FRI BRIEFINGS

Bovine Spongiform Encephalopathy

An Updated Scientific Literature Review

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Summary

Although the number of cases of BSE has decreased dramatically since peak incidence in 1992, this disease is still of concern as cases continue to be diagnosed in Europe and Japan and two cases have been reported in North America. Recent reports from France, Italy, and Japan presented evidence that more than one strain of BSE exists in cattle. Of particular interest in the UK are the animals born after the 1996 ban on animal protein in animal feed — the so-called BARB (Born After the Real Ban) cohort. According to the latest statistics from the UK there are 70 such cases, including 4 born in 1999.

Another concern in the UK is the possibility that BSE was transmitted to sheep by contaminated feed and the potential for spread of BSE among sheep similar to scrapie transmission. Unlike BSE-infected cattle that have infective prions confined to the nervous system, disease-associated prions have been detected in a variety of tissues in sheep with scrapie or BSE. Most recently, experiments demonstrated that infectious prions are present at low but consistent levels in muscles of some scrapie-infected sheep. Relative prion concentrations indicate that infectivity is about 5000-fold lower in muscles than in brain tissue but these prions were detectable in muscles several months before clinical signs of scrapie appeared.

In North America, chronic wasting disease in farmed and wild deer and elk has been reported from 12 states and two Canadian provinces. Recent experimental data demonstrated that CWD infectivity from decomposed carcasses of deer with CWD and from fecal matter or other material excreted from deer with CWD persists in the environment for over two years. This material has caused CWD in deer newly introduced to infective paddocks. However, cattle grazing in areas inhabited by CWD-infected deer do not acquire this disease. Investigations of 12 TSE cases in persons known to have consumed deer meat concluded that none were related to CWD. Conversion of the human prion protein by CWD-associated prions has been observed in vitro but it appears that CWD is not easily transmissible to humans or cattle.

Variant CJD cases continue to be diagnosed in the UK but the rate of increase in cases is not increasing, leading some experts to revise downward predictions of the future extent of the epidemic. However, all of the vCJD cases tested to date have one specific form of the prion protein. It has been observed in sheep that animals with less susceptible prion genotypes can eventually develop scrapie but the incubation period is longer. Whether this will be the case for vCJD is not known. Screening of 12,674 appendectomy samples in the UK revealed that 3 contained vCJD prions. This suggests that 237/1,000,000 persons may have a subclinical infection and reinforces recommendations to prevent iatrogenic transmission of vCJD.

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http://fri.wisc.edu/docs/pdf/BSE04update.pdf

In early 2004, some published research provided evidence that human vCJD may be transmitted by blood transfusions. Comparison of data on blood donors and recipients and on cases of vCJD in the UK revealed that 48 individuals received a blood transfusion from a person who later developed vCJD. One person developed symptoms of vCJD 6.5 years after a transfusion of red blood cells at 68 years of age (significantly older than the usual vCJD cases). It is possible that both the donor and recipient contracted vCJD by eating contaminated beef but the researchers estimate that the probability of this is about 1/15,000 to 1/30,000. Brain homogenates from monkeys that had been infected with BSE were used to infect other monkeys either orally or by intravenous injection. Survival times were 14 to 26 months shorter for animals infected intravenously, and much higher concentrations of prions were present in spleen and tonsils of these animals. This demonstrates that the intravenous route is a highly efficient means of transmission.

Topical application of TME prions to the tongue following a superficial wound also resulted in a high incidence of disease and a short incubation period in hamsters. This suggests that animals, including livestock and humans, with tongue abrasions may have an increased susceptibility to TSEs when exposed orally to prions.

Although sporadic CJD is generally considered to be distinct from vCJD and unrelated to BSE, some recent data cast doubt on these assumptions. Transgenic mice containing the human prion protein gene with methionine at codon 129 were inoculated with BSE prions, vCJD prions, or prions from cases of sporadic CJD. In some mice the BSE inoculum produced disease symptoms that were indistinguishable from one type of sporadic CJD. This suggests that some sporadic CJD cases may result from BSE exposure. Sporadic CJD cases in Switzerland nearly doubled in 2001 as compared to the previous five years. Analysis of prions indicated that these were not vCJD cases but the authors note that Switzerland had the highest incidence of BSE in continental Europe during 1995–1998.

