

Transmissible spongiform encephalopathies: The threat of BSE to man

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Transmissible spongiform encephalopathies in mammals (TSEs) are due to ultrafiltrable agents of prodigious resistance to physical agents. The exact nature of the infectious agent is not known, possibly being a virus-like (virino) structure or a proteinaceous agent (prion). Some of the proteins found in tissues from infected animals may result from the host response to the infectious agents. The resultant diseases are slowly developing dementias and paralyses, although the infective agent is found in many tissues. The route of acquisition is thought to be mainly through ingestion, with some evidence of vertical transmission. The host range of each agent is modified unpredictably by passage in a new species. The infective agent responsible for Bovine Spongiform Encephalopathy (BSE) was acquired through incorporation of animal products into cattle feed. The source of this product has not been identified, nor has its host range. There is a distinct possibility that man could acquire spongiform encephalopathy from consumption of contaminated beef.

Introduction

Transmissible spongiform encephalopathy (TSE) is an accurate description of a group of inevitably fatal diseases in several mammals. Those said to be naturally affected include sheep, man, cattle, mink, deer and cats. It is debatable whether the mode of acquisition of bovine spongiform encephalopathy (BSE) by cattle, kuru by man, and spongiform encephalopathy (SE) by cats is in reality 'natural'. The disease does seem to be confined to mammals, and both the clinical features and the understanding of the nature of the presumed infective agents justifies the placing of these into one group. The clinical features result from slowly developing alterations and destruction of neurons. Although these diseases are primarily seen as brain diseases, the infective agents have been found in many tissues (*vide infra*).

The timescale of the disease in each species is characteristic: there is a long incubation period, usually of some years, followed by a progressive sub-acute illness over many weeks or months.

In most cases, three cellular changes are evident in brain material from infected animals. These are a degeneration of neurones, the cells actually responsible for brain function, a hypertrophy or enlargement of the supporting astrocytes, and a diffuse sponge-like appearance sometimes referred to as status spongiosus. In some cases the diagnosis is not clear cut and there are problems in distinguishing TSE from other diseases (Zlotnik and Rennie 1965, Hadlow et al. 1982, Collinge et al. 1990). The clinical pictures of the disease can also be variable. These two factors contribute to some of the uncertainties over the actual incidence of TSEs.

Scrapie in sheep has been the TSE most studied in animals. It was first described with confidence by Leopold in 1759 in Germany. This disease is found in many countries, including Britain and France and the incidence is said to be high in Iceland, and exceedingly low in New Zealand. Caution must be made in accepting some estimates of the incidence of scrapie because of the shortcomings in procedures for recording cases. Sigurdsson (1954) made the important observation that after eradication of the whole sheep population in the area where the disease is endemic in Iceland, scrapie appeared a few years later in the new flocks which were imported from a region where scrapie had never existed, with the same annual loss as before eventually. This does raise the possibility that the new flocks were infected from residual agents on the ground which has some disturbing implications for the long term elimination of BSE from cattle.

Scrapie generally presents as one of two clinical types, although the features can overlap. In the scratching form, there can be frenzied rubbing of the back legs by the head or scraping the body against walls, posts or trees. The animal is often weak, tending to fall easily. It shows enhanced fear to stimuli. In the other type, the infected sheep shows stubborn reactions to stimuli, jerky movements of limbs or ears or stretching back of the head. The muscles may visibly tremble (fasciculation) and the gait may resemble that of a donkey ('cuddly trot'). Sometimes an extreme thirst sets in. By the time that the symptoms are advanced, the animal is destroyed. Scrapie appears to have been prevalent in the UK since the eighteenth century (Critchley et al. 1972).

As will be discussed below, the remarkable observation is that BSE did not appear in cattle on a major scale until the last few years despite sheep and cattle

sharing the same pastures for centuries. Any hypothesis on the aetiology of BSE must account for this. One of the human TSEs, Creutzfeldt-Jakob disease (CJD), was described independently by these two authors (Creutzfeldt 1921, Jakob 1921). It was first known as 'spastic pseudosclerosis' or 'subacute spongiform encephalopathy' and various other workers' names have been associated with disease. The illness occurs mainly sporadically, although a familial incidence is described in about 15% of cases. Most cases occur in late middle age (50-60 years) in both sexes. Some younger people have been afflicted, although the confidence with which the diagnosis is made varies. There is an understandable reluctance for some pathologists to perform post-mortem examinations on patients thought to have succumbed from CJD (Critchley et al. 1972, Report 1981).

The first symptoms are pains and trembling in muscles, and loss of coordination (particularly in walking) may occur. Uncontrolled twitching of the eyes (nystagmus) and tremors are common. Double vision, difficulty in speaking, and muscles spasticity are often seen. Mental changes include depression, loss of memory and confusion. Blindness and epilepsy are frequent. Towards the end of the illness, the patient is confined to bed, is incontinent, helpless, and requires constant nursing. Death usually occurs between 3 and 9 months after the onset of the illness. However, this can vary from a few weeks to 5 years.

Another human TSE, kuru, occurred in the Fore tribe, a stone-age civilisation in the remote highlands of New Guinea. The disease kuru was prevalent during the first half of this century. Most authorities have attributed cannibalism, notably eating brain, as the cause of the disease, although it is possible that the infective agent can enter the body through cuts of the skin while hand

s for centuries. Etiology of BSE is the same as that of the human disease (CJD), typically by these two (L, Jakob 1921). The disease is a chronic progressive spongiform encephalopathy, named after the workers' names disease. The illness is typically, although not always, described in cases occur in years) in both people have been confidence with made varies. The reluctance to perform post-mortem autopsies thought CJD (Critchley 1972). Claims and loss of coordination (walking) may occur. Drooping of the eyes is common. In some cases, speaking, and depression are seen. Mental changes, loss of memory, and epilepsy are the end of the disease, and is confined to the brain, and requires surgery usually occurs after the onset of the disease, this can vary from weeks to months.

The disease, occurred in the early stages of civilisation in New Guinea. It is a zoonotic disease, prevalent during the 19th century. Most cases were attributed to cannibalism, as the cause of the disease, it is possible that people entered the disease through the hand-

ling brains in funeral rituals. As with CJD, the clinical picture of the disease involves progressive damage to musculoskeletal functions and those of intellect.

The disease is now rarer, as a result of education. Before the 1950s, women, who were mostly involved with cannibalism, predominantly suffered from the disease. The age at which the illness typically developed was often between 20 and 40 (e.g. Critchley et al. 1972).

The relationship between the potential for infectivity of the host through oral ingestion with findings from experimentally induced infection through intracerebral inoculation requires clarification. Thus, if an agent generates an infection through intracerebral inoculation, it may not do so necessarily orally or, if it does, the incubation period may be longer. Similarly, the failure to establish an infection intracerebrally in a few animals of a species cannot be taken to assume that every member would resist oral challenge.

BSE: Clinical and statistical aspects

Because of their generally greater longevity, most cases of BSE have been reported in cows rather than cattle. A good description of the clinical disease comes from a farm that has had a large number of cases (Winter et al. 1989). Initially, the cow appears mentally alert, but unusually anxious and apprehensive. It takes a wide base stance, and the abdomen is drawn up. The gait becomes abnormal and exaggerated, and the animal splay its hind limbs when turning sharply, especially on wet surfaces, which gives rise to tumbling and skin wounds. Appetite is the same as unaffected cows but faeces are firmer. However, the animal loses weight and produces less milk. Fine muscle fasciculations involving small muscle groups over the surface of the neck and body are seen and occasionally myoclonic (i.e. repetitive and vigorous

jerks) occur (Hope et al. 1989, Scott et al. 1989). The tone of the 'moo' can change and apparently aimless head butting is seen with other anxious and frenzied movements.

Changes are seen in the electroencephalogram pattern of the cow's brain activity and also in the CSF chemistry (Scott et al. 1990), but no antibodies specific to the disease are produced and no specific tests are available while the animal is alive, except of course unpracticable brain biopsy.

