MINIREVIEW

Origin and Implications of Bovine Spongiform Encephalopathy* (43975)

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Abstract. **All spongiform encephalopathies in animals, including humans, are slow-developing infectious diseases. The current working theory links the origin of bovine spongiform encephalopathy (BSE) to the feeding of cattle with meat and bone meal prepared from scrapie-infected sheep remains. Recycling of cattle meat and bones (MBM) essentially resulted in the selection of a single strain from the "wild type", a mixture of 20 strains. The BSE agent is easily transmitted through ingestion, with some evidence of vertical transmission. Paradoxically, cattle have selected a major new strain which appears to be more virulent than an unselected strain found in scrapie sheep. The same strain of BSE agent is implicated in the occurrence of spongiform encephalopathy in domestic cats, tiger, and some exotic species of ruminants in zoos. The properties of BSE and its spread into cattle are still disputed. Since our understanding of the disease and its transmissibility in humans must await observations that will be made over some years to come, it is important to keep a reasonable perspective and ensure that any speculative comment is consistent with fact. In risk assessment in such circumstances, it is tempting give too much credence to persuasive parallels when direct relevant information is not available. On the other hand, it would also not be wise to assume that the disease will die by itself and will have no effect on humans.** [P.S.E.B.M. 1996, Vol 211]

History

A group of chronic, progressive, and always fatal infections of the central nervous system (CNS) of animals and humans have been shown to be caused by a slow-replicating agent, which requires many months to reach high titers (1, 2). The most important pathological hallmark of these infections is the strikingly similar histopathological lesions with a widespread vacuolation of the CNS without the involvement of any other organs. Hadlow (3) revealed the importance of vacuolation both in humans and animals in a letter to the *Lancet*, entitled "Scrapie and kuru." Because of similar features, eight degenerative CNS disease are grouped together as spongiform encephalopathies (SEs): scrapie of sheep and goats (4), Creutzfeldt-Jakob disease (CJD), kuru (1), and Gerstmann-Sträussler-Scheinker syndrome (5), transmissible mink encephalopathy (TME) (6); and wasting diseases of mule deer and elk (7). All these disorders have been known for a number of years (1, 8).

Emergence of Spongiform Encephalopathy in Other Species

Bovine spongiform encephalopathy (BSE) (9), and similar SEs have been found in other animal species, including domestic cats (10) and captive wild animals of eight species at or from seven zoological collections in the British Isles (11, 12). The affected animals included members of the subfamily bovinae: one nyala *Tragelaphus angasi*, four eland *Tragelaphus oryx*, and

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six greater kudu *Tragelaphus strepsiceros;* members of subfamily hippotraginae: one gemsbok *Oryx gazella,* one Arabian oryx *Oryx leucoryx,* and one scimitar-horned oryx *Oryx dammah;* and members of the family felidae: three cheetah *Acinonyx jubatus* and 1 puma *Felis concolor.* Furthermore, three cases of SEs have been reported in ostriches *Struthio camellus* from two zoos in North West Germany (13). The characteristic features of all the SEs are similar to those observed in scrapie; that is, functionally, clinical symptoms become apparent after a long incubation period at terminal stages of the disease.

The BSE agent, like the agents of other SEs, has unusual biological properties, such as resistance to heat and irradiation with ultraviolet light, and exceptional physicochemical stability. The agent's apparent lack of specific antigenicity made it an unconventional replicating agent (8). Furthermore, since the emergence of BSE we have witnessed in numerous experimental results that the scrapie agent is a novel pathogen which is naturally transmitted through food from one species to another, jumping species barriers. The potential relationship between experimentally induced infection through intracerebral inoculation (ic) and oral ingestion requires clarification, and it is also necessary to review the facts of possible oral transmission of scrapie/BSE to humans.

Scrapie

One of the most common examples of an SE is scrapie. This chronic wasting disease of sheep had been known in Europe under various names even before the middle of the eighteenth century. A German writer, Kuers, found a passage referring to the disease by an agricultural writer in Roman times (14). At present, scrapie is distributed widely in Europe, America, and Asia. The disease is caused in sheep by a natural endemic infection. The epidemiological evidence indicates a predominantly maternal pattern for the transmission of the infection (4). Precise knowledge of transmission and possible routes of the natural scrapie infection in sheep remain a mystery. The disease is readily transmitted experimentally by the oral administration of scrapie-infected sheep tissue, (15) including the fetal membranes from an infected animal (16). In sheep, the oral route seems to be one of the important routes of infection (17, 18). The simple infection hypothesis, however, does not explain why lambs from scrapie-infected mothers are highly affected compared with lambs from apparently healthy mothers, on the same pasture.

Discovery of Bovine Spongiform Encephalopathy

A farmer, in 1985, noticed that one of his cows was exhibiting changing behavior, which was leading to a lack of coordination of gait with an unexplained nervousness. A diagnosis of a brain abscess or tumor was suspected. The vet did not think any more about this case, until he was called back to the same farm to see a second, third, and fourth case exhibiting similar strange symptoms. At this stage, the vet became suspicious of his original clinical diagnosis. Histopathological examination of these and six other similar cases in 1986 revealed spongiform changes in the brain and spinal cord. These first 10 cases of BSE were reported from four herds in southern England (9). The lesions seen were very similar to those described in scrapie and Creutzfeldt-Jakob disease (CJD). Thus, a novel neurological disease, bovine spongiform encephalopathy, was discovered.

The Clinical Symptoms of BSE

The ages of cattle affected by BSE ranged from 1 year 10 months to 15 years; however, the majority of cases occurred in 3- to 6-year-old age group. The clinical signs begin with anxiety, nervousness, apprehensive behavior, and aggression (9, 19, 20). The symptoms and the intensity vary from case to case, from normal to unmanageable behavior. Although anxious and nervous at the initial stage, most of the affected cattle appear mentally alert. They show difficulty in rising from the normal lying posture and an increased reaction to sound, and may repeatedly grind their teeth. At some stage, animals take a wide base posture, and the abdomen is drawn up. Gait is swaying, with both the fore and the hind leg of one side moving together, and postural abnormalities associated with high stepping of the feet are evident in the hind legs. In many cases, any excitement may result in posterior ataxia, often with dropping of the pelvis, kicking, and a general nervousness. There is evidence that clinical signs accelerate due to separation or transportation stress. Some animals become so difficult to milk that they have to be dried off. The signs may progress to frenzy with aggression; weakness, which causes trembling, becomes apparent. The animals splay their hind legs and thus have difficulty in turning, particularly on concrete, which may be observed accompanied by stumbling or even falling. Fore leg ataxia is not usually reported. Loss of the body weight has been reported despite an apparently normal appetite, except at the terminal stages. As the clinical signs progress, milk yield is reduced in the absence of mastitis. The fall in the milk yield varies considerably; in some cows a reduction of 5–10 l/day over 2- to 8-week period to a dramatic drop of 10–20 l/day in 1–2 weeks. The condition of the animals progressively deteriorates, and they become unmanageable, dying within 2 weeks to 6 months from the onset of symptoms.

Identifying Preclinical Cases of BSE

BSE, like other SEs, has a very long asymptomatic incubation period and protracted clinical course to

be reckoned in months or even years. At present, we have to way of identifying experimentally or naturally infected animals during the long incubation period. It is vitally important that this persistent infection be detected at preclinical stage of the disease. The present methods available for confirming a clinical diagnosis include histopathological examination and detection of scrapie-associated fibril (SAF) (21) and tubulofilamentous particles by electron micrography (EM) (22–25) in surgically accessible tissue.

