

## Scrapie—a personal view

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Scrapie disease of sheep and the closely similar kuru disease of the Fore people of New Guinea have been extensively documented. Preparation of review articles on scrapie and kuru has been a safety valve for workers frustrated by the maddeningly slow pace of research on these diseases; the literature on both has been exhaustively analysed and summarized. Anyone seeking up-to-date information should consult the excellent reviews by Field (1969) and Thormar (1971).

When the organizers of this Symposium very kindly invited me to speak about scrapie, I wondered what on earth I could present that had not already been recorded, again and yet again. Had I any unique contribution? Perhaps so. It happens that I have been associated with research on scrapie for twice as long as anyone else still active in the field, and three times as long as most. This does not make me more knowledgeable than other people, but it means that I have been privileged to be personally involved, as participant or onlooker, in a long and still unfinished story. At the risk of being dubbed a bore, I would like to tell that story.

My first contact with scrapie was in 1939 when I was still a veterinary student, working part time at Moredun Institute in Edinburgh. I learned that the disease was a slowly progressive, fatal, non-inflammatory degeneration of the central nervous system, characterized clinically by incoordination of the hind-quarters, a bewildered expression, and, in many cases, compulsive rubbing (scraping) against fixed objects. It was impossible not to become involved with scrapie at Moredun Institute at that time, because large-scale experiments were in progress to confirm the exciting claim by Cuillé and Chelle (1936) from France that the disease had been reproduced by intraocular inoculation of healthy sheep with a preparation of spinal cord from an affected sheep. When first made, this claim had been viewed with a scepticism that has from time to time been applied to observations made by scrapie research workers since the days of Roche-Lubin (1848) who suggested that the cause of the disease was sexual excess or lightning. Scepticism in 1936 was not unreasonable, because Cuillé and Chelle had re-

ported that the disease had not appeared until between 14 and 22 months after inoculation—and that took some believing.

By the time I arrived at Moredun Institute, Cuillé and Chelle were riding high. Not only had they again reproduced scrapie after a long incubation period, but they had also shown that material passed through an antibacterial Chamberland L3 filter would reproduce the disease. In 1938 they suggested that the cause of scrapie was a virus. I was just in time to help with the histological diagnosis of scrapie in the experiments at Moredun Institute that proved beyond doubt that what Cuillé and Chelle had claimed was correct. The occasion was unique. For the first and last time, scrapie workers were unanimous. The disease, they said, was caused by a virus.

In the early 1940s all of those involved in scrapie research at Moredun Institute except the late D. R. Wilson went their various ways. Wilson remained at the Institute and during the next 10 years carried out pioneer work on the disease. Wilson's achievements have been greatly undervalued. He was a shy, reticent person, incapable of exaggeration, honest to a rare degree. At that time scrapie was of limited economic importance (compared with many other sheep diseases), and of no special scientific interest. Wilson accepted the conclusion reached by Cuillé and Chelle that it was a virus disease, and he set about looking for the virus. He published very little: a paper in 1950, and a brief general article on scrapie in 1952, but Stamp, Brotherston, Zlotnik, Mackay, and Smith (1959) and Stamp (1960) have recorded a good deal of his unpublished work.

Wilson's achievements were remarkable when one remembers that his only method of detecting the transmissible agent of scrapie was by inoculating sheep only about 25% of which were susceptible to the disease after up to a year's incubation period. By carrying the disease through nine serial passages he showed beyond doubt that scrapie is an experimentally transmissible disease. He was first to demonstrate the high resistance of the transmissible agent to heat (100°C for 30 minutes), formalin, phenol, and chloroform. He showed that it would pass filters of APD 650  $\mu\text{m}$  and 410  $\mu\text{m}$ , that it could