Claims have been made that this is not a new disease, it having been seen in approximately one cow in 20 000-30 000 in the past (Eddy 1990b). Similar clinical symptoms can be seen with other conditions (e.g. magnesium deficiency), but these respond to treatment and are generally not fatal. The discussion as to whether it is a new disease has continued (Marr 1990) and it has been claimed that the condition previously had a different name (P. Hayes, pers. commun.), being called 'stoddy' in Yorkshire.

BSE was first comprehensively documented in the UK in 1986, and the British Isles remain the only certain source, although two calves were exported to Oman that subsequently developed the illness (Carolan et al. 1990). The disease infects Holstein Friesian breed milking cows and other breeds. There is little reason to believe that any one breed is particularly susceptible although, as with many infections, a genetic predisposition may occur among individual animals (Wijeratne and Curnour, 1990).

Epidemiology suggests (Editorial 1988, Southwood 1989) that BSE initially infected animals from 1981/2 and that the majority of animals became infected in calfhood (Wilesmith et al. 1988) through their food (Morgan 1988), giving an incubation period of approxi-

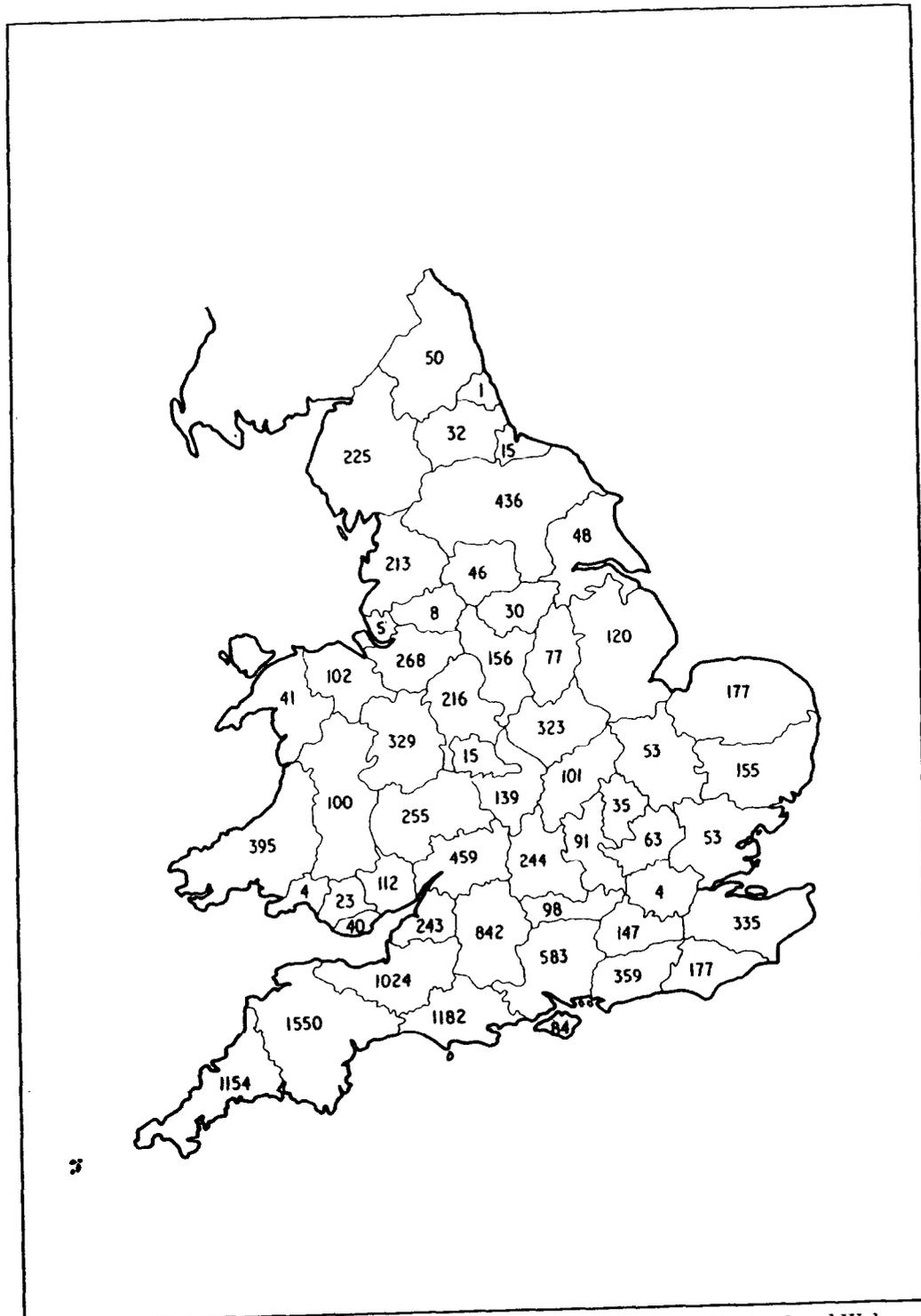


Fig. 1. The number of confirmed cases of BSE in different counties in England and Wales up to 16 April 1990.

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Table 1. The age of cows (when available) at time of slaughter due to BSE in England and Wales in 1989 (Hansard 1990g).

Age (years)	Number of cases
1-2	1 ^a
2-3	28
3-4	586
4-5	2138
5-6	1874
6-7	667
7-8	125
8-9	37
9-10	8
10-11	3
11-12	1
12-13	1
15-16	1

^a22 months

mately 3-6 years (Table 1). The continuation of this mode of infection of cows should have stopped following the prevention of rendered mammalian tissue being incorporated in cattle food in 1988. Some veterinarians are fearful that the disease will be passed from the cow to the calf and hence may not be eradicated (Tyrrell 1989, Aldhouse 1990, Grant 1990), whereas the British Parliamentary Agricultural Committee does not share this worry (Report 1990). It is also possible theoretically that the capacity of the infectious agent to survive rigorous environmental extremes (*vide infra*) will enable it to persist on grassland and infect new generations of cattle.

Relatively few cases have been reported from Scotland, Northern Ireland or the Republic of Ireland. The distribution in England and Wales is shown in Fig. 1 (Hansard 1990e). Apparently no cases have been reported from the Isle of Man. On 15 February 1990 the largest number of cases of BSE on a farm was 29, the average number in an infected herd was 1.75, the proportion of herds with a single case was 63%, the proportion of all dairy herds that have had at least one case was 10%, and 0.7%

of all beef herds have had one or more cases (Hansard 1990b). The concentration of cases in the South of England means that these figures can only be a guide. For instance, the annual incidence of BSE in the total population of adult cattle in the UK is 0.2% but a veterinarian in the South of England may ultimately see 10% of the dairy cows on large local farms with the disease (M. Winter, pers. commun.).

It should not be forgotten that bulls with BSE have been reported (Hansard 1990c), that Scotland may have low numbers but is also affected (Hansard 1990h), and that the mounting compensation costs to the Government are not small [more than 6 million pounds to February 1990 (Hansard 1990f)].

Diagnostic methods for BSE are developing rapidly through PrP detection techniques (*vide infra*) (Hope et al. 1988, Farquhar et al. 1989) but currently histological examination of brain tissue is used for this purpose. Currently we do not know the percentage of cattle that are being slaughtered that are infected with BSE (Hansard 1990a) and research into this has not been undertaken (Tyrrell 1989, Southwood 1989). We do not know the time at which the animal becomes infective during the incubation of the disease and we do not know how it is passed, or to which animals it will cause disease.

History of BSE

The numbers of cows reported to the British Government with BSE is shown in Table 2 (Hansard 1990e, g).

BSE was first reported by the technical staff at the Central Veterinary Laboratory, Weybridge, Surrey, in November 1986 (Tyrrell 1989). The small initial numbers of reported cases quickly increased as more farmers realised the relevance of neurological symptoms in cows on their farm. It is probable that

Table 2. Cumulative number of cases of BSE reported per year in England and Wales.