BSE in Cattle Born after the Feed Ban

The ban of contaminated food from July 1988 should have stopped infection through cattle feed. Up until March 1995, over 17,000 BSE cases had been histologically confirmed in the United Kingdom in cattle born after the ruminant feed ban was imposed. However, this figure represents less than half of the 35,000 suspected cases reported by vets (26). In the remaining 18,000 cases, the characteristic SE legions were not observed in the brain, and no alternative diagnosis has been made for this large number of cattle. The Ministry of Agriculture Fisheries and Food (MAFF) accounted for some of these early cases of BSE with the "Bin end" theory that old-style foodstuff had been hanging about on farms or that the feed compounders had taken a while longer than anticipated to reformulate their product. The number of new cases born after the feed ban would suggest either that the practice of contaminated feed is still ongoing or that there is horizontal/vertical transmission.

Postmortem Histopathological Studies

In many instances, the diagnosis of BSE has presented difficulty in farm animals showing clinical signs of the disease. Like all other known SEs of animals or humans, postmortem examination of BSE cases revealed no gross surface lesions of any of the internal organs.

The diagnosis of BSE is based on the light microscopic neuropathological examination of the CNS which relies on the demonstration of systematic scrapie-like vacuolation in the form of spongiform changes (27). Several neuropathological studies have been performed in Great Britain since 1987 on clinically suspected BSE cattle (9, 28–34). The vacuolation of neurones and neurites is confined to the central nervous system, giving the distinctive appearance of spongiform changes within the grey matter. Spongiform encephalopathies typically have a systematic and bilaterally symmetrical distribution of vacuoles. In one series of 657 clinically suspect BSE cases, minimal lesions were reported in 3%, where an unequivocal diagnosis was obtained in 86% (30). Vacuolar changes in the spinal cord in BSE were most severe in the dorsal horn and intermediate grey matter (34). Moderate changes in the solitary tract nucleus and severe lesions in the spinal tract nucleus have also been described (9, 30–32). The degenerative changes in the basal ganglia were more striking in the cerebellum. These observations are considered important pathological distinguishable changes, which are not recognized in healthy cattle. The vacuolation and degenerative pathological changes seen do not support the diagnosis of any previously recognized disease of cattle.

Histopathological Studies in Cattle Born after Feed Ban

In clinically diagnosed BSE cases born after the feed ban (BAB), histological studies have revealed an increasing number (39%–57%) of brains have no significant lesions or vacuolation (35). In Scotland, the number of cases with no significant lesions in cattle BAB has gradually increased from 26% in 1988–1989 to 41%. However, in BSE suspects born after the meat and bone meal (MBM) ban between July 1988 and December 1991, the negative rate had increased to 82% (35). This new pattern of low grade vacuolation may reflect either the emergence of a new strain of BSE, or the possibility that the infection occurred by a different route.

The rising number of cattle showing the typical BSE symptoms with negative histopathological results in cattle BAB, where no alternative diagnosis has been established, obviously raises a number of serious concerns. These include the question of whether all cases of BSE, including atypical BSE, arise from contaminated feed, or *via* maternal or horizontal transmission. Atypical cases may be caused by other strains of the scrapie agents with a different distribution of lesions. This feature has been observed in experimental transmission of scrapie to cattle: where inoculated calves developed clinical symptoms, none of their brains had SE vacuoles though these brains were immunopositive to protease-resistant protein (PrP) (36). Since a significant number of clinically suspected cases of BAB have no characteristic vacuolar pathology, it is important that a second line of tests be used to determine the nature of the disorder.

Immunohistochemical Studies

Through immunohistochemical studies, two types of plaques can be demonstrated in the brains: (i) amyloid β-protein–positive plaques and (ii) protease-resistant protein–positive plaques. Amyloid β-protein positive plaques (sometimes referred as amyloid plaques) are seen in Alzheimer's disease (AD), a noninfectious CNS disease sharing many clinical pathological features with the SEs. Amyloid β-protein–positive senile plaques are also seen in Down's syndrome and in some normal aging humans and in cases of CJD, kuru, Parkinson's disease, and some strains of

experimentally induced scrapie in mice (37–39). In a limited series of cases, amyloid plaques have been observed in 55% of natural scrapie of sheep (40) and in 62% of mule deer (41). Amyloid plaques have been observed in only one BSE case out of 20 examined (31).

Protease-resistant protein–positive plaques are seen in CJD, kuru, natural scrapie of sheep, and some strains of experimentally induced scrapie in mice. Specific PrP antibodies have been used to demonstrate PrP plaques in SEs (42). PrP-positive plaques have not been observed in AD or Down's syndrome. In BSE, the analysis of several different brain areas has confirmed the specificity of PrP, but the sensitivity of detection was low (46.5%) (43) and, therefore, the test has not been used for routine BSE diagnosis in the United Kingdom. Recently, in a configuration and topographic distribution of PrP in the central nervous system in a BSE immunochemical study, the density of neuropil immunostaining approximated inconsistently to the severity of vacuolar changes (34). This correlation was not seen in experimentally inoculated cattle with sheep scrapie brain: where the calves developed clinical BSE, their brain sections were negative for vacuolation and positive immunohistochemically for PrP (36).

Ultrastructural Studies

Ultrastructurally, the pathological findings in BSE-affected cows resembled those in natural and experimental scrapie, and in CJD-infected animals. Numerous membrane-bound intracellular vacuoles were present. Some vacuoles contained abundant membrane bodies. Occasionally, some of the processes contained a network of branched intercalating cisterns and tubules with entrapped mitochondria. Astrocytic reaction and the tubulofilamentous particles termed nemavirus (44–46), similar to those seen in natural and experimental scrapie of sheep and CJD, have been observed in BSE brains (47).

A simple grid touch negative staining method by EM has been developed to demonstrate both tubulofilamentous particles and SAF (24–26, 44). This touch method has been successfully applied to the diagnosis of human CJD (48, 49). Three BSE brains and three normal cow brains were examined from five different areas by the negative touch method for both tubulofilamentous particles and SAF. From each area, five grids were prepared and examined. Tubulofilamentous particles and SAF were revealed in all BSE cases (Fig. 1). Some of the grids prepared were negative, suggesting a patchy distribution; therefore, it would be advantageous to prepare grid samples from different regions of the brain.

In a blind trial, 10 cow brain were examined by the touch technique, as previously described (24–26), while conventional SAF preparations for EM and histological examination were carried out independently by MAFF. Each grid was examined for about 15 min by the EM. The results of this study are shown in Table I. Examination of the grids revealed both typical nemavirus particles and SAF in three of the five suspected BSE cases. There were no false positive results. Two of the specimens (± results) were tested by only one, not both, of the participating laboratories due to an administrative error, so the false negative result in two cases may be due to a mixup of specimens. The simple touch method can provide a rapid means of diagnosis of BSE with very little handling and risks of exposure. In another study, 27 cow brains from apparently healthy cattle over 4 years old were collected from a local abattoir for examination by the touch technique. Out of 27 brains examined, eight were positive for SAF by the EM. This is about a 29% positive rate in cattle over 4 years old being processes for the human food chain.

Epidemiological Study

The disease was made compulsorily notifiable disease in countries of the European Union. Over 99%

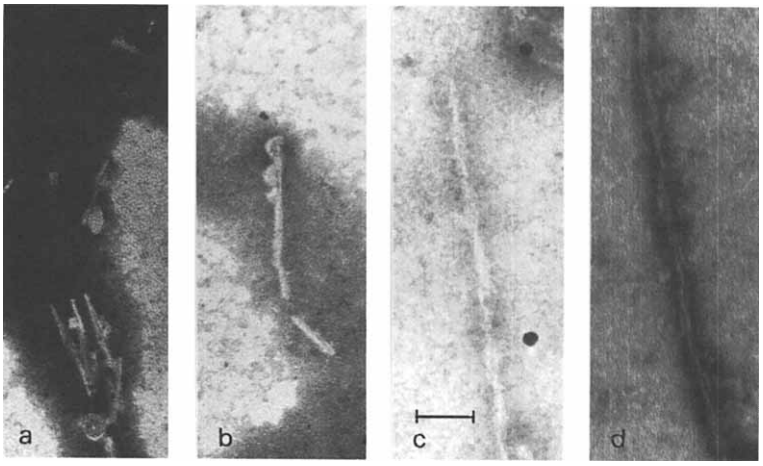

Figure 1. Electron micrograph of negatively stained cerebral cortex from 10- (a), 18- (b), and 60-day (c) post-inoculated brains of a scrapie hamster prepared with grids soaked in 1% SDS showing typical SAF. (d) Electron micrograph from a negatively stained grid prepared from cerebral cortex from a BSE. Grids were soaked in 1% solution of SD. Note typical SAF. Bar, 150 nm.