Research on eradication and control of TSEs in animals has focused on elimination of potentially infectious material from feed, new detection methods for surveillance of live animals and culling of infected animals, and, for sheep, selection for more resistant animals in breeding programs. Strategies proposed to minimize human exposure to TSEs include testing of cattle and sheep, particularly downer and older animals, use of slaughter methods that will not spread central nervous system tissue through a carcass, and use of some disposable surgical equipment. Practical methods for disinfection of equipment and treatment of meat will not destroy all prions. But some procedures reduce levels of infectious prions by several logs and may effectively reduce the possibility for infection from materials with low concentrations of prions.

Numerous new and improved procedures have been described for detection of TSE diseases in animals and humans, disease-specific prions and nervous tissue in foods, and ruminant and other animal proteins in feed.

Bovine Spongiform Encephalopathy

Bovine spongiform encephalopathy (BSE) was first recognized in British cattle in 1986. The peak reported incidence in Great Britain was in 1992, with 36,680 confirmed cases, but cases continue to be diagnosed to this date (1039 cases during 2002; 549 in 2003; 98 as of April 2004). Approximately 180,000 confirmed cases of BSE have occurred in Great Britain (*113*). BSE has also been reported from cattle in several European countries, Israel, Japan, Canada, and the U.S. Current updates on BSE are available on the following websites: Animal and Plant Health Inspection Service (APHIS) (*378*), Office International des Epizooties (*289*), and Department for Environment, Food, and Rural Affairs, UK (*113*).

BSE Cases Reported in:

Country	2004*	2003*	2002	2001	other yr.
Austria		–	0	1	
Belgium	7	15	38	46	
Canada		1	0	0	
Czech Republic	1	4	2	2	
Denmark	0	2	3	6	
Finland		–	0	1	
France	14	137	239	274	
Germany	14	51	106	125	
Greece		–	0	1	
Ireland	47	183	333	246	
Israel		–	1		
Italy		29	38	48	
Japan	2	4	2	3	
Liechtenstein		–	0	0	2 (1998)
Luxembourg	0	0	1	0	1 (1997)
Netherlands	4	18	24	20	
Poland	5	5	4	0	
Portugal	36	133	86	110	
Slovakia	2	1	6	5	
Slovenia	1	1	1	1	
Spain	47	167	127	82	
Switzerland	0	21	24	42	
United States		1			

*These numbers were posted to the Office International des Epizooties web site on May 24, 2004. Actual reporting dates for different countries ranged from May 2003 to May 2004.

BSE surveillance and detection

Cattle are subject to a variety of neurological disorders caused by parasites, bacteria, viruses, mycotoxins, plant toxins, chemicals in the environment, heat stroke, and, of course, prions. These disorders were described and procedures for the differential diagnosis of diseases were recommended for cattle with suspected BSE. The 10 most relevant behavioral signs of BSE were noted. These included hypersensitivity, reluctance to enter and kicking in the milking parlor, head shyness, temperament changes including a panic stricken response, reduced milk yield, and teeth grinding. Ataxia was not a specific sign (*324;325*).

An overview of the European diagnostic hierarchy of officially approved methods for rapid testing and confirmation of suspect cases has been provided. This includes a discussion of the coordination of numerous laboratories involved in large scale testing and quality control measures used to ensure consistent performance of commercially available rapid test kits (*74*). Analytical procedures for specific detection of BSE-related prions are summarized here in the section on Diagnostics (p. 24).

The Office Internationale des Epizooties has developed standards to prevent spread of BSE between and within countries (*272*). However, these recommendations are not always followed (*234*).

A systems dynamics simulation model has been developed to model the risk of BSE in different cattle populations and evaluate the potential for various control measures to mitigate this risk (*163*). Effects of control measures, particularly with respect to cattle feed, were calculated by estimating the importance of different transmission routes and the number of new infections induced by one initial infection. Meat and bone meal from rendered, BSE-infected cattle were a major route of transmission but perhaps not the only route (*109*). Other modeling studies assessed the age distribution of BSE cases and the impact of eliminating animals older than 30 months from the human food chain (*22;110*). Effects of potential changes to current control measures for BSE in the UK were also discussed (*126;139*).