Year	Number of cases
1986	7
1987	413
1988	2247
1989	6420
1990	17 434 ^a

^a Estimated from figures to April 1990 (Hansard 1990e, g) However, by the end of June 1990, nearly 20 000 cases had been reported, suggesting that the total by the end of 1990 will be around 25 000.

an apparent BSE epidemic arose because large numbers of cows were being fed a protein supplement which contained the brain tissue or other offals of animals (possibly other cows or sheep) that were suffering from either scrapie or from another TSE (Tyrrell 1989, Report 1990). The British Government banned the use of ruminant tissue in food supplements to cows in July 1988 (Report 1990) but the same animal food could still be exported to Europe despite widespread demands that this should not happen (Hansard 1989). This may have led to large numbers of cattle in Europe being infected (Horizon 1990), although it is likely that few will have already shown signs of disease. Also in July 1988, the British Government introduced legislation demanding compulsory reporting of animals that are considered to have BSE, compulsory slaughter and compensation to the farmer at 50% of the market value and disposal of their meat and milk (Report 1990). The rapid rise in numbers of affected cows, particularly in the South of England (Southwood 1989, Hansard 1990g), gave rise to increasing interest from the press and, when the report of the Working Party of Bovine Spongiform Encephalopathy (the 'Southwood'

Report) appeared in January 1989, the public interest in the condition increased dramatically.

The relative paucity of infected animals in Scotland has not been explained by published information. Presumably, the degree of contamination of the feed by disease-inducing material varies with product. Details of this are presumably available to MAFF, but not to the authors of this article! One possible factor may have been the need to use protein supplements where the availability of grass and silage was least. The climate in Southern England could therefore have been a decisive factor.

The Southwood Report recommended that a second committee should look into the research that was needed to investigate BSE: The Consultative Committee on Research into Spongiform Encephalopathies (the 'Tyrrell' Committee). This committee, soon after its initiation, asked the British Government to ban all bovine tissues that had been shown in other species to be infective for SE (the gut, lymphoid and nervous tissues) from human consumption. This was announced in June 1989 but not enacted until November 1989 and this gap allowed the potentially infected nervous, gut and lymphoid tissue of approximately 2 million cattle to reach human food. The report of this committee was submitted to the government in June 1989 but not published until January 1990 (Tyrrell 1989).

By June 1989, 150 cows a week were being reported as having BSE. When the amount of compensation was raised to the full sale price of the cow in February 1990, the numbers of cows reported with BSE initially rose by a further 73% (Hansard 1990i). Hence, it is likely that prior to this many of the cows with BSE were not being notified.

The report of an SE in a five-year-old male Siamese cat in May 1990 (Wyatt et

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of infected animals has not been explained. Presumably, the transmission of the feed material varies with the source, but are presumably not due to the One possible factor is the need to use products where the availability is at least. The clinical course could therefore be different.

The committee recommended that the government should look into the need for a consultative Committee on Spongiform Encephalopathy. The 'Tyrrell' Commission was set up in 1989 after its report on the Government's policy on diseases that had caused concern, such as scrapie and BSE, and their transmission to humans.

In June 1989 but in November 1989 and in December 1989 and in January 1990, the committee reported that the incidence of BSE had increased and that the transmission of BSE to humans had been confirmed.

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al. 1990) and the further reports of SE in zoo animals (Gibson 1990, Hansard 1990d) brought into doubt the main credence in the 'Southwood' report that BSE was not a threat to man. It had been hoped that the cow would be a 'dead end' host for BSE as there was no evidence that it would be transmitted to other hosts. However, there appeared to be few resources other than BSE that could have infected the cat and reports of further cases have followed this. The possibility that Southwood was not correct and that the beef was infective was taken up by the media and the demand for beef slumped.

The demands by the French and German governments (although many other European Governments were also involved) in May 1990 that British beef should not be imported to their countries led to the agreement that only beef from farms that had no history of BSE (specifically, no clinical case within the previous 2 years) would be exported to them. At the current time the auction price of a cow for slaughter from a BSE free herd is 142% that from a herd with BSE. That the value of a cow from a herd with a history of BSE is much lower than one from a BSE free herd means it is very much against the farmer's interest to report a new case.

The media activity, the loss of profits of farmers and the difficulties within the European Community led the House of Commons Agriculture Committee to produce a report on BSE in July 1990. The report declared that British beef was safe to eat, but questioned the activity in abattoirs of splitting bovine heads to remove the brain, the breeding of cattle that may be infected with BSE, and the inclusion of potentially infected meat in pet food. The report also asked that calves also be included in the ban on brain and lymphoid tissue being taken for human consumption, and it

asked that mechanically recovered meat and the use of saws in the cutting of animal carcasses that may be infected be stopped (Report 1990). In the evidence of this committee, Tyrrell stated that he expected that one in a thousand cows that came to slaughter for human consumption should be considered infected (Report 1990). The tight measures to control BSE are expected to revive the demand for British beef. However, further announcements in the veterinary press concerning the infectivity of the meat of scrapie infected goats (Pattison 1990), the possibility of infection being passed accidentally between cows by needle injection or by surgical procedure (Lees 1990), may have blunted this hope.

By May 1990 the number of reported cases had reached around 300-400 per week; higher than the ability of the Ministry of Agriculture Fisheries and Food (MAFF) to dispose of the carcasses by incineration. Animals have been reported having been dumped into open waste sites for disposal (Keighley News 1990). This practice is apparently within current legislation.

British farmers now declare that beef farming is not profitable at current prices (which have dropped by approximately 20%), but despite this land prices have stayed steady and farmers are moving into other produce. The increased cost of cows and their offspring being registered in order to prevent the calves of infected cows being used for mating may be born by the Government but beef farming is currently uneconomic despite this.

The suggestion that cases of BSE have appeared in France and in the United States (Horizon 1990) have not been confirmed in the scientific literature. However, the continuing practise of feeding animal protein to cows in European countries may represent a threat. The

export from Britain of calves which may be infected with BSE raises the possibility of similar epidemics in other countries.

Epidemiology of TSE in other animals

It has been established that scrapie can be transmitted between sheep within the same flock, that lambs of an infected ewe were more likely to become ill than those of an uninfected one, and that sheep that had grazed on land that had previously supported sheep with scrapie also might acquire the disease. Some sheep seemed to develop scrapie spontaneously, with no contact with infected animals.

The disease can be transferred by injection of brain material from an infected to an uninfected animal. Subsequently, much of the experimental work has been performed in laboratory animals, such as hamsters and mice on account of their short incubation periods (often less than 1 year). However, in passing the scrapie agent to different species, the incubation period can increase, the histopathology changes, and the infected animal shows atypical clinical signs (Pattison 1988). This has been termed the 'species barrier'. Moreover, once the infection was passed into a different species, there was also a 'species barrier' in attempts to pass the disease back to the original donor species. When passed onto another animal of the same species, however, the incubation period decreased, and the histopathology became the same as with further generation infections, as did the clinical signs. These findings suggest that passage through a new mammalian species changes the properties of the infective agent.

Such transfer of TSE has been achieved by intracerebral injection (Kimberlin and Walker 1979), percutaneously (Eklund et al. 1967, Kimberlin and Walker 1979, Matthews 1981), intraperitoneally (Kimberlin et al. 1971), intraocularly

(Kimberlin and Walker 1986), intragastrically (Kimberlin and Walker 1989), intranervously (Field and Hill 1974, Fraser 1982) and through injections of infected tissue. Unfortunately this happened by accident due to the contamination of louping ill vaccine with scrapie (Brotherston et al. 1968) and concern has been proposed about the possible contamination of human growth hormone derived from human pituitary (Report 1990).

One animal ingesting infected material derived from another has been considered to be the cause of infection when different animals are kept in the same cage and the disease is passed between them, e.g. mice (Pattison 1964) and mink (Hadlow et al. 1987). This was thought to be due to bites or scratches of one animal by another as it might occur between two animals in adjacent cages (this work has yet to be confirmed).