Table I. Comparison of Scrapie Sheep and BSE Strains

Animals inoculated	Source of inoculation	
	Scrapie sheep	BSE
Mice	80% +ve variable ic and oral routes 381–809 days[a]	100% +ve both by ic and oral routes 328–537 days
Mink	ic route (poor)[b] Oral route −ve	100% +ve both by ic and oral routes 12–14 months
Sheep positive line	ic route +ve Oral route +ve	ic route 724 days Oral route 538+ days
Sheep negative line	ic route −ve Oral route −ve	ic route 440+ days Oral route 734+ days

Note: +ve = develop disease; −ve = do not develop disease.
[a] Inoculation periods.
[b] Marginal success (50).

(140,000 cases) of laboratory-confirmed cases of BSE have occurred in Great Britain. This includes over 17,000 confirmed BSE cases of cattle BAB. It is commonly accepted that the disease in cattle has been transmitted by feed containing the infectious agent from scrapie and BSE cattle brains. Cows are herbivores deliberately turned into carnivores and, more importantly, into cannibals. It is believed that contamination during cannibalistic ritual was the sole source of transmission of kuru from human to human and possibly the original source of a spontaneous case of CJD (51). For the epidemiological studies, the retrospective clinical evidence collected strongly suggested that BSE appeared as a new disease and that it did not exist in Great Britain before 1984. The prevalent hypothesis on the origin of the epidemic is food-borne infection, caused by an increase in the absolute numbers of scrapie-infected sheep entering the rendering chain combined with changes in rendering technology which led to the clinical infection of cattle (52). The majority of animals became infected in calfhood through their food (19).

Feeding of Cow on the Farm

Calves as young as 2 weeks old are introduced to proprietary calf pencils (dried pellet food), and they become accustomed to food other than mother's milk at 4 weeks old. Grass silage and calf pellets are fed *ad libitum* post-weaning until turnout in mid-April the following year. About 12 kg/head of animal protein of ruminant origin had been incorporated into the proprietary compound foodstuff which was fed to the calves from 2 weeks to 6 months old. These observations suggested that infection of the calves occurred during this period. After the ingestion of the infective agent by feeding, the incubation period is between 2 and 5 years before clinical signs appear.

Preparation of Feed

Prior to the 1970s, the majority of material was processed in the conventional batch cooker, a steam jacketed vessel with an agitator placed in the machine. After cooling, fat and solids were crudely separated. Fat collected during this process was further refined and sold for various uses: this is known as tallows. Tallows is used in the cosmetic industry for the preparation of soap and other cream-based products. The solids went through a solvent extraction process, similar to a dry-cleaning operation, where fat was leached out and collected by a distillation process, and the solvent driven out by blowing inert gas or steam. At the end of the 1970s and the beginning of the 1980s, most renderers moved way from the solvent extraction procedure, replacing it with a continuous mechanical method with high pressure, which left 10%–12% fat in the solids compared with 5%.

A ban on the feeding of ruminant protein to ruminants would probably be the single most effective measure to eliminate any feed-borne infections of transmissible SEs agents. To prevent further transmission of the infective agent by this route, a ban on the use of ruminant-derived protein in ruminant foodstuff was introduced in July 1988. The continued epidemiological studies have not revealed any evidence to refute MBM involvement. However, crucial proof experiments have not been carried out; that is, contaminated feed has not been fed to the cattle to establish the hypothesis that bone meal in proprietary cattle foodstuffs was the vehicle of infection.

Origin of BSE

Why did this disease not appear till the 1980s? The feed renderers had altered their methods, in order to cut costs. Instead of high-heat batch production, manufacturers had switched to continuous processing at lower temperatures. According to MAFF, the newer rendering processes of the 1970s were no longer killing off the scrapie agent. It was originally estimated that the BSE epidemic would peter out at 20,000 cases. In fact, the total number has claimed seven times than estimated. Initially, the cause of the epidemic was attributed to the relaxation of laws governing the heat treatment of animal MBM preparations at rendering plants and the discontinued use of solvents.

Endemic BSE

The scale of the BSE epidemic in Britain increased very considerably from the latter part of 1989, when a greater proportion of cattle became infected with a strain with a shorter incubation period (52–55). There was a spectacular increase of the disease in the United

Kingdom during 1988 and 1989; the total number of cases in June 1988 was 687, in December 1988, 2160, in June 1989, 5375, and in November 1989, 8100 (56). The number of suspected cases now being reported in 1994 (400–500 cases/week) is significantly less than at the same time in 1992 (600–900 cases/week) and 1993 (800–1000 cases/week). Large differences in the incidence of BSE have also been observed between herds. The peak monthly incidence of BSE in the United Kingdom in 1993 represents 1/100 adult cattle, similar to that seen in the kuru epidemic in the Fore Tribe in the 1950s. This was an important change and was attributed to the recycling of infected and subclinically affected cattle tissues in MBM (57). Sheep-to-cow infection would represent a species cross over; the disease would have a long incubation period in the cow. A small number of sporadic cases of BSE might have occurred before 1985, with a very low incidence of the order of 1/100,000 adult animals/year. At some stage, cattle and sheep remains were mixed together (although at the time of killing, the affected cattle appeared clinically healthy). This was a very common practice used at many abattoirs in England designed to clear away unwanted meat. No one saw any danger in this practice of mixing the remains from one species with those of another. Recycling the scrapie agent created a situation equivalent to serial passage which was likely to select a single strain of the scrapie agent pathogenic to cattle (57). Although, the cow infected with the scrapie agent from sheep may not have developed the clinical disease in their lifetime, transmission of the agent from cow to cow selected a unique strain of pathogenic BSE, reducing the incubation period to a level where the clinical disease became apparent in the lifetime of an average cow.

The number of BSE cases rose every year, and total numbers in March 1995 reached over 140,000. In herds with adult breeding cattle, 30.9% have experienced at least one case of BSE, while 49.8% of dairy herds, but only 12.5% of beef suckler herds, have also done so. From an adult cattle population of around 4.5 million, the current annual incidence of confirmed cases of the disease is 0.75%. A reduction in the number of new cases has been reported in animals 3 years old and under, while there has been a continued rise in incidence in five-year-old animals (58).

In the earlier study, a marked geographical variation was reported in the incidence of BSE from south to north, in particular, relatively few cases were reported from Scotland. However, the number of BSE cases diagnosed in Scotland increased gradually. This variation of BSE cases in different parts of the country has been considered to be related to geographical differences in the market share of the cattle foodstuff, compounding dissimilarities between companies and the rate at which the MBM was used within the herd.

