvement of the central nervous system and long incubation periods (if indeed multiple sclerosis is due to an infective agent). It seems most unlikely that the same agent could be responsible for such dissimilar diseases unless as the precipitating factor of two different conditions. There have, however, been suggestions that sheep might play some part in the natural history of multiple sclerosis (Campbell, Daniel, Porter, Russell, Smith, and Innes, 1947; Sutherland and Wilson, 1951; Campbell, 1963) and in view of

the findings in sheep reported by Field *et al.* (1962) and Palsson *et al.* (unpublished data) we decided to try to repeat their experiment.

In addition to testing the brain material (SELL) which initiated scrapie in sheep in Iceland we also inoculated sheep with material from the central nervous system from six other fatal cases of multiple sclerosis. At the same time we inoculated monkeys intracerebrally with aliquots of the central nervous system suspension which we tested in sheep. Finally we inoculated monkeys with suspensions of scrapie-infected brain from sheep, goats, and mice in an attempt to reproduce the disease in these animals.

### MATERIALS AND METHODS

Portions of brain and spinal cord were obtained from seven fatal cases of multiple sclerosis all of which were confirmed by post-mortem examination. One of the specimens (SELL) was from the patient described by Field *et al.* (1962) which initiated the scrapie-like illness in sheep in Iceland (Field *et al.*, 1962; Palsson *et al.*, unpublished data). It was shipped to Belfast on dry ice. The sources of the other specimens were as follows:

Name	Patient		Duration of Illness (yr.)	Town	Physician
	Sex	Age (yr.)			
SMI	M	24	3½	Bristol	Dr. A. M. G. Campbell
MAL	F	28	7½	Bath	Dr. A. M. G. Campbell
RIC	M	55	4	Belfast	Dr. R. S. Allison
BLA	F	33	6 8/12	Belfast	Dr. H. Millar
ROB	M	57	25	Belfast	Dr. H. Millar
HAR	F	27	1 8/12	Belfast	Dr. H. Millar

The specimens from the above cases were stored in a mechanical  $-70^{\circ}\text{C}$ . cabinet as suspensions or portions of whole brain taken immediately after necropsy, except SMI and MAL, which were transported on dry ice before storage. The suspensions of material from the central nervous system were made by grinding portions of the white matter showing acute plaques of multiple sclerosis in balanced salt solution to make approximately 20% wt./vol. suspensions. Immediately after thawing, the suspensions were inoculated into lambs and a few sheep (Table I) and into rhesus monkeys of 7 to 9 lb. in weight. The lamb and sheep inoculum was 1.0 ml. which was

injected towards the anterior end of the sulcus exto-lateralis into the fronto-lateral region of the brain. Monkeys were inoculated intrathalamically with 0.5 ml. into each thalamus (Bodian, 1952).

All of the sheep with the exception of three Blackface × Leicester and two Blackface × Suffolk were horned Blackface. They were kept as two separate flocks. The first consisted of lambs inoculated with SELL brain and of control lambs, and the other flock, which had no contact with the first, consisted of lambs and sheep inoculated with the other multiple sclerosis brain suspensions.

The suspensions of scrapie brain material for the inoculation of monkeys were kindly supplied by Dr. I. H. Pattison, Agricultural Research Council, Compton, Berkshire, and consisted of (1) whole brain from Swaledale sheep no. U. 878WY; (2) 'sleepy' goat brain homogenate and (3) 'scratching' goat brain homogenate from the 'standard pool', and (4) mouse brains nos. S23FS and S23F6. The sheep and mouse brain materials were each made up as 20% wt./vol. suspensions and all suspensions were stored at -70°C. till inoculated.

Approximately 24 months after inoculation half of the monkeys which were inoculated with one or other of the suspensions of scrapie brain material or human multiple sclerosis brain were given a course of cortisone consisting of 28 daily intramuscular injections of 50 mg. of cortisone inj. B.P. (Cortisyl).

All glassware, syringes, pipettes, etc., used in each experiment were disposed of immediately after use in view of the alleged stability of the scrapie agent (Stamp, 1959).

Representative blocks of brain and cord were examined histologically from all but a few of the inoculated monkeys.

## RESULTS

The results of the inoculations of the sheep and lambs are summarized in Table I.

TABLE I

RESULTS IN LAMBS (AND SHEEP) INOCULATED INTRACEREBRALLY WITH SUSPENSIONS OF CENTRAL NERVOUS SYSTEM MATERIAL FROM FATAL CASES OF MULTIPLE SCLEROSIS

Specimen	Age of Animals when Inoculated (days)	Duration of Observations (months)	No. of Symptom-free Survivors/No. Inoculated
SELL	<1, <1, 1, 1, 1, 1, 7, 9, 16, 31	35	9/9
Normal	7, 9, 16, 31 <sup>1</sup>	35	4/4
SMI	<1, 1, 1, 2	36	3/4 <sup>2</sup>
MAL	<1, <1, <1 Ewe	36	4/4
RIC	1, 1, 1	36	3/3
BLA	3, 5 Ewe	38	3/3
ROB	<1, <1	36	1/2 <sup>3</sup>
HAR	1, 1, 1 Ewe	36	4/4

<sup>1</sup>Twins of SELL group

<sup>2</sup>Died 21 days after inoculation: brain showed no abnormality

<sup>3</sup>Killed by police dog about eight months after inoculation: no abnormality found

Throughout an observation period of three years no sign of any abnormality was observed in any of the inoculated lambs or sheep. One lamb died

and one was killed accidentally 21 days and eight months respectively after inoculation. In neither of these lambs was any abnormality of the central nervous system found.