Disease transfer has been presumed to be oral in the natural state in sheep (Pattison and Millson 1961, Pattison et al. 1972) and the newborn lamb has not been shown to be infected, but the placenta is (Hadlow et al. 1982). Scrapie can be produced in a sheep by feeding the foetal membranes of an infected animal (Pattison et al. 1974) and this is a favoured method by which scrapie is thought to pass to offspring. The placenta is often eaten by the ewe. The contamination of the pasture and the resilience of the infective agent have meant that this has been thought to be the method of lateral transfer between sheep (Pattison 1964, Morgan 1988) although this has been disputed (Wilson et al. 1950). It has also been suggested that contact is all that is required to transfer scrapie between animals (Brotherston et al. 1968).

Oral transmission of TSEs has been achieved with scrapie in sheep (Pattison et al. 1972), mice (Carp 1982, Kimberlin

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and Walker 1989), goats (Pattison and
Millson 1962), hamsters (Prusiner et al.
1985) and mink (Hanson et al. 1971).
Transmissible mink encephalopathy has
been transmitted orally to mink (Bur-
gher and Hartsough 1965), CJD to squir-
rel monkeys (Matthews 1981) and BSE
to mice. The full scope of the potential for
the oral route of transfer of TSEs be-
tween mammals is not yet established.
The scarification of the gums may change
the likelihood of passing the TSE by
mouth (Carp 1982), but it is clear that it
can pass orally between species without
this.

Effect of host passage on the prop- erties of the infectious agent

Attempts to produce prion infections
(*vide infra*) in animals have not always
been successful. Some animals appear
to be more vulnerable than others and
some laboratory strains seem to possess
variations in susceptibility so that only a
proportion of the animals when given
what was anticipated to be an infective
dose actually develop the disease.

Strain variation as shown by alteration
in general biological properties has been
seen in scrapie (*vide supra*). Propagation
of such agents on a new host could well
modify the agent's potential infectivity for
other hosts. This results from the possible
incorporation of host cellular material in
the surface of the particle. Transmissible
mink encephalopathy (TME) is thought
by some to have been derived from scrapie,
and yet its host range differs from that of
scrapie (Davanipour et al. 1986) (Table 3).

It follows from this that even if it is
assumed that scrapie did transfer to
cattle as BSE, and the scrapie agent in
sheep was not infectious to man, it is not
possible to exclude the possibility that
the BSE agent from cattle is infectious
to man.

Approximately 50% of the animal
species that have been inoculated or fed

with the agents responsible for scrapie,
CJD, TME and kuru have been shown
to become infected subsequently (See
Table 3) (Davanipour et al. 1986). This
has occurred through a variety of routes,
but that each of the 'natural' encephalo-
pathies has a different range of infection
to any other species suggests that we
should assume that BSE has a charac-
teristic and a large undefined host range
specificity. Thus, TME is thought to have
been derived from the feeding of scrapie
to mink (Burgher and Hartsough 1965,
Hartsough and Burgher 1965, Hanson
et al. 1971, Hadlow et al. 1987) but it
has a clearly different range of in-
fectivity. In particular, the agent from
sheep has no affinity for rhesus mon-
keys but that from mink has (Hanson et
al. 1971). The species barrier has been
shown much more clearly in rats by
Pattison and Jones (1968), where strain
types of 'scrapie' infecting the rats
changed after infecting them. The
recent reports of a feline spongiform
encephalopathy (FSE) (Wyatt et al.
1990) may indicate that BSE may have
infected cats (Report 1990). Scrapie
infected meat has been fed to cats for
many years and there have been no
reports of FSE. Similar reports of novel
SEs in zoo animals such as antelopes
(Gibson 1990) are highly relevant. The
alternative proposal that FSE and enceph-
alopathies in zoo animals are not truly
novel, but just newly recognised cannot
be ruled out, but seems most unlikely.
We must assume that the agent causing
BSE will have a different associated
proteinacious structure from that of the
agent causing scrapie (Endo et al. 1989,
Goldman et al. 1990); it will also have a
different range of infectivity in the same
way as the causative agent of TME. The
possibility that BSE was derived not
from scrapie but from another SE would
also be consistent with this. BSE may
well have been due to the feeding of offal

Table 3. Range of animals to which SE from various animals can be transmitted (see text; in particular Prusiner 1984, Davanipour et al. 1986, Hope et al. 1989, Dawson et al. 1990)

Host	CJD	Scrapie ^a	TME	Kuru	Cow
Human	+	NT	NT	NT	NT
Sheep	-	+	+	-	NT
Mink	-	+	+	-	+ ^b
Cow	NT	+	-	NT	+
Chimpanzee	+	-	-	+	NT
Gibbon	-	-	-	+	NT
New-world monkey					
Capuchin	+	-	NT	+	NT
Marmoset	+	NT	NT	+	NT
Spider					
Squirrel	+	+	+	+	NT
Woolly	+	NT	NT	+	NT
Old-world monkey					
Cynomolgus	-	+	NT	-	NT
Managabey	+	NT	NT	-	NT
Rhesus	-	-	+	+	NT
Pig-tailed	+	NT	NT	+	NT
Bonnet	NT	NT	NT	+	NT
African green	+	-	NT	-	NT
Baboon	+	NT	NT	NT	NT
Bush baby	+	NT	NT	-	NT
Patas	+	NT	NT	NT	NT
Stump-tailed	-	NT	+	-	NT
Talapoin	+	NT	NT	NT	NT
Goat	+	+	+	-	NT
Ferret (albino)	+?	NT	+	NT	NT
Cat	+	NT	-	-	+ ^c
Raccoon	NT	NT	+	NT	NT
Skunk	NT	NT	+	NT	NT
Mouse	+	+	-	-	+
Rat	-	+	NT	-	NT
Hamster (golden)	+	+	+	-	- ^d
Gerbil	+	-	NT	-	NT
Vole	NT	+	NT	NT	NT
Guinea-pig	+	-	+	-	NT
Rabbit	-	-	-	-	NT
Pig	NT	NT	NT	NT	+

^a Various primates inoculated with mouse adapted scrapie failed to develop the disease.

^b Presumed from outbreak of TME at Stetsonville, USA.

^c Presumed from epidemiology experimental work to follow.

^d Current findings at Central Veterinary Laboratory.

+ denotes transfer of infectious agent.

NT, not tested.

NB: relatively little work has been done on chronic wasting disease of deer, which can be transmitted to the ferret. The British zoological animals: kudu, oryx, nyala, gemsbok and eland have also acquired SE but the origin is unknown.

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derived from a cow which was suffering from a sporadic case of SE to other cows, and then the feeding of their tissues to further animals. At each passage this would multiply the infectivity of the feed.

If BSE follows the general potential of the TSEs to infect about 50% of other mammalian species, then those with responsibility for control of infection should assume that humans are included until this is proved not to be the case. The prion chemistry of sporadic cases of CJD are apparently the same and this may suggest that they are not sporadic mutants but true infections. This is considered in more detail below. There are few animals that we eat which live long enough to become infective with SE. The possibility that CJD represents human infections with the previous rare sporadic or subclinical cases of BSE has not been investigated but would suggest that human beings are at risk of catching BSE orally from infected bovine tissues. There appears to be little association between the incidence of scrapie in sheep and CJD (Southwood 1989). Pig products are generally consumed from animals less than 6 months old. The only other mammals commonly consumed at an age for such an infectious agent to be present in large amounts are cows and cattle.

Infective tissue from TSE infected animals

To find out which tissues from an animal suffering from SE are infective is very expensive and time consuming. Small amounts of tissue from the infected animal are injected intracerebrally into an animal strain that is known to be vulnerable to infection. This can be done at different points during the progress of infected animals' disease in order to find out at what point the tissue becomes infective, or may be done when the ani-

mal shows clinical signs of infection. Neither of these processes has been carried out systematically for BSE but they are planned (Tyrrell 1989). Hence, in the absence of any contrary data it should be assumed that BSE is similar to SE in other animals (see Tables 4 and 5).