not be completely sedimented by centrifugation at 40 000 rpm for two hours, and that it remained viable in dried brain for at least two years, and he noted resistance to a considerable dose of ultraviolet light. He found that the disease could be transmitted not only by the intracerebral and intraocular routes, but also subcutaneously, intravenously, and intradermally. He searched extensively for, but was unable to find, antibodies in scrapie-affected or scrapie-inoculated sheep. He could find no abnormality in the cerebrospinal fluid of affected animals, and he noted that the characteristic vacuolated nerve cells of scrapie in sheep were less numerous in experimental than in field cases. He recognized the enormous difficulties of working exclusively with sheep, and in 1952 he wrote wistfully. 'The inability to infect a small laboratory animal greatly increases the difficulties of scrapie investigation . . .' Not that he had not tried to infect laboratory animals; he had done so extensively, but his luck was out. That advance was still nine years into the future.

Scrapie was first diagnosed in Canada in 1939 and the United States of America in 1947, and an extensive programme aimed at complete eradication of the disease was started in the USA in 1952. It is highly likely that scrapie in Canada and the USA was related to importation of sheep from Britain. The disease was diagnosed in Australia in 1952, in sheep imported from Britain some 12 months earlier. Not surprisingly these countries, together with New Zealand, placed a complete embargo on the importation of sheep from Britain unless they could be guaranteed free from scrapie. That embargo is still in force, and more recently has been applied also by South Africa. This sudden closure of export markets for British sheep created a new importance for scrapie research. In 1953 the Agricultural Research Council decided that investigation of the disease must be intensified, and that the work should be done at Moredun Institute and at the Institute for Research on Animal Diseases (at that time called the 'ARC Field Station') at Compton. The late W. S. Gordon, who had worked with scrapie in the prewar days at Moredun Institute, was by this time Director at Compton. And this was when I again became actively involved. Wilson visited Compton in 1953 to inoculate sheep and start our experiments. The strain of scrapie agent that we—and many others—still use is often called the 'Compton' strain, but in fact it is Wilson's strain, established by his technical skill, not ours.

Our first experiments at Compton were aimed at extending Wilson's observations, and it was really fortuitous that as early as 1954 we examined further an observation recorded in 1939 by Cuillé and Chelle that both of two goats had developed scrapie

26 months after intraocular inoculation with a homogenate of lumbar cord from a scrapie-affected sheep. I happened to be inoculating a batch of sheep with scrapie on a day when 12 goats were available; I inoculated these animals also, intracerebrally with the suspension of scrapie sheep brain I was putting into sheep. To my surprise, all these 12 goats developed scrapie between 15 and 22 months after inoculation (Pattison, Gordon, and Millson, 1959). This 100% take was entirely unexpected, because we had become resigned to being unable to produce the disease in more than about 25% of inoculated sheep. When this high susceptibility was recognized, goats were used for more and more experiments, both by ourselves and others, and a lot of information was obtained that would have been difficult to acquire with sheep because of the large numbers of animals that would have had to be involved.

Looking back to the period 1953 to 1960, at Compton and elsewhere, I recall it as a time of steady but unspectacular progress. Some might call it pedestrian. But the fact remains that many of the original observations out of which the facts of scrapie have emerged were made during that time. The disease was passed from sheep to goats and back to sheep again, and the high take in goats was confirmed; the disease was shown to be non-febrile throughout its course; the presence of the transmissible agent in various body tissues was followed from the time of inoculation onwards, and its very wide distribution in cellular tissues in the advanced clinical disease was recognized; transmission of the disease was achieved by many routes of inoculation, and by oral dosing; the remarkable resistance of the agent to many adverse physical and chemical treatments was confirmed, including heat, formalin, repeated freezing and thawing, and DNase and RNase; also confirmed was an earlier observation, reported by Gordon (1957), that the agent was active in the brain to a dilution of at least  $10^{-6}$ , and a clear indication was obtained of a relationship between length of incubation period and amount of agent in an inoculum. The pathology of the disease in the sheep and goat was studied by several people, myself included, but it turned out that the pathology of goat scrapie was very similar to that of sheep scrapie, and all we did really was to dot a few *is* and cross a few *ts*, because the detailed observations on the pathology of sheep scrapie by Besnoit and Morel (1898), Stockman (1926), Bertrand, Carré, and Lucam (1937), and Brownlee (1940) left little new to be discovered. During this period, also, extensive but unsuccessful attempts were made (especially at Moredun Institute) to grow the transmissible agent in tissue culture, and to find antibodies in the natural and experimental disease. Also, the first attempts