Host Genetic Variation is not a Factor in the Occurrence of BSE

Beginning in 1985, BSE has emerged simultaneously in widely differing geographical areas throughout the country, on an epidemic scale. There is no supporting evidence that the occurrence of BSE varies in different breeds of cattle (59). BSE had occurred in one of each of nine sets of female twins, of which seven were confirmed (19). Epidemiological studies show a substantial amounts of familial aggregation of the disease, with 99% of BSE cases having affected first- or second-degree relatives, suggesting genetic effects (60). The problem is separating the effects of the vertical and "first" infection hypothesis in a common shared environment. Aggregation of cases could be due to shared environments or to the highly structured nature of the pedigree herds. In one study, 501 BSE cases were identified and their records examination revealed that 239 were from different sires. Furthermore, a logistic regression analysis of data on 30 pedigree herds to investigate possible sire effects provided little difference in disease incidence among the progeny of 302 different sires including sires of different sire groups (60). In this study, herd effects varied from 3% to 28%, which were statistically significant, but sire effects failed to reach the 5% significant level. The incidence within breed groups did not differ significantly by a simple chi-square test: 15.5% for Holstein, 11.9% for Friesian × Holstein, and 12.1% for Friesian (60). These results suggested that there were no host genetic factors contributing to the susceptibility to the SE agent or outbreak of BSE. These authors ignored and did not investigate the possibility of horizontal or vertical transmission. The number of sires involved and the absence of any breed association militated against an autosomal mode of inheritance as a cause of BSE. Specific analysis of these cases in one of the herds further eliminated the possibility of BSE being exclusively determined by simple Law of Mendelian inheritance (19). There is a need for further careful studies, particularly of affected animals born after feed ban, and these studies must include the possibility of material transmission.

Role of PrP Gene

The PrP precursor protein of protease-resistant protein contains 254 amino acids (61). It has been widely accepted that the PrP gene plays a major role in determining the susceptibility to and incubation period of all the diseases caused by SE agents (62). Certainly, the primary structure of the PrP gene in a healthy animal does not differ from that in experimentally scrapie-infected animals (61). However, there are striking differences in the mammalian PrP gene sequence and a great deal of allelic complexity in both

PrP-coding regions and in its flanking regions in sheep (62–64). Host genetic variation is obvious in both natural and experimental scrapie in sheep, and experimental scrapie in mice. The same does not seem to be true of bovine PrP gene.

There are two polymorphisms in the bovine PrP gene-coding region (62). In a study of around 400 cattle, in both BSE affected and healthy cattle no difference was found between the two groups in frequencies of these variant PrP alleles. Transmission of BSE experimentally to mice and to sheep both to "positive" (susceptible) and "negative" (resistant) lines strongly suggest that BSE is not preferentially influenced by certain PrP genotypes. The pattern of lesions seen in BSE is consistent with individual models of experimental scrapie in which mice of a single PrP genotype were infected with a single strain of agent by one route of injection (34). The genetic hypothesis could not explain the outbreak with a simple spontaneous mutation occurring either in the cattle or the scrapie agent. The evidence strongly suggests that the strain of the scrapie agent has a major influence, and maybe there are reasons other than simple association between PrP genotype.

Host range of the Scrapie and BSE Agent

Transmission of BSE to Cattle. Dawson *et al.* (65, 66) inoculated calves from Holstein/Friesian and Jersey (including one Jersey × Limousin). They observed clinical abnormalities in all cattle between 37 and 78 weeks postinoculation. In this study, a uniformity of the experimental disease in all cases indicated that the source of the four inocula, each prepared from a separate case of BSE, and the breed of cows had no significant influence on the experimental outcome. BSE was confirmed in the experimentally inoculated cows by histological examination, thus demonstrating the presence and the transmissible nature of the agent in BSE brains.

Transmission of BSE to Sheep and Goat. BSE was readily transmitted to both sheep and goats both by intracerebral (ic) and oral routes (67). In a wide range of sheep breeds, a single sheep gene called Sip with alleles sA and pA was considered to exert precise control over the timing of appearance of symptoms and incidence of natural scrapie. Based on their response to the scrapie agent challenge. Cheviot sheep at the Neuropathogenesis Unit (Edinburgh, United Kingdom), are divided into "positive" and "negative" lines (68). Both susceptible and resistant lines of sheep developed the disease after inoculation by ic and oral routes with the BSE agent without significant or clear difference in efficiency or incubation periods between the routes or lines. Thus, allelic complexity in sheep PrP gene failed to identify any genotype resistance to BSE (67). Both ic and oral route transmission experiments in mink also revealed a greater efficiency to the agent of a bovine origin compared with the scrapie sheep (36). Comparison of direct transmission to mice from BSE-infected tissues gave the same incubation period to those observed in experimentally passaged BSE cases into sheep, goat, and pig (69).

Transmission of BSE to Monkeys. Experiments on four marmoset monkeys were devised to determine the transmission of BSE. Two of the monkeys were injected with scrapie material, and the other two, with BSE. The two monkeys inoculated with scrapie-infected tissues developed the clinical disease a few months earlier than the BSE monkeys (70).

Transmission of BSE to Mice. BSE has been experimentally transmitted to mice both by intracerebral inoculation and by feeding infected tissues. The recipients of the BSE agent developed the clinical disease at high efficiency and more rapidly than those that received a comparable transmission of sheep scrapie (67, 71, 72). RIII mice had the shortest incubation periods, and there were reproducible differences between mice of different genotypes (67). Transmission of BSE results are interesting: BSE cases transmitted easily to 100% of the mice. The pattern of pathology and incubation periods in standard mouse strains was remarkably similar for all transmissions (73). In comparison, the three transmission attempts from natural sheep scrapie showed no such uniformity, had long incubation periods, and in some cases even failed to produce either clinical disease or pathology.

Comparison of Scrapie and BSE Strain. Many strains of scrapie have been characterized in genetically defined *Sinc* gene mice which has been shown to exert a major influence on the incubation period of experimental scrapie (74, 75). Two alleles s7 and p7 of the *Sinc* gene have been identified with three possible combinations homozygote and heterozygote (76). For strain-typing, BSE was transmitted to mice of different *Sinc* genotypes from seven unrelated BSE cases, collected at different times from separate geographical locations (73). There were large reproducible incubation period differences between mice of different *Sinc* genotypes, but also, unexpectedly, between mouse strains of the same *Sinc* genotype (i.e., the incubation period in RIII mice [*Sinc* s7], 328 ± 3 days, was about 100 days shorter than that in C57BL mice [*Sinc* s7], 438 ± 7 days, while in the F1 cross between C57BL and VM mice [*Sinc* p7], the incubation period was over 700 days, well beyond the incubation period in the two parental mouse strains) (73). The authors compared these results with transmission attempts set up with three brains collected from natural sheep during the same period. The incubation period in RIII mice was 381 ± 11 days, and in C57BL mice, 404 ± 5 days, while in the F1 cross between C57BL and VM, the incubation period was 611 ± 8 days (73). Mean incu-

bation periods in transmission of BSE and sheep scrapie to mice in the *Sinc* p7 gene allele was about 300 days shorter with BSE than with sheep scrapie (73). Further, the two strains of BSE isolated by serial passage in mice of different *Sinc* genotype also differed from all mouse-passaged strains previously characterized (73).

Similar reproducible incubation periods and lesion were observed in transmission studies from cats, greater kudu, and nyala with SEs. They also used brain homogenates from pig, goat, and sheep, all of which had developed clinical disease after experimental infection with BSE, to inoculate mice and compared these with a direct source from BSE (69). All mice developed clinical disease with reproducible incubation periods as in direct transmission from BSE cases (Fig. 2). However, BSE appears to be a new strain of a scrapie-like agent, probably from sheep, having undergone mutation by serial passage in cattle, or it could be that the inadequately high temperatures involved in rendering process have selected relatively heat-resistant strain from sheep scrapie.

Cattle Have Selected a Major New Strain. Transmission to and between inbred mice, and between mice and hamster, has shown that many strains of the causal agents of the scrapie disease exist (75, 76). Transmission experiments of both ic and intraperitoneal (ip) infections show a diversity of animal species that can be infected with the scrapie agent directly or indirectly. Previous studies have revealed that scrapie strain passage among hamster, rats, and mice show the species barrier, which results in a reduced efficiency of infection on interspecies transmission, reduction in incubation period between first and subsequent passage in a new species, and the selection of strains of agent that replicate more quickly in the new host species (77). Comparative transmissions experiments have revealed that the BSE agent shows no change in incubation period between first and subsequent passage in a new species. This would suggest that cattle have selected a different strain of the agent, and this appears to have further infected several other ruminant and nonruminant species without change in phenotype.