These figures may give an impression of every type of tissue that has been tested being found to be infected. Indeed, the low sensitivity [often 10³ infective units (IU) per gram of tissue] of the test technique used by some researchers may mean that these are underestimates of the infectivity of the tissues. However, the relevance of these to the possibility of human infection via the oral route must be cautioned. Many researchers have not been able to reproduce other work in showing the infectivity of some tissues (Eklund et al. 1967, Pattison et al. 1972, Hadlow et al. 1974, Casaccia et al. 1989). Thus meat, blood, milk, faeces and urine are considered either not infective at all or to a relatively low degree (Eklund et al. 1967, Pattison et al. 1972, Hadlow et al. 1974). However, in the abattoir it is difficult to stop nervous or lymphoid tissue being present in meat for human consumption (Report 1990). Approximately one in five cattle that go to slaughter for human consumption are dairy cattle, and hence at greatest risk of being infected with BSE. It is unclear which tissues of these animals should be considered potentially infective to humans.

Infective period of animals with a spongiform encephalopathy

The infective units (IU) are first found in the spleen and lymphoid tissue of the animal that has been infected (Table 4). (Eklund et al. 1967, Hadlow et al. 1974), and this is followed by increasing numbers of infection-generating particles in nervous tissue (Eklund et al. 1967, Hadlow et al. 1974). It has been claimed that peripheral nerves carry the infective

Table 4. Stage of incubation period at which tissues were first found to be infectious. (See Pattison and Millson 1961, 1962, Marsh et al. 1967, Eklund et al. 1967, Hadlow et al. 1974, 1987)

Tissue	% of incubation period									
	30	40	50	60	70	80	90	100 ^b	110	
Brain				m,g, mk						
Pituitary					g					
Spinal cord		m		g,mk						
Peripheral nerve					g,mk					
Spleen	m ^a		g			mk				
Adrenal					g					
Lymph node	g,mk									
Thymus	m ^a ,mk									
Lung		m								
Liver/kidney						mk		m		
Muscle									g	
Gut	m,g							mk		
Bone marrow						m				
Salivary gland				g		mk				

^a Infectivity started at 14% of incubation period.

^b 100% incubation period coincides with onset of clinical disease.

m, g and mk represent the incubation period percentage at which the tissue infective in mice, goats and mink.

agent towards the central nervous system and that the spinal cord becomes infected before the brain (Kimberlin and Walker 1979, 1986, 1989). The spleen starts to contain IU soon after intravenous injection of infective material in the animal (Eklund et al. 1967). This is also shown with local injection into the gastric wall (Kimberlin and Walker 1989). Other tissues become infective more slowly and it is probable that lymphoid tissue, all abdominal organs, and nervous tissue become infective between one-half and two-thirds of the duration of the incubation period. Therefore, it is clear that clinically well cattle might contain the infectious agent, and this is obviously important with respect to food safety (*vide infra*).

The experiments with SE in many species have the problem of the delay before intracerebrally injected animals show signs of disease. This may be as little as 3 months in mice or it may be 5

years in larger mammals, depending on the species. When SE passes from one species to another the incubation period can increase (Pattison and Millson 1961, Zlotnik and Rennie 1965, Pattison and Jones 1968, Hanson et al. 1971, Kimberlin and Marsh 1975, Kimberlin and Walker 1979, Hadlow et al. 1987). For primates this may be many years but experimenters cannot be expected to wait 20 years to find out if an infection has taken place and this may make the experiments difficult with BSE.

Human kuru may sometimes have an incubation time of more than 30 years and this may represent the low number of infective particles that gain access to the nervous system as the incubation period is also inversely related to the infecting dose (Pattison and Millson 1961, Kimberlin and Walker 1978, Prusiner 1980, 1982, 1984). Because *Homo sapiens* lives so long, we may be open to infection by relatively low numbers of IU.

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Table 5. Tissues found to be infected by their ability to infect further animals

Tissue	Animal ^a					
	Sheep	Goat	Mouse	Mink	Man	Cow
Brain	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	
Peripheral nerve	+	+	+			
Eye					+	
Adrenal		+				
Lymph node	+	+	+	+		
Tonsil		+				
Salivary gland	-	+	+	+		
Spleen	+	+	+	+	+	
Gut	+	+	+	+		
Liver		+	+	+		
Kidney	-	-	+	+		
Bladder				+		
Pancreas		-				
Heart	-	-				
Lung	-	-	+	+		
Thyroid	-					
Thymus			+	+		
Testis			-			
Ovary		-				
Uterus			+			
Blood/serum	-	-	-	+	+	
Bone marrow		-	+	-		
CSF	-	+			+	
Urine		-		-	-	
Faeces				+	-	
Saliva	-	-			-	
Milk	-					
Muscle ^b	-	+		+		
Mammary gland	-					

^aSheep (Hadlow et al. 1982); goat (Pattison and Millson 1962, Hadlow et al. 1974); mouse (Pattison and Jones 1968); mink (Hadlow et al. 1987, Marsh et al. 1969); man (Matthews 1981); cow (Hope et al. 1988).

^bScrapie infected hamsters also have infective muscle tissue

Destruction of the agent

Most disinfectant chemicals (e.g. domestic bleach) do not appear to neutralise the infectivity of the agent (Taylor 1989), and neither do proteinases (specifically those found in the animal gut, Prusiner 1984), DNAase (Pattison 1988), RNAase (Prusiner 1984), ultraviolet light (Prusiner 1982), ionising irradiation at usable doses (Fraser et al. 1989), or heat (cooking temperatures) (Dickinson and Taylor 1978). Protease K has been shown to decrease infectivity as have specific chem-

icals that react with protein (Prusiner 1984). However, the chemicals that react specifically with DNA or RNA (psoralen photoadducts, hydroxylamine) do not have any effect (Prusiner 1984). Autoclaving at 134°C for 1 h decreases the infectivity of CJD or scrapie material to low levels (Rohiner 1984) but there is still evidence that full destruction has not taken place. Some infectivity of the scrapie agent can survive baking for 24 h at 160°C in dry heat (Taylor 1989) or at 360°C for 1 h (Brown et al. 1990). The only ways of ensuring destruction of these agents are

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The American current standard for autoclaving for decontamination of CJD is 132°C for 1 h (Rosenberg 1986). In Britain several methods are recommended for decontamination; the most common one used is 136–138°C for 18 min (Report 1981). This is aimed to prevent the transfer of the infective agent for CJD between patients by metal electrodes, corneal transplants (Behan 1982), dental procedures (Adams and Edgar 1978), or in the laboratory (Manuelidis et al. 1985, Miller 1988). Pathologists are often unwilling to undertake postmortem examinations of patients considered as possibly having died of CJD and when they do, gloves, autoclaving gowns and cap, overshoes, and masks are worn along with a disposable plastic apron under the operation gown (Report 1981).

The MAFF sent out 'Guidance for veterinary surgeons handling known or suspected cases of BSE' to all District Veterinary Officers giving directions as to how to deal with cows that are thought to be infected with BSE. This did not state, however, that many cows that showed no sign of the illness might be infected and therefore a possible risk to other mammals, including man. As an example of this, in 1989 14 cases of BSE occurred in a herd in Surrey. In 1990 there were 60–80 (Winter, pers commun.), suggesting the probable existence of 46–66 infected animals that remained undetected in 1989. The British Veterinary Association recommended to its members that, contrary to the MAFF's suggestions, no veterinarians should take part in calving or caesarian sections of infected cows (Cooke 1990, Editorial 1990).

Abattoir workers and farmers have been treated in a different light in that animals have been split open mechanically and sawn using circular saws, ani-

mals have had their heads removed to be sent to MAFF in Weybridge, Surrey, and headless cows have been carried in blood-dripping lorries to sites of incineration. Farmers have not been warned about the possible danger of infective products to other cows (or to themselves) during calving, killing or eating of an infected animal. In large farms in parts of the South of England, BSE is now reaching 10% of the dairy stock and abattoir workers have not been warned as doctors or veterinary surgeons have.