were made to detect the agent by electron microscopy, and a great deal of work was done on the epidemiology of the disease. Two distinct types of clinical syndrome, called at that time 'nervous' and 'scratching', were noted in scrapie-affected goats (Pattison and Millson, 1961), and these subsequently played an important role in the transfer of scrapie to mice by Chandler (1961).

The late W. S. Gordon, Director at Compton for 25 years, was an investigator in the grand manner, only marginally interested in experiments with fewer than 50 animals and rejoicing to work with hundreds. But even he excelled himself with what has come to be known as the '24-breed experiment', an audacious plan that established once and for all the importance of genetic make-up in susceptibility to experimental scrapie. Gordon assembled at Compton between 30 and 57 sheep of each of 24 different breeds (1.027 in all), and on a balmy day in July 1957 all were inoculated with a suspension of scrapie sheep brain. I have cause to remember that day, because over a period of 12 hours I injected 250 animals intracerebrally; the remainder were inoculated subcutaneously. The sheep were held under observation for two years, and the occurrence of scrapie was noted. The results of this experiment (Gordon, 1966) are given in the Table. The differences in incidence of scrapie between breeds represented differences in genetic make-up, and detailed analysis of the results

showed that within breeds there were resistant and susceptible families.

I still feel an urge to genuflect as I pass the spot at our Institute, beside the boiler house, where my colleague R. L. Chandler paused one day in 1960 to suggest to me that he might inoculate three strains of mice with brain material from the two clinical types of goat scrapie that G. C. Millson and I had called 'nervous' (now usually called 'sleepy' or 'drowsy') and 'scratching'. Chandler had found a difference in susceptibility between C57, CBA, and Swiss mice to the slowly progressive disease caused by *Mycobacterium johnei*; might these strains show different susceptibilities to scrapie? So he injected C57, CBA, and Swiss mice intracerebrally with 'drowsy' goat scrapie brain, or with 'scratching' goat brain. Seven months later Swiss mice inoculated with 'drowsy' brain developed scrapie (Chandler, 1961). Some weeks later C57 and CBA mice inoculated with 'drowsy' brain also developed scrapie; mouse-to-mouse passage was achieved with 100% susceptibility and an incubation period of about four months (Chandler, 1962). Thus occurred the greatest single advance in scrapie research since experimental transmission of the disease to sheep by Cuillé and Chelle in 1936. It had now become possible to carry out experiments involving large numbers of animals, particularly titration experiments that had been virtually impossible with sheep or goats. Above all

<i>Breed of Sheep</i>	<i>Number<sup>1</sup> Inoculated</i>	<i>Scrapie<sup>2</sup></i>	<i>%</i>	<i>Breed of Sheep</i>	<i>Number Inoculated</i>	<i>Scrapie<sup>2</sup></i>	<i>%</i>
Herdwick	36	28	78	Dorset Horn	45	6	13
Dalesbred	43	31	72	Suffolk	51	6	12
Swaledale	46	25	54	Leicester	42	5	12
S.S.	45	16	36	Welsh Mountain	42	4	10
Cheviot				Hampshire Down	30	3	10
Derby Gritstone	46	16	35	N.S.	45	4	9
Exmoor Horn	41	14	34	Cheviot			
Border	42	11	26	Southdown	38	3	8
Leicester							
Scottish Blackface	44	8	18	Wiltshire Horn	57	4	7
South Devon	35	6	17	Shropshire	41	2	5
Romney Marsh	43	7	16	Kerry Hill	41	1	2
Welsh Cheviot	40	6	15	Clun Forest	52	1	2
Ryeland	34	5	15	Dorset Down	48	0	0

Table *Comparative susceptibility to scrapie of 24 different breeds of sheep*<sup>1</sup>

<sup>1</sup>Inoculum 10% suspension of scrapie brain tissue in saline. Period of test: July 1957 to July 1959.