Furthermore, selection for a new strain is supported by the uniformity of the incubation periods and lesion profiles in transmission of BSE to each of four inbred strains and one F1 hybrid strain of mice inoculated with seven sources of BSE (67, 73, 78). The distribution of the degenerative lesions "spongiform changes" and the regional intensity of vacuoles appear to be controlled by a number of factors, of which the strain of scrapie agent is the most important. The sustained uniformity of the lesion profile observed in BSE throughout the epidemic to date (67, 73) strongly suggests that the selection of a single major strain of the BSE agent resulted from recycling and had already occurred by the time the disease was first recognized. The argument for selection of a single major stable strain is further supported by transmission of experimentally sheep-goat-pig-passaged BSE in mice, which has given results similar to those from direct transmissions of BSE from cattle (Fig. 2). These transmission studies also revealed that the BSE agent remains unchanged when passaged through a range of species and that "donor" species has little specific influence on the disease characteristic in mice. In mink, sheep, and goats, and on mouse-to-mouse passage of a range of scrapie strains, it has been demonstrated that the ip incubation periods are consistently longer than the ic incubation periods. In contrast, when BSE is transmitted to mice, sheep, and mink there is very little differ-

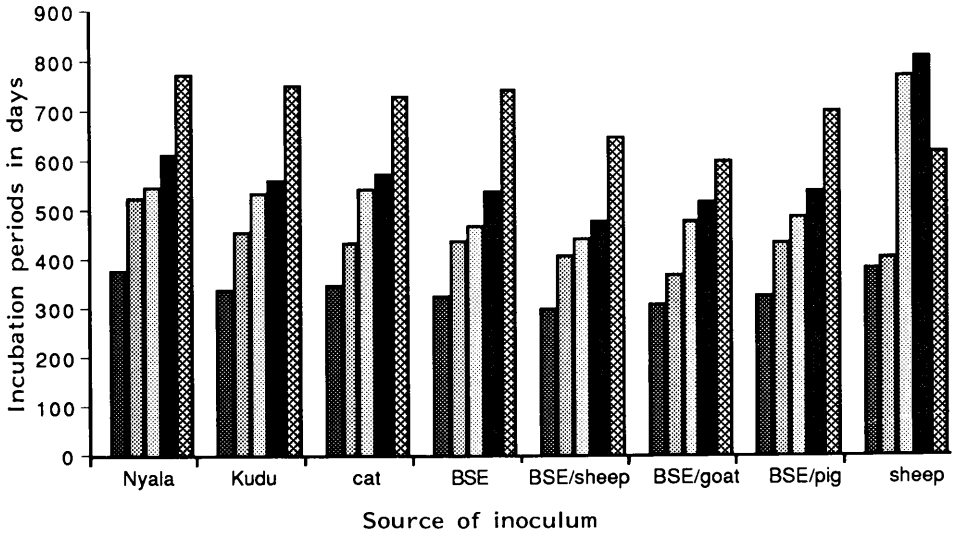

Figure 2. Incubation periods in transmission of spongiform encephalopathy from different sources in different strains of mice. Columns 1 to 5: RIII Sinc s7, C57BL Sinc s7, VM Sinc p7, IM Sinc p7, C57BLxVM. Note difference in the incubation period between the scrapie sheep and from the other source including BSE. (Data take from the following sources Refs. 12, 62, 67, 73).

ence in incubation period between the ic and oral routes (Table I).

All these findings demonstrate that host variations in the host PrP gene or other bovine genes is not a major factor in the susceptibility of BSE. Furthermore, severity and distribution of lesions in BSE brains revealed a uniformity which is not seen in natural scrapie of sheep, suggesting that the cattle-selected strain may have persisted in the epidemic after the initial exposure. The transmission results of all these studies are also dissimilar to those observed from any previous primary transmission of scrapie from sheep or goat source (73). Unique properties of the cattle-selected strain of the BSE agent, which has not been previously identified in scrapie-like pathogen strain, with its relative uniform susceptibility, particularly when there is no change after passaging in various species, and the fact that its ic and ip incubation periods in some interspecies transmissions are not greatly different. From these inoculation studies, one can conclude that almost every mammalian species can be infected by the BSE agent to produce spongiform encephalopathy, and, therefore, humans are at a greater risk of being infected than previously thought. This does not automatically mean that humans will become part of the epidemic. Great care needs to be taken in order for the BSE epidemic to become self-sustained the way that scrapie is, and humans will escape infection.

Selection Process of the BSE Strain

Since in the U.K. rendering process both sheep and cattle remains were included, it is difficult in epidemiological studies of BSE in the United Kingdom to distinguish between the two alternative hypotheses, namely, (i) bovine origin, with an unrecognized reservoir of infection existing in U.K. cattle before the outbreak of BSE and (ii) ovine origin, with the agent having jumped species from scrapie-infected sheep remains to cattle. MBM has also been prepared by rendering in other countries. However, it is important to document whether in other countries cattle offal and their heads with brains were used in their rendering process. It is difficult to determine when cattle remains were included in the rendering process, but during visits in the early 1970s to local abattoirs, the author personally saw the heads of cattle along with sheep heads in the waste skips. However, Wilesmith (55) postulated that the major effect of this would have commenced earlier in 1984/85 and would have continued until the statutory ban on the feeding of ruminant derived protein to ruminants in July 1988.

Bovine Origin Hypothesis. Theoretical possibility exists that there was an unrecognized reservoir of infection in U.K. cattle long before the outbreak of the BSE epidemic. The clinical symptoms of BSE, with the eventual fatal outcome within 6 months, would not have gone unnoticed. Unlike sheep, dairy cows come into daily contact with herdsmen, and even though the disease was not known, from the clinical symptoms that occur at later stages of incubation, it would have been very obvious that a cow with BSE was abnormal, and the disease would have been hard for the farmer or herdsman in daily attendance to miss. Although the disease was unnamed, the clinical symptoms are so distinctive that it would have been difficult to forget the herd history. Furthermore, the death of a cow for unexplained reasons would not have been considered an insignificant loss.

Endemic BSE infection of cattle would have had to be geographically widespread, naturally maintained with a very low incidence by a non-feed source of infection, and may currently exist in other countries where MBM is used in cattle feed. Previously, a single isolated suspected case of BSE was reported in the United States (79). Further support for the bovine origin hypothesis comes from outbreaks of TME on some ranches in the United States. TME is a food-borne disease of mink and use of scrapie-infected sheep in mink feed has been postulated as one possible source of TME (80). Another possible source theory is that cattle acted as the silent host for TME, as the animals which developed mink encephalopathy had consumed food that contained raw meat from dead, sick, or paralyzed "downer" cows as part of their ration (81). Based on the TME outbreaks, using several assumptions it was calculated that in Wisconsin among adult cattle incidence of BSE could have occurred at a rate of 1:900,000 per year (36). This prediction should be investigated by surveillance programs focused on examining brains of extremely old animals. Since not all animals infected with the scrapie agent show typical SE pathology, the bovine brains should be examined by all possible methods available.

Experimental efforts to reproduce TME by intracerebral inoculation of sheep scrapie had marginal success, and oral transmission experiments were not successful (50). More recently, the susceptibility of mink to agents of bovine origin was tested by inoculating and feeding a homogenate made from affected 10% (w/v) bovine SE brain tissues (36). In this study, all the mink, both inoculated or fed developed the clinical disease with incubation periods of 12 and 14 months, respectively. The results of this study indicated that mink, like the "negative line" of sheep, are relatively more susceptible to the BSE agent through both ic and oral transmission of a bovine origin of the agent (36).