Chemical structure of TSE infective agents

The two main structures that are considered the likely candidates for the actual infectious agent are the virino and the prion (Prusiner 1982).

(1) *Virino*.

This is made up of a small fragment of DNA associated with the proteinase material (Kimberlin 1982, Prusiner 1982, Collinge et al. 1989, Southwood 1989). The DNA would be involved in induction of the protein from the DNA of the brain. However, no DNA has been found associated with the infective fragments (except by Narang et al. 1988), and DNAase, psoralens and amidines (Prusiner 1984) do not decrease its infectivity, nor does ionising radiation (Fraser et al. 1989).

(2) *Prion* (see Prusiner 1989).

This is a sialoglycoprotein derived from the DNA of the infected cell but which is changed by the infecting particle. This protein has been isolated from infected species and evidence for its role is mounting. The major problem with this hypothesis is its inability to account for variation among the different strains of scrapie. Some of these variants infect some species that others cannot. There is also variation in the length of incubation

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periods. It is not clear how such varia-
tion can be generated if the infecting
particle was derived from the DNA of
the host genome (Pattison 1988).

The argument that the infective par-
ticle must contain its own DNA particle
(Kimberlin 1982, Manuelidis 1985, Pru-
siner 1989) or need not (Kimberlin 1982,
Prusiner et al. 1983, Bockman et al. 1985,
Braig and Diringer 1985, Basler et al.
1986, Gabizon et al. 1988), continues.

The chemical structures of the virino
and prion are well reviewed by Prusiner
(1989), Manuelidis (1985), and Griffin
(1985).

Scrapie associated fibrils (SAF) and
prion rods are found by electron micro-
scopy only in the brain of SE infected
animals, and the PrP (prion protein) is
found in all animals with SE disease but
is produced by the genes of the host ani-
mal. After concentration these can be
studied microscopically and chemically.

Concentration of infectivity from scrapie infected tissue

This can initially be carried out using
molecular properties, partial purifica-
tion and assay of the tissue using ham-
sters (Prusiner 1980, Manuelidis et al.
1987, Sklaviadis et al. 1989). In order to
find the infective particles however, various
methods have been attempted. When anti-
bodies to the prion protein (PrP) are at-
tached to the particles in a sephadex col-
umn and infective tissue passed through
it, it is found that infective particles also
stick to the antibodies (Gabizon et al.
1988). It has also been found that if the
infected tissue is subjected to isoelectric
focusing, the same band that carries the
PrP also carries the infectivity (Ceroni
et al. 1990). There is strong evidence
that the PrP is infective but this does not
rule out the possibility that a small
amount of DNA is present inside PrP
which is acting as a protective protein.

PrP

This is the prion protein (PrP) associ-
ated with SE. A normal form of the pro-
tein is found in the membrane of normal
nervous tissue cells and is coded by a
specific gene. This gene, from chromo-
some 2 (Sparkes et al. 1986, Prusiner et
al. 1987) is highly conserved between
strains (Hope et al. 1988) and is altered
only between species (Endo et al. 1989,
Goldman et al. 1990) and in the GSS
syndrome (Collinge et al. 1990). This gene
also encodes the scrapie PrP messenger
RNA (Cheesebro et al. 1985) and hence
the PrP protein (Wietgreffe et al. 1985,
Basler et al. 1986, Narang et al. 1988,
Borchelt et al. 1990) but it does so in
much greater quantities in infected cells.
Initially, the protein appears identical to
the natural protein, but it becomes modi-
fied after production (Basler et al. 1986,
Hay et al. 1987, Manuelidis et al. 1987,
Caughey et al. 1989). This modification
gives rise to the infective form (Endo et
al. 1989), and renders the protein resis-
tant to most proteases (Borchelt et al.
1990). When the protein is treated with
proteinase K, particles of 27-30 kDa are
produced, the structure of which is com-
plex (Barry and Prusiner 1986, Meyer et
al. 1986, Bockman and Kingsbury 1988,
Hope et al. 1988, Dor-uru et al. 1989,
Serban et al. 1990, Yost et al. 1990), and
it varies between species (see above; Kim-
berlin and Marsh 1975, Bockman et al.
1985) with glycoside residues being added
to the molecule (Prusiner et al. 1987,
Sklaviadis et al. 1989). Also it appears
to vary with scrapie strain (Carlson et al.
1989, Lowenstein et al. 1990), and may
vary between strains of animal of the
same species (Lopez et al. 1990). In the
normal cell the protein is found in the
periplasmic membrane and, as such, it
has an affinity for joining liposomes or
membranes (see above; Prusiner 1982,
Gabizon and Prusiner 1990). The modi-

fied form however, may be found inside the cell, in the membrane, or as a secretory form (Hay et al. 1987). When antibodies against PrP are made in rabbits to produce polyclonal (Bendheim et al. 1984, Barry et al. 1986, Roberts et al. 1986), or monoclonal antibodies (Barry and Prusiner 1986, Kascsak et al. 1987). These have been used to demonstrate the presence of PrP in the amyloid of some infected tissues (Prusiner et al. 1983, Bockman et al. 1985, de Armond et al. 1985, Roberts et al. 1986, Wiley et al. 1987) and with the scrapie associated fibrils (SAF) (Kimberlin 1989, Liberski et al. 1989a,b) although this is not always successful (Bode and Diringer 1985). The use of PrP as an indicator of tissue infection (Farquhar et al. 1989) and its finding in other tissues that are themselves infective but do not contain large amounts of neurological tissue (Kitamoto et al. 1989) have suggested that PrP has indeed a crucial role in infectivity. This issue is at present under fierce scientific debate (de Armond et al. 1989, Manuelidis et al. 1987). Because of these uncertainties, the use of serological reagents to diagnose specific infective agents is fraught with difficulty, but is being studied by several groups.

Prion rods

It has been suggested that PrP will polymerise (de Armond et al. 1985, McKinley et al. 1986) to form prion rods that might be visible under the electron microscope (Prusiner 1982, Prusiner et al. 1983, 1987), similar to SAF, but this is not accepted by all workers.

There are many minor differences in appearance which can be stained by Congo red histochemical stains. They possess the same diameter and limited twisting as the shorter rod shaped particles observed in purified preparations of prions (de Armond et al. 1985). Immunological staining has been carried out using anti-

bodies specific for PrP and they themselves have been used to make antibodies (Barry and Prusiner 1986). It has, however, been claimed that there is poor correlation between the presence of prion rods and the site of tissue that is infective. They are not the same as the SAF, which many researchers feel are the infective particles.

Scrapie associated fibrils (SAF)

These are twig-like structures, 12–16 nm in width and 100–500 nm long (Merz et al. 1981, Diringer et al. 1983) seen under the electron microscope (Bode et al. 1985, Liberski et al. 1989a). They are only found in TSE infective tissues (Merz et al. 1984, Hope et al. 1988), and they copurify with infectivity (Bode and Diringer 1985). The fine structure under the electron microscope is different from prion rods (Prusiner et al. 1987, Liberski et al. 1989a,b) but they may be made of a normal or abnormal form of PrP (Hope et al. 1986). Some consider that a form of PrP is present in SAF (Hope et al. 1986) but others disagree (Manuelidis et al. 1987). SAF can be concentrated (Bode et al. 1985), and antibodies made against them (Diringer et al. 1984, Cho 1986, Rubinstein et al. 1986) including monoclonals (Kascsak et al. 1987). Staining techniques generally require the presence of these antibodies (Hope et al. 1986) and the amount of SAF is proportional to the infectivity of the tissue (Merz et al. 1984). No variation in antibodies has yet appeared against different scrapie strains of SAF (Fraser et al. 1989).