<sup>2</sup>Incubation periods ranged from three and a half to 23 months.

it was now possible for people with only limited animal accommodation to become involved in scrapie research.

Over almost the same period of time as that covering transfer of scrapie from goat to mouse, other highly significant events occurred that brought scrapie into the medical field, and again Compton was privileged to be intimately involved. Early in 1958 the Agricultural Research Service of the United States Department of Agriculture assigned a veterinary pathologist, W. J. Hadlow, to Compton to create close liaison on scrapie research between the two countries. In September 1959 a letter written by Hadlow, entitled 'Scrapie and Kuru', was published in *The Lancet*. In this letter Hadlow drew attention to close epizootiological, aetiological, clinical, and pathological similarities between scrapie and kuru. He concluded by saying, 'Thus it might be profitable, in view of veterinary experience with scrapie, to examine the possibility of the experimental induction of kuru in a laboratory primate, for one might surmise that the pathogenetic mechanisms involved in scrapie—however unusual they may be—are unlikely to be unique in the province of animal pathology.' What a prophetic statement! Seldom can medical research workers have received so valuable a directive from a veterinary colleague! D. C. Gajdusek, already deeply interested in kuru, visited Compton in June 1961 to learn about scrapie. We gave him a 'drowsy' goat brain, and in due course he confirmed Chandler's finding by successfully producing scrapie in mice with this brain (Morris and Gajdusek, 1963). With great diligence, he and his colleagues at the National Institutes of Health then followed Hadlow's suggestion that an attempt should be made to induce kuru in a laboratory primate. After a long incubation period, success was achieved with the chimpanzee (Gajdusek, Gibbs, and Alpers, 1966).

Scrapie research in 1961 and 1962 was largely devoted to establishing that Chandler's disease in mice was indeed scrapie. This was done by passing it back to sheep and goats, and onwards through mice, and by studying the clinical and pathological features of the mouse disease in detail. By 1963 the mouse was ready to play its full part. So began the modern era of scrapie research, and I daresay no one at that time believed that the problem of scrapie would be still unsolved in February 1972.

Helpful though the mouse has been, however, its use in assaying scrapie activity still leaves much to be desired, and there have been many attempts to find an alternative method, especially by tissue culture techniques. Gustafson and Kanitz (1965), Field and Windsor (1965), and Haig and Pattison (1967) observed abnormal properties in cells from some

tissues of scrapie-affected animals, and these studies stimulated further work on culture *in vitro* of scrapie tissues. Within the past two years my colleagues at Compton, D. A. Haig and M. C. Clarke, have established a cell line from scrapie mouse brain in which the agent apparently multiplies in synchrony with, and as an integral part of, the cells; apparently there is only one active site in each affected cell. Very recently Haig and Clarke have suggested that the 'target cells' in scrapie may be of reticuloendothelial origin (Haig and Clarke, 1971). These studies have opened up new lines of research on scrapie that bid fair to become of increasing importance in the future.

As was said at the beginning of this paper, published work on scrapie has been extensively summarized. This applies particularly to the modern era. What may not be immediately obvious to the casual reader, however, is why there should be such a wide divergence of opinion on the nature of scrapie itself and of the transmissible agent. After all, the same experimental evidence is available to everyone; why should its interpretation be so widely different? In particular, why should some authors refer to the transmissible factor as a 'virus', and others studiously avoid the word—and substitute 'agent'? Authors who support a more or less conventional virus aetiology have been influenced by the necessity to explain apparent multiplication of the agent in terms of nucleic acid. Those who have abandoned a virus theory believe that the physicochemical properties of the agent are inconsistent with the presence of nucleic acid.