BSE in the UK

Some recent reviews discuss the epidemiology of BSE and its relation to the outbreak of vCJD in humans (*53;65;200;345;346*).

The low level persistence of BSE in the UK despite stringent feed control laws has generated discussion about the origin of these cases. Of particular interest are the animals born after the 1996 ban on animal protein in animal feed — the so-called BARB (Born After the Real Ban) cohort. According to the

latest statistics from the UK, there are 70 such cases: 4 born in 1999, 17 born in 1998, 35 born in 1997, and 15 born after July 31, 1996 (*113*). An epidemiological analysis of 16 of the first BARB cattle reported that 15 were in dairy herds and 8 of these cases were suspect because of clinical signs and 7 were slaughtered as casualties. The one beef cow was apparently healthy but was tested because it was over 30 months of age. Incubation periods for these cattle were longer than was typical of previous BSE cases perhaps reflecting a lower dose exposure. None of the dams or siblings of the cases developed BSE indicating that there was no maternal transmission. The source of infection remains unknown but the author suggested that meat and bone meal imported from other countries may have been incorporated into feed supplements before these countries adopted strict feed controls (*394*). Others have suggested that genetic factors may have a role. Either these cases are "sporadic" mutants of the normal bovine prion or these animals may have a genetic predisposition to acquiring BSE from very low doses of infective material that may be present in their environment (*97;141*).

BSE in Canada and the United States

In 2003, BSE was detected in one cow in Canada and another in the U.S. A summary of the investigation of the Canadian case indicated that the animal was born in Alberta or Saskatchewan, was a 6- to 8-year-old Black Angus, exhibited no neurological symptoms, and was slaughtered in January as a "downer" cow after it contracted pneumonia. During the resulting inquiry into the animal's history and her offspring, 18 farms were quarantined and more than 2700 animals were killed and tested. No other cases of BSE were identified. No meat from this animal entered the human food chain; rather, its remains were rendered and may have ended up in pet food, nonruminant animal feed and fertilizer. It was considered likely that this animal consumed infective feed prior to the 1997 ban on feeding ruminant meat and bone meal to other ruminants (*233*).

Approximately eleven months later, a single downer dairy cow in Washington state tested positive for BSE. The cow was 6.5 years of age, born in Alberta, Canada, and believed to be suffering complications from calving. Its carcass was released for use as food for human consumption. Brain, spinal cord and intestine of the animal were sent for inedible rendering and all potentially infectious rendered products were recovered before commercial distribution. FSIS recalled beef from cattle slaughtered at the same plant on the same day as the case animal (*84*).

An epidemiological perspective on these North American cases of BSE suggests that if these cows acquired BSE from contaminated feed, then it is likely that there were other cattle exposed to this feed in the past and that some other cases of BSE may have occurred. Nevertheless, the number of cases was likely small and the ban on use of ruminant meat and bone meal in feed prevented amplification of the disease. It has been suggested that occasional sporadic cases of BSE may occur in cattle. Increased levels of testing, strict regulations on feed production, and an extension of the ban on use of brain and spinal cord from cattle in human food were recommended but have not yet been enacted (*125*).

BSE in other countries

BSE has been reported from a number of European countries and from Japan. Reports of cases and epidemiological investigations from France (*83;99;124;132*), Italy (*56;75;76;279;381*), Switzerland (*122;123*), Germany (*103;332*), Japan (*376*), and Spain (*29*) have recently been published.

BSE prions and pathogenesis

Disease-specific prions are present in the greatest concentration in the central nervous system of BSE-infected cattle. Analyses of tissues from naturally occurring and experimental orally infected cattle with BSE demonstrated that BSE prions were present in macrophages and Peyer's Patches of the distal ileum of the experimental animals but not in the naturally infected animals. This may be a result of the higher dose inoculum ingested by the experimental animals (*365*).