The identity of infective agent is still unclear but it is now unlikely that spiroplasmas, AIDS (with which some PrP may immunologically cross-react), or the infective agent in Alzheimers disease are involved. In summary, it is not possible to formulate a unitary hypothesis to account for the nature and mechanism of pathogenicity of the agents

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structures, 12–16 500 nm long (Merz et al. 1983) seen by electron microscope (Bode et al. 1989a). They are only found in nervous tissues (Merz et al. 1989), and they copurify with PrP (Dingler and Diring 1985). Under the electron microscope, they appear as thin rods (Bode et al. 1989a). They are made of a normal form of PrP (Hope et al. 1986) and a form of PrP^{Sc} (Hope et al. 1986). Manuelidis et al. (1984) concentrated (Bode et al. 1984, Cho 1986, 1987) including monoclonal antibodies (1987). Staining of PrP require the presence of PrP^{Sc} (Hope et al. 1986). SAF is proportional to PrP tissue (Merz et al. 1983). Antibodies has yet been raised against scrapie strains (1989).

The infective agent is still unknown. It is unlikely that spirochetes (which some PrP cross-react), or the zoonotic agents (such as the zoonotic disease agents), it is not possible to support the hypothesis of a zoonotic and mechanical transmission of the agents.

causing TSEs. It is likely that some of the conflicting findings will be capable of resolution by further molecular work.

Food and infective dose

Bovine brain was used in many foods, generally meat pies, sausages and beefburgers, until November 1989 when this was banned by the British Government. Brain is defined as 'beef' and not 'beef offal' according to European Community (EC) regulations. Hence, if it was used in the manufacture of beefburgers, the final product could legitimately be known as '100% beef'.

The figures are based on numbers that have been found in other animals with SE. This is an attempt to identify whether potential infective quantities might have been achieved previously.

Bovine brain from animals with clinical BSE (and possibly from those incubating it) is expected to have 10^6 – 10^{10} infective units (IU) per gram of tissue (Marsh et al. 1969, Hadlow et al. 1974, Kimberlin and Walker 1979, Kimberlin et al. 1983, Prusiner et al. 1985, Kitamoto et al. 1989, Robinson et al. 1990). If it is assumed that the brains of 1000 cows (Tyrrell's most optimistic incidence) were mixed together and one of them had been obtained from an animal with BSE, then the mixture would be expected to contain 10^3 – 10^7 IU g^{-1} . If 10% of a beefburger was brain tissue then the burger would be expected to contain 10^2 – 10^6 IU g^{-1} . Animal infection has been shown to take place orally at 4×10^4 IU with a spongiform encephalopathy agent (Kimberlin and Walker 1989). In this case the infective dose would be contained in 0.04–400 g of beefburger. Other experiments have showed, however, an oral infective dose to be 10^9 IU (Prusiner et al. 1985). The lower infective dose is supported by the very wide distribution of BSE in cows and cattle in the UK (Southwood 1989). An

infective dose may indeed be much higher but at present this is not known. If the total number of cows infected with BSE was substantially higher than Tyrrell's estimate of 0.1%, then the risks would also increase. The most gloomy prediction of the number of animals infected is 5–10%. The possibility that cows were infected with scrapie in their food could mean that the infective dose for the cow had come from sheep (i.e. crossed the 'species barrier'). One farmer (Winter et al. 1989) reported that 14 of his herd of 500 animals acquired BSE and that each had not had more than 12 kg of protein supplement. It is reasonable to assume that around 1% of this would be brain tissue and that perhaps 1% of the sheep brains would have been infected with scrapie. It should be said at this point that a short survey of Yorkshire sheep farmers showed that only one out of 20 claimed to have seen a case of scrapie in his lifetime, that veterinarians near to Leeds claimed it to be a rare disease and that a well known researcher into the subject (who has asked not to be named) had never seen more than ten cases in a flock. It has been claimed that the number of cases of scrapie in Britain have been increasing but there are no statistics to support this. Hence, we consider that our estimate of 1% of sheep being infected with scrapie is probably an overestimate. If the sheep brain was infected at approximately 10^7 IU g^{-1} (Robinson et al. 1990) then, given these suggestions, his cows would have eaten 2×10^6 IU. For human beings to eat this number of IU from, say, beefburger meat (and hence cross the species barrier from cows to humans), would require them to eat 2 g to 20 kg, assuming that the agent has a specificity to us at all. Such consumption is certainly feasible in practice. Infection with SE prions may be cumulative, i.e. an infective dose can be achieved by taking in small

parts over a long period. There is little evidence for this except that there are no antibodies formed in the body against other prion, and hence it might always remain infective.

If this is so then the dose of beef-burger mentioned above could have been eaten by many people over the period of a year before November 1989.

Since November 1989, the eating of British beef has continued but under the demands of the British Government, this should not contain any brain, spinal cord or lymphoid tissue (Report 1990). In order for this to be possible the animal may be cut with a saw (Report 1990) so that the brain and spinal cord tissue can be removed (often using suction or irrigation equipment). This may be a dangerous procedure considering that brain tissue has the consistency of blanc-mange and may be spread over any meat tissue. A piece of bovine central nervous system the size of a sugar grain could convey 10^3 – 10^7 IU. Also, peripheral nerves have been shown to contain infective prions in goat scrapie and TME (Pattison and Millson 1962, Hadlow et al. 1987) so it is not surprising that meat has been shown to be infective in some animal experiments, although not with BSE as yet (Marsh et al. 1969, Pattison et al. 1972).

The risk of BSE to man

Because of the fragmentary knowledge over the agents responsible for TSEs, it is not possible to make any firm prediction as to whether, or on what scale, man might be infected by the BSE infective agent. There is not even sufficient information to calculate any probabilities and their limits of reliability. Some reports have relied upon the optimistic view that any risk to man is 'remote' (Southwood 1989, Tyrrell 1989). However, the first authors to provide firm reasons for the potential hazard of BSE

for man were Holt and Phillips (1988).

In this section, we discuss the information which favours a pessimistic or an optimistic outcome, and give our assessment of the relative certainties of these factors. We will not be tempted into making an arithmetical prediction.

The study of CJD provides a useful starting point. Formally notified cases are of the order of 30–40 cases annually in the UK. The real numbers are thought to be between 1500 and 9000 (Roberts 1990), the discrepancy arising from the failure to diagnose accurately the nature of fatal dementing illness. If BSE did infect man, it is by no means certain that the resultant disease would be identical to CJD. Degenerative neurological diseases do not always form precisely defined entities. There are some, such as motor neurone diseases, which could also have an infective basis.

There is incontrovertible proof that the infectious agent for CJD can be transmitted from human tissues to experimental animals (*vide supra*). It is virtually certain, therefore, that CJD is caused by the acquisition of that infectious agent. However, it is theoretically possible that the infectious agents associated with or causing CJD evolve *de novo* in an infected individual as a result of genetic disposition or mutation (Goldfarb et al. 1990). If for example, PrPs were the causative agent, and these arose from congenital or environmental affect on a host cell DNA, then fears over the transmissibility of the BSE agent to man would rescind to some extent; CJD could just possibly not be acquired from meat. The familial incidence of the disease is said to be approximately 15% and might be consistent with this view. However, the familial nature of the disease, particularly in Libyan Jews, could be explained by the genes in question conferring a specific disposition to acquiring an exogenous infection. Furthermore, such an apparent

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genetic basis could result from exposure of members of families to uniform environmental sources such as idiosyncratic diets. It has always been difficult to identify the true biological origin of any infectious agent, whether novel or not. On grounds of probability we must consider that the evolution of such an infective agent *in situ* in so many individuals at any one time must be unlikely.