The first published reference to the transmissible agent as something other than a virus was by Parry (1962), who called it a provirus, defined as something that was formed inside an affected animal but had no independent outside existence. The next published suggestion that the scrapie agent might be odd was my own (Pattison, 1965) when I said that '... if the transmissible agent of scrapie is a living virus, it is a virus of a kind as yet unrecognized.' This conclusion was based on a long series of unsuccessful attempts to inactivate the scrapie agent by heroic methods, including treatment with 12% formalin for 28 months. But what is a 'virus'? If by 'virus' is meant something that will negotiate an antibacterial filter and can be passed indefinitely through animals, apparently increasing in quantity as it goes, then the scrapie agent is a virus. If, however, a virus has to contain nucleic acid, then I believe that D. A. Haig and M. C. Clarke at Compton and their collaborator Tikvah Alper at the Hammersmith Hospital have shown beyond reasonable doubt by their studies with ultraviolet irradiation that the scrapie agent does not contain nucleic acid and is not a virus (Alper, Haig, and Clarke, 1966; Alper, Cramp, Haig, and Clarke,

1967; Haig, Clarke, Blum, and Alper, 1969; Latarjet, Muel, Haig, Clarke, and Alper, 1970).

Scrapie is one of four closely similar diseases, the others being kuru, Jakob-Creutzfeldt disease, and transmissible mink encephalopathy, all of which will be discussed at this Symposium. All are difficult to handle experimentally, because their undefined transmissible agents can be detected only by animal inoculation after an incubation period of many months. The common link between them is the clinicopathological one of a slowly progressive, fatal, spongiform encephalopathy. Research on scrapie was responsible for recognition of this group of diseases, to which others may be added in due course, and knowledge of the vagaries of scrapie has been of great value in planning research on them all, for in planning a complicated journey it is reassuring to know that similar ground has already been covered.