Ovine Hypothesis To test the feasibility of the "scrapie sheep" hypothesis it must be first shown whether or not cattle can be infected by the scrapie agent from sheep. Previously, susceptibility of scrapie from sheep and goats to cattle was demonstrated by

simultaneous ic, ip, intravenous, and oral dosing of brain homogenates from scrapie sheep (82). Three out of 10 cows developed typical clinical signs of BSE, and later histological examination of brains confirmed the disease. Recently, the experimental transmission of scrapie to cattle was independently confirmed (36). None of the calves revealed histopathological changes seen in SE; however, the brains were positive for immunohistochemistry for PrP (36). The transmissibility of scrapie to cattle by parenteral exposure shows the susceptible nature but does not address the issue, because the study was carried out with an "American strain" of the scrapie agent, which may not be representative of the scrapie sheep strain in the United Kingdom. Important questions about the interspecies transmission of these diseases are beginning to emerge.

In the same study, four calves each inoculated with different strains of TME isolate showed clinical signs approximately 15 months postinoculation (36). Histopathological examination of brains revealed both astrocytic response and vacuolar degeneration, which were much more extensive than that seen with BSE compared with negative SE pathology in the calves inoculated with the scrapie sheep (36). The extensive vacuolar lesions in the TME-inoculated calves suggest the strains or source of infection can give different pathology in a new host species. The negative histopathological results in significant numbers of clinically suspected cases of BAB suggest that they are indeed BSE cases, infected with a different strain mutant of the agent.

Other Factors Involved in the Origin of BSE

A number of factors have been identified which when combined have some significance to the occurrence of BSE. In order to maximize production of beef, calves, and milk during the Second World War, cattle were fed cheap high-protein chiefly abattoir waste, including sheep brains, processed into meal. In the last decade, MBM has been traded to a great extent on the international market. The total increase from 1960 to 1980 in the amount of compound feed per cow would have been, at maximum, about 50% (83), resulting in a lot more animal protein being fed to cows and a lot more milk being produced. Since 1981, the national sheep flock has increased by 16% due to the introduction of the sheep meat regime (84). There has been a more gradual increase in the number of flocks infected with scrapie. These two changes could account for an increased and wider distribution of the source of infection (85). It is also possible that the species barrier was crossed because of increased exposure to the scrapie agent. However, this experiment has never been done: contaminated ruminant-derived protein feed, MBM theoretically responsible for the infection, has not been used experimentally in laboratory animals to produce the disease. It is hard to understand why this vital experiment has been neglected.

Enzymes in Foodstuff Renderers take the remains of animals from the butchery trade, process the material into tallow or lard, with the residue becoming MBM. Tallow and MBM are supplied to feed compounders for use as ingredients in livestock feed. The function of the feed compounders is totally different from that of renders of animal proteins. The role of the feed compounders is to blend raw materials to provide livestock with balanced rations that will give them the necessary protein energy. A range of raw materials used includes: homo-grown cereals, cereal by-products from the food industry, oil cake, fat, and animal proteins. However, there was no association of the disease with a single compounder of proprietary food.

It appears that all the affected animals received proprietary foodstuff. At the end of the feed preparation process, the solid commercial concentrates, either as finished rations, such as pellets of calf feed and dairy cow cake, or as protein supplements, are combined in home-mixed rations. In order to improve milk production, some feed suppliers supplemented the dairy feed, at a rate of 30 g/head of cattle kg/head/day with enzymes (i.e., α-maylase, β-glucanase, cellulase complex [including pentosanase, xylanase, pectinase, and cellobiase], hemicellulase, protease, lipase anise flavor, and carriers). The enzymes are added in the compounding of the feed stuff to improve the overall digestibility and to release valuable feed nutrients from most of the raw materials. These enzymes are used extensively, while their side effects, if any, on human or animal health in general remains unknown. It would certainly appear that the quantities used will break up the food during digestion, but it is also possible that the aggregates of the scrapie agent, protected in a hard protein shell and present in the feed, would be freed. Further, the enzymes might alter the structure of the intestinal wall, creating portal a route for the agent. This process would help in the agent +'s adaptation from one species to another. Once the infection has been established, the practice of cannibalism would produce the epidemic disease.

Spongiosis Due to Toxins It was considered that the extensive widespread neuronal degeneration might be due to the toxic effect of a plant locoweed, which includes leguminus plants of the genus Astragalus and Oxytropis, and causes chronic locoisum in livestock, including cattle, horses, and sheep. BSE's clinical signs are not like those observed in locoweed poisoning in the United States. The disorder is characterized by motor and sensory nerve damage re-

sulting in peculiarities of gait, impairment of vision, and extreme excitement, sometimes with confusion. Locoweed does not grow in England. Furthermore, an epidemiological study of BSE cases revealed that there was no evidence of any toxic agent being involved in the etiology of the cattle disease (86).

The Organophosphate Link In Britain, the epidemic was identified in the areas that were designated by MAFF as "warble fly eradication zones" about three years previously (87). The farmers in these zones were legally obliged to treat their cattle with one of the three pouron quasi-systemic "phenylphosphorothionate" type of organophosphorus insecticide known as Phosmet, Famphur, and Fenthion (88). In support of the hypothesis that there is a link between the treatment and BSE, Purdey states that he was able to chart the history of cows that died of BSE, the cows having been treated with organophosphorus during their lives, usually 3 years prior to the onset of the disease (87). Furthermore, he gives an example of a farmer who had one of his three farms included in warble fly zone in 1985. The replacement cattle that were moved onto the two farms outside the zone remained BSE free, while a high incidence of the replacements that were moved onto the farm within the zone developed BSE a few years later. Purdey also found farms where cattle had been treated with organophosphorus but had no cases of BSE. He considered that it might be a coincidence that those farmers who avoided the use of organophosphorus substances had no incidence of BSE in the cattle they reared. With time, BSE cases also occurred in all parts of England, Ireland, and other countries, which would discount the possibility that organophosphorus substances were solely responsible for BSE.

Hexachlorohane (Hexachlorophene) Toxicity Hexachlorohane is a bacteriocide and anthelmintic, was marketed as disinfectant, and was used in cosmetic antiseptic soap, talcum powder, and toothpaste. In animals, it had a limited use as an extremely efficient skin antiseptic, particularly as an under wash. Reports of its toxicity in cattle were reported in calves over a period of 3 years (89). The calves had become intoxicated by drinking milk from buckets which had at times been contaminated with under wash containing 9.6 W/v hexachlorohane. Essentially, clinical signs were muscular tremor and nervous degeneration (89). Brain histology revealed no abnormalities, and no cases resembling BSE were recorded in adult cattle.

An important difference between viral encephalopathies and neuro-toxin substances is that the tissues from the former after inoculation or feeding produce a similar predictable disease. Compared with nonliving toxin, after inoculation of a new host, the SE agent replicates and its titer increases with the incubation period. From the known properties of the BSE and scrapie agents, it is unlikely that hexachlorohane is the sole cause of the disease.

Treatment Remission, or Recovery of BSE Cases

There are not a great deal of experimental studies where cattle have been treated for BSE. In experimental animals at all stages of the illness, particularly those in the early phases, placed on a variety of therapeutic regimens, none of the drugs employed could influence the course of the disease. Purdey (87) treated one of his BSE cows after this cow calved in January 1991. She went down with a severe relapse of milk fever, due to complex biochemical disturbances, and the symptoms were considered due to chronic magnesium deficiency (87). The cow was twice injected with calcium borogluconate and magnesium sulphate before she regained health. During the following spring, the cow gradually developed the classic initial symptoms of BSE, including apprehension, an uncoordinated gait and loss of milk yield. In June of the same year, the cow was injected with two bottles of magnesium sulphate. This caused a dramatic remission of the majority of neurological symptoms approximately 30 hr after the treatment. However, after a short remission the symptoms of BSE worsened in the cow and she also suffered weight loss. Diagnosis of BSE was confirmed by histopathological examination of the brain.