Moreover, the epidemiology of BSE has produced overwhelming evidence of the existence of an infective agent acquired through the oral route. Whilst it can be argued whether or not BSE is unique to the British Isles, the scale of the epidemic in cattle is manifestly so. Therefore, we believe that the TSEs should be considered to have an infectious basis at the present. Likewise, CJD should therefore be considered an infection. The potential routes of acquisition of the infectious agent—transplacental, mucosal, inhalation, injection, sexual or oral—are all possible. Certainly vertical transmission of scrapie in sheep is known, and vertical transmission of the causal agent for CJD may occur, but at least one other route must be postulated in order to account for the presence of the putative infective agent in so many sporadic cases. Again, the most impressive evidence for the importance of any route of acquisition of these agents comes from BSE in cattle, where the oral route would appear to offer the only plausible explanation. Experimentally, such infections can also be transferred to mice through the oral route (*vide supra*). Whilst there is no proof of this, it would seem likely that oral route in man provides the most likely portal of entry. This is at the very least, the most obvious working hypothesis for most of the cases, but seemingly impossible to prove or disprove. That the spleen is the first organ to be infected in sheep acquiring scrapie experimentally (*vide supra*)

is consistent with the view that the infectious agent is capable of penetrating the gastrointestinal tract. Some slight reservation has to be made here because, theoretically, animals fed the infective agent either naturally or experimentally could eliminate it in their faeces, and then acquire the infection through mucosal or skin penetration.

Since all the TSEs have been identified in mammals, then one or more of these must be considered the source of CJD. Until very recently the disease had not been reported in domestic pets. Some contact with say, goats, might on occasion cause the infection. Deer could also be a minor reservoir. The three major sources of animal meat throughout the world must be scrutinised very carefully—i.e. products from cattle, sheep and pigs. The possibility that sheep scrapie might be responsible for CJD has been considered by several workers. Because the incidence of scrapie in sheep varies substantially throughout the world, being highest in Iceland and lowest in New Zealand, it has been possible to look at the varying incidences of the two diseases in a number of countries. No association has been found (e.g. Southwood 1989, Tyrrell 1989). These negative findings do not establish that scrapie never inflicts man, but make an ovine origin most unlikely to be the major source.

Pigs might provide such a reservoir, but a number of factors argue against this. First, pigs apparently do not suffer naturally from SE. Secondly, pig meat is generally eaten when the animal is 5 or 6 months old: that is an age when it would be expected not to be infectious, even if SE did develop subsequently. Thirdly, a high incidence of CJD occurs in Libyan Jews, whose diet presumably excludes pig products.

Thus, through the exclusion of other sources, cattle may be the most likely

source of CJD. Consistent with this proposal is the frequency with which beef cattle and cows are slaughtered at the end of their lactation (between 3 and 10 years). Age must be a highly significant factor in the availability of the infectious agent. Urgent research is needed to study the relationship between the consumption of beef products by communities and the coincidence of CJD. If cattle are the sources of CJD, then this implies the presence of an infectious agent long before the recognition of BSE. The first cases of BSE were identified in 1985-6, so it is unlikely that the infectious agent was present in meat products before 1982-3. If the incubation period of CJD is 15-20 years, then current CJD cases cannot be caused by the BSE agent in the 1980s. It is certainly possible that a BSE-like infectious agent could have been present in cattle for many years either without producing clinical infection, or indeed responsible for occasional clinical disease. There are rumours of the existence of cattle suffering a BSE-type disease many years ago in Yorkshire, UK or more recently in other countries and these would be compatible with this view.

The above thesis is of course speculative, but we believe that cattle are the likely major source of the infectious agent for CJD that is acquired through food. There is no proof, but these ideas should prompt further work.

If the putative 1500-9000 cases of CJD in the UK annually have indeed been caused by consumption of beef products some years ago, the potential danger from BSE comes disturbingly obvious. The most worrying hypothesis must suppose that the infectious agent from BSE-infected cattle is responsible for CJD. If this is the case, the high prevalence of infected animals—estimated at between 1 and 10%—must present a phenomenal danger to man. There is no

reason to exclude the possibility that the BSE epidemic represents an 'amplified' natural infection as a result of rendered cattle offal being fed to cattle between 1981 and 1988. This is a different interpretation from the much publicised belief that the sheep scrapie agent was responsible for the BSE epidemic. This proposal was supported by the rearing of increasing numbers of sheep in this country, and an alleged increasing incidence of scrapie in this animal. There is however no direct evidence to support this, illustrating the poor data base that exists for animal infections in this country as a whole. Those who claim that scrapie is the likely source of BSE (e.g. Southwood 1989, Tyrrell 1989) must account for the failure of scrapie to infect cattle previously, despite the sharing of common grazing land for centuries (Morgan 1988). A third possibility has been proposed, namely that the BSE agent represents a mutated scrapie agent whose host range has become altered (Lacey and Dealler, in press).

Beef as a potential hazard to man

The possibility of danger to man from cattle brain and other offal material incorporated into processed items has already been considered (*vide supra*). There is little reason to believe that the agents responsible for transmissible spongiform encephalopathies are found actually within or around muscle fibres, although occasionally the agent has been transferred from muscle (Tables 4 and 5). However, the main danger from beef products is due to the presence of adventitious material, including: peripheral nervous tissue that may contain infectious agent, lymphatics in channels and nodes around beef tissue that may be infectious, and the contamination of the carcass with spinal cord, brain and other 'high-risk' tissues during the processing. In particular, the use of mechanical

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the possibility that represents an 'amplifier' as a result of rennet being fed to cattle in 1988. This is a difference from the much published sheep scrapie agent and the BSE epidemic supported by the rear-ers of sheep in an alleged increasing scrapie in this animal. There is no direct evidence to corroborate the poor data for animal infections in a whole. Those who believe is the likely source of the BSE (od 1989, Tyrrell 1989) the failure of scrapie to spread previously, despite the use of grazing land for centuries (38). A third possibility is namely that the BSE agent is a mutated scrapie agent which has become more infectious (Dealler, in press).

Environmental hazard to man

The danger to man from other offal material processed items has been considered (*vide supra*). It is reasonable to believe that the BSE agent for transmissible spongiform encephalopathies are found around muscle fibres, and possibly the agent has been found in muscle (Tables 4 and 5). The main danger from beef is the presence of adventitious material including: peripheral nerves that may contain infectious agents in channels and tissue that may be in contact with contamination of the carcass, brain and other organs during the processing. The use of mechanical

saws to remove bone could be hazardous in this way.

In discussing the possible effect on man, the most pessimistic view has been taken so far. Any one of the following could substantially reduce the risk.

The first reassurance could come from the failure of the BSE to gain access to the milieu of the human body. It may not adhere to mucous membranes or be able to penetrate the gastrointestinal mucosa; it might also be inactivated by blood or proteolytic enzymes in the gut (although this is unlikely). If the BSE agent was heterogeneous in its properties, then at least some of these factors could reduce the risk of entry to the body. The agent may not survive or multiply in the reticulo-endothelial or nervous system. Finally, it might do so so slowly that its adverse effects would not be manifested during the human lifespan.

The concept of an infective dose may not truly apply to the potential of the BSE agent to infect man. It is not known why a certain number of infectious particles of TSEs are required to induce experimental infections. The crucial question remains unanswered. Is a high infectious dose required to provide the certainty of the infectious agent entering a

cell at a specific receptor? If this were the case, then the consumption of small amounts of agent on numerous occasions could provide the same risk as consumption of the total number of particles on more than one occasion.

The more optimistic explanation for the requirement of a certain number of particles to generate an experimental infection is that cellular or other immune mechanisms may satisfactorily eliminate the agent up to a critical number at any one time. If this was the case, the repetitive consumption of small numbers of particles over a long term should provide little infectious risk. Exceptions to those would be people with impaired immunity. With either explanation, pregnant women might be most vulnerable.

In conclusion, the scale of the BSE outbreak in the British Isles presents major problems. More research is required to assess the risk to man, and to develop methods for early detection of TSEs before the onset of clinical symptoms. Whilst the evidence that the feed is responsible is compelling, the host range of BSE is not defined. Generally, TSEs are capable of transfer experimentally to about half of the mammals tested.

Addendum

The SE infection of one out of eight pigs which had been parenterally injected with 10% suspensions of BSE in saline after 69 weeks incubation has recently been shown (Dawson et al. 1990). The possibility that pigs fed with BSE infected meal became infected prior to this being banned in September 1990 must be considered (Medrum 1990) and that these animals may be infective to man (Mills 1990).

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