#### References

- Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C. (1967). Does the agent of scrapie replicate without nucleic acid? *Nature (Lond.)*, **214**, 764-766.
- Alper, T., Haig, D. A., and Clarke, M. C. (1966). The exceptionally small size of the scrapie agent. *Biochem. biophys. Res. Commun.*, **22**, 278-284.
- Bertrand, I., Carré, H., and Lucam, F. (1937). La 'tremblante' du mouton. *Ann. Anat. path. méd.-chir.*, **14**, 565-586.
- Besnoit, C., and Morel, C. (1898). Note sur les lésions nerveuses de la tremblante du mouton. *Rev. vét. Toulouse*, **23**, 397-400.
- Brownlee, A. (1940). Histopathological studies of scrapie, an obscure disease of sheep. *Vet. J.*, **96**, 254-264.
- Chandler, R. L. (1961). Encephalopathy in mice produced by inoculation with scrapie brain material. *Lancet*, **1**, 1378-1379.
- Chandler, R. L. (1962). Encephalopathy in mice. (Letter). *Lancet*, **1**, 107-108.
- Cuillé, J., and Chelle, P. L. (1936). La maladie dite tremblante du mouton est-elle inoculable? *C.R. Acad. Sci. (Paris)*, **203**, 1552-1554.
- Cuillé, J., and Chelle, P. L. (1938). La tremblante du mouton est-elle déterminée par un virus filterable? *C.R. Acad. Sci. (Paris)*, **206**, 1687-1688.
- Cuillé, J., and Chelle, P. L. (1939). Transmission expérimentale de la tremblante à la chèvre. *C.R. Acad. Sci. (Paris)*, **208**, 1058-1060.
- Field, E. J. (1969). Slow virus infections of the nervous system. *Int. Rev. exp. Path.*, **8**, 129-239.
- Field, E. J., and Windsor, G. D. (1965). Cultural characters of scrapie mouse brain. *Res. vet. Sci.*, **6**, 130-132.
- Gajdusek, D. C., Gibbs, C. J., Jr., and Alpers, M. (1966). Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature (Lond.)*, **209**, 794-796.
- Gordon, W. S. (1957). Discussion to Palmer, A. C. Studies in scrapie. *Vet. Rec.*, **69**, 1324-1327.
- Gordon, W. S. (1966). Review of work on scrapie at Compton, England, 1952-1964. In *Report of Scrapie Seminar, Washington D.C.*, ARS 91-53, pp. 19-36.
- Gustafson, D. P., and Kanitz, C. L. (1965). Evidence of the presence of scrapie in cell cultures of brain. In *Slow, Latent and Temperate Virus Infections* (NINDB Monograph No. 2), pp. 221-236, edited by D. C. Gajdusek, C. J. Gibbs, Jr., and M. Alpers, National Institute of Health, Washington D.C.
- Hadlow, W. J. (1959). Scrapie and kuru. *Lancet*, **2**, 289-290.
- Haig, D. A., and Clarke, M. C. (1971). Multiplication of the scrapie agent. *Nature (Lond.)*, **234**, 106-107.
- Haig, D. A., Clarke, M. C., Blum, E., and Alper, T. (1969). Further studies on the inactivation of the scrapie agent by ultraviolet light. *J. gen. Virol.*, **5**, 455-457.
- Haig, D. A., and Pattison, I. H. (1967). *In-vitro* growth of pieces of brain from scrapie-affected mice. *J. Path. Bact.*, **93**, 724-727.
- Latarjet, R., Muel, B., Haig, D. A., Clarke, M. C., and Alper, T. (1970). Inactivation of the scrapie agent by near monochromatic ultraviolet light. *Nature (Lond.)*, **227**, 1341-1343.
- Morris, J. A., and Gajdusek, D. C. (1963). Encephalopathy in mice following inoculation of scrapie sheep brain. *Nature (Lond.)*, **197**, 1084-1086.
- Parry, H. B. (1962). Scrapie: a transmissible and hereditary disease of sheep. *Heredity*, **17**, 75-105.
- Pattison, I. H. (1963). Resistance of the scrapie agent to formalin. *J. comp. Path.*, **75**, 159-164.
- Pattison, I. H., Gordon, W. S., and Millson, G. C. (1959). Experimental production of scrapie in goats. *J. comp. Path.*, **69**, 300-312.
- Pattison, I. H., and Millson, G. C. (1961). Scrapie produced experimentally in goats with special reference to the clinical syndrome. *J. comp. Path.*, **71**, 101-108.
- Roche-Lubin (1848). Mémoire pratique sur la maladie des bêtes à laine connue sous les noms de prurigo lombaire, maladie convulsive, trembleuse, tremblante, etc. *Rec. Méd. vét. Sér 3*, **5**, 698-714.
- Stamp, J. T. (1960). Scrapie and scrapie research at Animal Diseases Research Association, Moredun Institute, Edinburgh. In U.S. Department of Agriculture *Special Meetings on Scrapie*, ARS 91-22, pp. 13-22.
- Stamp, J. T., Brotherston, J. G., Zlotnik, I., Mackay, J. M. K., and Smith, W. (1959). Further studies on scrapie. *J. comp. Path.*, **69**, 268-280.
- Stockman, S. (1926). Contribution to the study of the disease known as scrapie. *J. comp. Path.*, **39**, 42-71.
- Thormar, H. (1971). Slow infections of the central nervous system. *Z. Neurol.*, **199**, 1-23 and 151-166.
- Wilson, D. R. (1952). Scrapie. *Vet. Rec.*, **64**, 468.
- Wilson, D. R., Anderson, R. D., and Smith, W. (1950). Studies in scrapie. *J. comp. Path.*, **60**, 267-282.