Some other farmers who suspected chronic magnesium deficiency produced a similar short-term alleviation of the initial symptoms by treating their cows with magnesium sulphate (87). These cows then went on to be diagnosed after slaughter as BSE positive. MAFF trails with magnesium treatment also showed a temporary remission of BSE symptoms. It is important to stress that true chronic magnesium deficiency cases would recover, while the temporary nature of the remission of clinical symptoms in BSE cases suggests that the underlying degenerative process of the central nervous system continues unhindered.

Eradication of BSE from the Herd

Wilesmith (2–54) states that, to sustain BSE in the cattle population through vertical transmission, each infected individual animal would have to pass on the disease to at least one other individual animal (i.e., 1:1). Most cattle develop the disease at around 4 to 5 years of age, by which time a cow would have produced a maximum of three calves. Given the current pattern of breeding and herd replacement, only one in four (25%) calves produced by an individual cow will enter the adult herd, bull calves and beef breeds being slaughtered before the clinical disease would develop at 18 months to 2 years of age. Therefore, each BSE cow will produce on average less than one female calf that will reach adulthood and herself produce off-

spring. Also, if BSE is maternally transmissible, it may not achieve an incidence of 100%, which in turn would reduce still further the likelihood of an infected cow passing the disease on to successive generations. The first case of vertical transmission where a cow died of BSE and its calf also developed BSE though it had not been fed on the protein food was confirmed in April 1991. In summary, breeding and herd replacement patterns, combined with the age at which BSE commonly develops, make it unlikely that BSE wold be self-sustaining in the national cattle population, even if maternal transmission took place (90).

Comparison of Congenital Mechanism in BSE and Scrapie

Although dissemination of the disease from parent to offspring (i.e., vertical transmission) has been talked about, the vertical transmission in the broad sense includes *utero* exposure. Maternal transmission has been used in many studies to mean vertical transmission, including both prenatal and neonatal infection (91). Attempts to detect the scrapie agent in fetuses of affected sheep have failed; however, the scrapie agent has been detected several times in the placenta from infected sheep. The scrapie agent has also often been detected in various parts of the ewe's reproductive tract (92). What role the placenta and reproductive parts of infected female play during gestation is not known, but the infected organ would form a regular source of extraneural infection in the environment. The influence of the mammary glands, colostrum, and milk in maternal transmission needs further evaluation.

The evidence implicating a hereditary or congenital mechanism in the spread of scrapie among sheep has been suggested throughout the literature. At present it is difficult to prove if there is vertical transmission in BSE, as seen in scrapie sheep. Although about 75% of calves are destined for slaughter for beef at 18 months to 2 years, will the remaining 25% of animals to be used for breeding replacement be free of the BSE agent? From the results available so far, the occurrence of maternal or horizontal transmission cannot be absolutely excluded. If this is the case, then like scrapie in sheep, BSE will become a permanent feature of British cattle and it will not be possible to eradicate the disease. A ban on breeding from affected offspring would slow the spread of the disease. Breeding from animals where BSE has not been confirmed will ensure continuation of the cattle offspring with fear of BSE.

BSE in Other Countries

Exposure of U.K. cattle to BSE has major implications for the risk of BSE's occurring in other countries. The first case outside mainland Britain was in 1988, in Northern Ireland, where over 1000 cases have occurred since. Epidemiological studies suggest that BSE in Northern Ireland did not arise from indigenous scrapie but from MBM imported from Britain (93). The possibility is considered that it is most likely that all cases of BSE outside the United Kingdom have been due to exports of preclinical infected cattle or infected MBM used in feed.

BSE in Ireland The first case of BSE from the Republic of Ireland was confirmed in January 1989, in a 4-year-old Friesian cow from a dairy herd of 25 cows in County Cavan. Up through the end of August 1993 there were 74 confirmed cases of BSE in the Republic of Ireland. Of the 74, ten were imported from the United Kingdom (94), and the remaining 64, with the possible exception of four would have received feed containing MBM.

In the Republic of Ireland, no case has been recorded in animals born since the introduction of a total ban on the feeding of MBM to ruminants. Costelloe (94) suggested that the situation in Ireland is very different from that in the United Kingdom. There is less scrapie, and the ratio of ovine to bovine material rendered is around 1:10. Further, the use of animal meal in feed is about 10 times less in Ireland than in Great Britain, because Ireland practices a traditional, highly extensive livestock production system based on grass and silage. In the Republic of Ireland, the Department of Agriculture and Food introduced compulsory notification of suspected cases of BSE, with restrictions on movement, the destruction of carcasses, payment of compensation, and a ban on the use of milk except to feed a cow's own calf (94). The progeny of affected dams in 1989 have been purchased and held at the Department's Veterinary Research Laboratory Farm at Abbotstown (94). Furthermore, movement restrictions were placed on the herds in which BSE had occurred (94). As these movement restrictions were causing severe financial and management problems for herd owners, a scheme of voluntary depopulation of the herds was introduced. Up to September 1993, 71 herds, comprising 9474 animals, have been depopulated at a cost of £6.7m and depopulation of the remainder is under way (94). The Government in the Republic of Ireland decided to kill all the cattle in a herd on the diagnosis of one BSE case, which removed any chance of vertical transmission in the Republic of Ireland. Depopulation of affected herds formed a major difference between the United Kingdom and the Republic of Ireland; therefore, BSE cases in cattle born after the feed ban in the United Kingdom might be due to vertical transmission.

BSE in Switzerland Since the initial recognition of BSE in Swiss cattle in November 1990, the cumulative incidence is 40 cases, among the total dairy population of 796,000 animals. Switzerland has hardly im-

ported any living cattle from Great Britain in the last decade, though most of the cattle were fed with imported, concentrated rations containing MBM (95). Switzerland has a relatively small sheep population of about 10 sheep/km^2 vs 175 in the United Kingdom. Furthermore, Swiss sheep are free from scrapie. There was one confirmed case of scrapie in a sheep in 1991, one in a goat in 1993, and two more in sheep in 1993. Since the major factors contributing to BSE did not exist in the country, the occurrence of the disease in Switzerland was considered unexpected.

Swiss animals waste renderers always used wet heat "pressure cooking system" at greater than atmospheric pressure (120° or 130°C for 30 or 20 min respectively). However, 22% of all MBM concentrate foodstuff that was imported from 1985 to 1990 came from the United Kingdom, and thus it was concluded that Swiss cases were the result of the importation and the use of contaminated ingredients in concentrated cattle feed (95). The population of cattle at risk comprised dairy cows and beef cows, all of which were exposed to MBM concentrate during calfhood before December 1990. The fact that beef cattle are slaughtered before 18 months of age while the minimum incubation period of BSE is about 2 years excludes the possibility that beef cattle would have developed the clinical disease, although they may have been infected. When the BSE survey was put into effect, out of a total of 215 suspect cattle only 40 (19%) revealed microscopical lesions of BSE (95). This confirmation for Swiss cases is relatively low compared with the relatively high correlation of about 88% between suspected and confirmed cases in the UK (96).

BSE cases in Oman, France, and Denmark Clinical cases of BSE in two Jersey cows were recorded in cattle imported by the Sultanate of Oman (97). These cases were confirmed histopathological by the Central Veterinary Laboratory, Weybridge, England. Both cows were part of a consignment of 14 pregnant cows imported from England in 1985. Investigation of the history of the animals suggested that the cattle were exposed to contaminated foodstuff in calfhood before the cows were exported from England. BSE in other countries has meanwhile been confirmed: six cases in France, one case in Denmark, and one case on the Falkland Islands. The six French BSE cases, which were confirmed out of 112 BSE suspect clinical cases, were in indigenous cattle (98), while in Denmark and the Falkland Islands the cattle were imported from United Kingdom.