Three recent reports presented evidence that more than one strain of BSE exists in cattle. Two old cattle (11 and 15 years) tested as part of routine surveillance in Italy were found to have an atypical distribution of protease-resistant prions in the brain and widespread prion-containing amyloid plaques in contrast to typical BSE. Prions isolated from these cases differed from the usual BSE prions in the predominance of the low molecular mass glycoform. This disease appears similar to one type of sporadic CJD (*78*). Three old cows (8–15 years) in France were

found to contain relatively low levels of a new form of a protease-resistant prion in brain tissue. These animals were tested as part of a national program of testing at slaughterhouses and rendering plants. They were not tested because they exhibited suspicious clinical signs (*54*). Atypical protease-resistant prions were also detected in two Holstein steers, 21- and 23 months old, slaughtered in Japan in 2003. These two animals were born after a complete ban on feeding meat and bone meal to ruminants in Japan (*404*).

Metallothioneins are metal binding proteins that may function to maintain homeostasis of essential trace metals in the brain. Analyses of brain tissue from cattle with BSE indicated that levels of metallothioneins I and II were elevated in these samples as compared to brain tissue from healthy animals (*169*).

BSE in sheep

Meat and bone meal from BSE-infected cattle was fed to sheep during the BSE epidemic in the UK and this has raised questions as to whether UK sheep are infected with BSE and whether scrapie and BSE can be distinguished in sheep. Although any sheep originally infected with BSE from meat and bone meal are probably now dead, it is not known whether BSE could spread in a flock of sheep as scrapie does (*198*). Experimental feeding of sheep with BSE-infected bovine brain does induce spongiform encephalopathy in susceptible animals (*209*). Naturally occurring BSE has not yet been identified in sheep with symptoms of scrapie but surveillance has not included large numbers of infected flocks nor has it targeted fallen stock (which are more likely to test positive). Estimates of the possible prevalence of BSE in UK sheep range from a "low prevalence" (*217*) to a possibility that 9% of scrapie cases are actually BSE (*159*). An estimate of the human health risk from possible BSE infection of sheep in the UK concluded that if sheep were infected with BSE then ongoing public health risks are likely to be increased unless there are additional restrictions on sheep products in the food supply (*140*). A recent review discussed data on experimental BSE in sheep, aspects of risk management, and possible techniques for distinguishing BSE from scrapie (*331*).

Sheep infected orally with BSE were found to contain infective prions throughout the central nervous system and also in the peripheral nervous system, lymphoid tissue and parts of the digestive tract (*145*). Blood transfused from sheep infected with scrapie or BSE to other susceptible sheep resulted in disease in 17–19% of recipient sheep within 538 to 737 days after transfusion (*199*). Histochemical analysis of brain and lymphoreticular tissue in scrapie- and BSE-infected sheep demonstrated that there were differences in the pattern of accumulation of prions in phagocytic and brain cells (*208*). Other molecular analyses of prions from BSE- and scrapie-infected sheep using different monoclonal antibodies demonstrated differences in the mass of the non-glycosolated form of the prion in the brains affected by the two diseases. This glycoform analysis could be used in distinguishing the two diseases (*282;356*).

Some sheep are genetically resistant to scrapie and apparently to BSE as well. Sheep with the scrapie-resistant genotype ARR/ARR did not develop BSE during five years of followup after oral inoculation. However, intracerebral inoculation of some ARR/ARR sheep with BSE-infective material did result in disease after nearly 3 years (*195*).

BSE in other animals

Concurrent with the BSE epidemic, transmissible spongiform encephalopathies occurred in domestic cats and in a number of zoo animals. Seven species of zoo ruminants (including bison), six species of wild cats, and four species of primates were affected in addition to 87 domestic cats in Great Britain and smaller numbers of domestic cats in other European countries. Analyses of the affected animals indicated that most were infected by consumption of meat and bone meal from BSE-infected cattle. Horizontal transfer may have occurred among some zoo ruminants (*340;373;414*). Analyses of infectivity of various tissues from infected greater kudu demonstrated the presence of low titers of infectivity in salivary glands, conjunctiva of the eye, and skin from the flank, in addition to relatively high titers of infectivity in nervous, gut, and lymphatic tissues (*414*).