The results of the inoculation of monkeys with scrapie brain material and with a suspension of SELL brain are recorded in Table II. In addition to the monkeys inoculated with human multiple sclerosis brain material from patient SELL, 18 monkeys were inoculated with aliquots of the human brains inoculated into sheep. Although there were some intercurrent deaths of inoculated monkeys from an outbreak of dysentery, monkeys in all groups were observed for periods of up to three years. No abnormal clinical signs referable to lesions of the central nervous system were observed. Histological examination of the brains of monkeys which died of intercurrent illness or were sacrificed showed no significant lesions. An occasional monkey showed the types of minimal lesions which are often observed in any extensive examination of the central nervous system of rhesus monkeys (see Jungherr, Cabasso, and Stebbins, 1963) but were in no way considered to be related to scrapie.

TABLE II

RESULTS IN MONKEYS INOCULATED WITH SCRAPIE BRAIN MATERIAL AND SELL BRAIN SUSPENSION

Material Inoculated	Duration of Observation of Each Monkey Inoculated (month)	Ratio of No. of Monkeys Showing Clinical or Microscopic C.N.S. Abnormality to No. Inoculated
Scrapie		
Sheep (Swaledale)	3, 5, 7, 12, 36, 36	0/6
Goat ('scratching')	5, 14, 26, 31, 36	0/6
Goat ('sleepy')	<1, 1, 5, 6, 36	0/5
Mouse	3, 3, 4, 25, 36, 36	0/6
Human		
SELL	<1, 5, 6, 6, 36, 36	0/6

## DISCUSSION

We have been unable to confirm the observations made in Iceland that inoculation of sheep with a suspension of brain (SELL) from a fatal case of multiple sclerosis induces a scrapie-like disease in sheep (Field *et al.*, 1962; Palsson *et al.*, unpublished data). Our results with SELL brain in sheep in Northern Ireland are entirely negative like those of Palsson *et al.* (unpublished data) in sheep inoculated with SELL brain in England. Similarly inoculation of sheep in N. Ireland with suspensions of brain from six other fatal cases of multiple sclerosis were entirely negative over an observation period of three years.

There is, as far as we know, no adequate published information on the natural incidence of scrapie in various breeds. We chose Blackfoot sheep since they

appeared to be from flocks free of natural scrapie and since they had been shown to be susceptible to inoculation with the scrapie agent (Gordon, 1946; Gordon, 1959; Stamp, Brotherston, Zlotnik, Mackay, and Smith, 1959). The negative experiments carried out in England with the SELL brain (Palsson *et al.*, unpublished data) were made in Herdwick and Cheviot breeds which are more highly susceptible to scrapie (Gordon, 1959). We do not think the failures in the U.K. to confirm the Icelandic experiment with SELL brain are due to the breed or the age of the sheep which were used.

A scrapie-like disease was not only initiated in Icelandic sheep inoculated with a suspension of multiple sclerosis brain but also in three out of four sheep inoculated in Iceland with a suspension from a case of subacute encephalitis (Campbell *et al.*, 1963) which bore no resemblance to multiple sclerosis. It would appear therefore either that Icelandic sheep are highly susceptible to the agent or agents of both multiple sclerosis and subacute encephalitis or else, as seems more likely, that the inoculation of human pathological material into Icelandic sheep may activate the disease called rida which appears to be a form of scrapie. It is of some importance to note that Sigurdsson (1954) reported that at one time areas over a large part of northern Iceland where rida was prevalent were cleared of sheep. The area was restocked with sheep from areas believed to be free from rida. Within a year rida had reappeared on farms where it had previously existed. Although Sigurdsson suggested that an intermediate host or vector might be involved it is also possible that the disease was introduced by the supposedly rida-free sheep. In the United Kingdom the true incidence of scrapie 'is often a closely guarded secret; the shepherd may even conceal the occurrence of the disease from his own flock master' (Stamp, 1962). The situation could be similar in Iceland and it seems possible that the sheep inoculated in Iceland were carriers of the rida scrapie-like agent which was activated by the inoculations.

Secondly we have shown that scrapie-infected sheep brain, goat brain, or mouse brain failed to produce any significant clinical or microscopical lesions in monkeys during an observation period of three years. In addition, prolonged observations of rhesus monkeys inoculated with the brain (SELL) material which initiated a scrapie-like disease in Icelandic sheep and with other multiple sclerosis brain suspensions were entirely negative.

#### SUMMARY

We have been unable to confirm the findings of Field *et al.* (1962) and Palsson *et al.* (unpublished data)

that a scrapie-like disease in sheep can be induced by the intracerebral inoculation of brain suspensions of patients with multiple sclerosis. It is suggested that the results of Field *et al.* and Palsson *et al.* may be explained by the activation of rida (a scrapie-like disease) in Icelandic sheep by the inoculation of pathological human brain material.

We have been unable to induce signs or lesions in sheep or in monkeys inoculated with six other brain suspensions from fatal cases of multiple sclerosis.

Monkeys inoculated with several strains of scrapie brain suspensions failed to show any evidence of involvement of the central nervous system.

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