Possible Risk of Exposure to BSE in United States and Spain Risk assessment of the possible occurrence of BSE in United States has been carried out (99), and, despite intensive surveillance efforts for the past 3 years, no variant of this disease has been detected in the U.S. cattle population (36). In the United States, 478 flocks of sheep have been diagnosed with scrapie. Marsh has cited the lower prevalence of scrapie in a smaller population of sheep and present practice of feeding MBM as reasons to believe that exposure to BSE has been less in the United States than in the United Kingdom (99). Furthermore, up until 4–5 years ago very little MBM has fed to cattle in the United States. More recently, the trend to feed nondegradable "by-pass" protein resulted in the use of more MBM, hydrolyzed feather meal, fish meal, poultry meal, and blood meal in cattle rations. These products are presently fed mainly to lactating dairy cows, which means that their exposure would be from 2 to 5 years of age on average (99). Since the incubation period for BSE is estimated to be 3–8 years (19), only a small proportion of American cattle would have time to develop BSE before being culled from the herd. It is important to know whether any cattle remains were included with sheep carcasses in the preparation of MBM in the United States or other countries, as appears to be the case in the British foodstuff. Exposure of cattle to MBM prepared from cattle subclinically infected with BSE will remain a threat in America for some years to come. In December 1989, American renderers decided to discontinue processing fallen and sick sheep, and some states have stopped rendering sheep material (99).

Spain in 1990 had the second largest sheep population, with 24.0 million heads, while Great Britain had the largest number of sheep, with 40.8 million heads. In Great Britain, only half the sheep are older than 1 year, whereas in Spain the majority of sheep are older than 1 year. In Spain, scrapie has been diagnosed in a total of 30 sheep flocks and one flock of goats, all from Aragon (100). In the country as a whole, 32% of sheep and goat carcasses go to the rendering industry. The application of solvents in the process of making MBM is not believed to be frequent in Spain, although its use is taken into account by the law (101). It appears that the amount of animal protein that goes to ruminant feed stuff is very low. Of animal protein, that most frequently used for animal feed is fish meal. MBM is used for the feeding of pigs, poultry, and trout.

Relevance of Scrapie to Humans

Scrapie is common in sheep and has been known to exist for at least 250 years, and, of course, sheep and cattle have often shared the same grazing field without the cattle being affected with BSE. Sheep are the only firmly established natural reservoir of SE infections in animals. A major part of the BSE epidemic has been the artificial recycling of the BSE agent, *via* infected feed, of bovine-adapted BSE in the U.K. cattle population. There is no epidemiological evidence

linking scrapie in sheep and CJD in humans. It is the occurrence of BSE (i.e., the crossing of the "species barrier" presumably from sheep to cattle) that raises the question as to whether circumstances have changed such that these agents can be transmitted from animals to humans. This uncertainty was one of the main important reasons that precautionary measures to protect the consumer and prevent the spread of the disease were brought to bear in England.

A number of questions have been asked, as to why and how a disease, particularly one so insidious as scrapie, suddenly and simultaneously erupted all over Great Britain, involving very large numbers of cattle on many farms, instead of emerging erratically in a few small foci? Given this wide range of species crossover, one has to ask, "Why now, and how?" If BSE was a selection or mutation with a changed range, humans like other ruminant and nonruminant species could be at risk.

After many years of research, we still lack the basic understanding of the main issues regarding the route of infection, source of transmission, and causative agent that makes the disease contagious. How much transmission is vertical and how much horizontal? How does the agent replicate and spread within the host body? We need to know answers to all these questions and many more, especially the nature of the agent, its diagnosis, and its control. This information is crucial to controlling the spread of the disease. Most experts will say scrapie had been common in sheep in the United Kingdom since the 1750s, and, of course, we have been eating sheep with no apparent harm to our health. BSE is the same as the scrapie agent in sheep. If it is all right to eat sheep, what harm could come from BSE? Since BSE occurred through the crossing of the "species-barrier" from sheep to cattle and other species, that raised the question as to whether there are circumstances in which these agents could be transmitted to other animals and humans. The most important feature is the selection of a single strain of the agent in cattle, which has a relatively short incubation period and high efficiency of primary transmission to mice. Furthermore, the fact that transmission was equally effective in "positive" and "negative" lines of sheep suggests that the agent selected is more virulent than that taken from the scrapie sheep.

Transmission experiments of BSE with animals, using nonhuman primates, sheep, goats, pigs, mice, hamsters, and many other species, have been carried out to determine susceptibility to the disease. Such experiments using humans would not be permitted. Humans do suffer from similar natural diseases. Creutzfeldt-Jakob disease and kuru, which are infectious. It would be hard to believe that, if other animals are susceptible to the agent of CJD, humans are not susceptible to the agents of scrapie and BSE. No one can predict with any certainty whether in the coming years BSE will infect humans.

Ban on the use of Brain and Offal in Human Foodstuff

Brains and offal must be removed from all cattle over 6 months, and these products must not enter the food chain. However, slaughtermen sawing down the length of potentially infected spinal cords would spray nerve tissue everywhere, and this could thus contaminate the clean meat.

Situation or Scenario

The public health implications of BSE are particularly difficult to address. There may not be evidence that BSE is a risk to humans, and in the short term there is virtually no way of finding out. But the possibility of this risk has to be seriously considered, and the major outcome of this consideration has thus far been the specified bovine offals ban, which was introduced specifically to minimize human risk. The situation arising from feeding cattle scrapie-contaminated feed is clear: cattle are at risk, or, more simply, they are affected. What if the scenario were the same for humans—if the agent of BSE could jump one more species? This raises two questions: how many humans might now be affected without clinical symptoms and how many might develop the clinical disease? Because of the long incubation period, we will not know for some time, maybe years. As in the case of growth hormone prepared from human pituitary bodies, the number of human that are subclinically affected and how many of these will develop the clinical disease remain unknown. It appears that British people are engaged in the world's first natural BSE transmission experiment from cows to humans. This experiment presumably started somewhere in the early 1980s, when subclinically affected cattle were slaughtered and the organs which might have contained high titers of the infective agent were put into human food. Thus, it would appear that a large section of the population has been exposed to the BSE-infected foodstuff for a long period.

In 1995, in the United Kingdom two CJD cases have been reported, one in a 16 and the other in a 18 year old (102, 103), and a third, another 18 year old, is also suffering from CJD. There are a few other unreported CJD cases, in people under the age of 40. Based on the previous experience with the worldwide occurrence of CJD cases, infected British teenagers bring a new twist. What is the source of infection in these young people? Epidemiological studies of the worldwide occurrence of CJD have revealed that there are three types: (i) familial, inheritance CJD cases (104),

(ii) iatrogenic cases, with infection caused by the process of accidental inoculation, and (iii) sporadic cases, which are scattered and spontaneous. Whatever the clinical, pathological form of CJD, be it familial, iatrogenic, or sporadic, a transmissible agent is present in all of them and can be transmitted like other SEs into susceptible hosts. The incidence of sporadic CJD in people under the age of 40 is about 1/20 million (105). In the United States, the first three cases of iatrogenic CJD in those under 30 occurred within 1 year (105). The diagnosis of these cases was considered, but rejected on the basis of the patients' ages. Further investigation of these cases produced vital clues in assessing the modes of transmission of the agent of CJD. In these young patients, the investigators discovered contaminated human growth hormone, prepared from human pituitary glands collected during autopsies, to be the cause of the disease. We must look at the dangers posed by the new strain of BSE. Cow brains were extensively used in sausage meat before the ban was introduced. Animal transmission studies of the oral route have revealed that the BSE agent has a much shorter incubation period; therefore, a possibility exists that humans have been infected.

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