BSE was passaged through a macaque monkey (*Macacca fascicularis*) and then a primate microcebe (*Microcebus murinus*). Both brain tissue and buffy coat protein (from blood) from an affected microcebe caused BSE in microcebes when inoculated intracerebrally (*57*). In early 2004, a research paper provided evidence that macaque-adapted BSE, which produces

a disease similar to vCJD in monkeys, may be transmitted both orally and by blood transfusions. Small amounts of brain homogenate from cynomolgus macaques with BSE were injected intravenously or fed to other macaques causing BSE. Incubation periods were 14 to 26 months shorter in macaques infected by injection. Infectivity was detected in the spleen, tonsils, the entire gut, peripheral, autonomic, and enteric nerves as well as the central nervous system of all macaques (*178*).

Undoubtedly pigs, poultry, and fish were also fed BSE-contaminated meat and bone meal during the UK BSE epidemic but no transmissible encephalopathies have been described in these species. Experimental feeding of chickens and pigs with high levels of brain tissue from BSE-infected cattle and scrapie-infected sheep (pigs only) produced no evidence of disease transmission. Intracerebral inoculation of BSE-infective material did not induce TSE disease in chickens but did cause TSE symptoms in pigs. Infective prions were detected in the brain, spinal cord, stomach, intestine and pancreas of affected pigs. No infectivity was detected in any tissues of pigs that were fed BSE-containing brain tissue (*257;390*). No published experimental data have been reported for fish although DNA sequences coding for prion proteins have been detected in salmon and pufferfish (*290*).

Transgenic mice containing the human prion protein gene with methionine at codon 129 were inoculated intracerebrally with BSE prions, vCJD prions, or sporadic CJD prions. BSE and vCJD inocula produced a similar neurological and pathogenic phenotype, providing further evidence that vCJD is derived from BSE. Interestingly, in some mice the BSE inoculum produced disease symptoms that were indistinguishable from one type of sporadic CJD. This suggests that some sporadic CJD cases may result from BSE exposure. Some mice inoculated with BSE developed a subclinical infection that was only diagnosed after death (*23*). In other experiments with four strains of mice, there was a longer incubation period for females than for males after intracerebral inoculation with BSE (*4*).

Other Spongiform Encephalopathies in Animals

Scrapie

Reviews. Clinical signs, pathology, and diagnosis of scrapie were recently reviewed (*396*). Another recent review updated information on the epidemiology of scrapie, its geographical distribution, transmission, dynamics within a flock, and risk factors for spread between flocks. Some current national scrapie programs were described (*119*).

Genetic Susceptibility/Resistance. It is well recognized that individual sheep vary in their susceptibility to scrapie depending on the amino acids present at three positions (*136;154;171*) in their prion protein. Data from sheep surveys indicate that there are five common alleles coding for the normal prion protein. The ARR allele (containing alanine, arginine, arginine at the critical positions) is associated with greatest resistance to scrapie while the VRQ allele (containing valine, arginine, glutamine) is linked to the highest susceptibility. Several studies have documented the prion alleles found in sheep with scrapie and in healthy flockmates and have derived risk estimates for different combinations of alleles (*42;131;285;372*). The EU has developed a framework for recognizing scrapie resistance in flocks depending on whether all sheep are ARR/ARR (Level I) or whether all rams producing progeny are ARR/ARR and ewes contain other alleles (Level II) (*2*). Published papers report variations in the prevalence of these alleles in different sheep populations, their potential susceptibility to scrapie, and prospects for breeding resistant flocks of sheep. Although scrapie has not been described from Portugal or New Zealand, sheep in these countries are susceptible to this disease (*8;129;194;276;291;379*).

Genotyping and immunoassay testing for scrapie prions in 25 clinical cases of scrapie from four flocks of sheep and in 159 cull sheep from the same flocks demonstrated that not all susceptible sheep come down with scrapie even when the disease is present in their flock. Of the clinical cases, 24 were ARQ/VRQ and one was VRQ/VRQ; of the cull sheep, none of 2 VRQ/VRQ sheep, 2 of 40 ARQ/VRQ sheep, and 3 of 23 VRQ/ARR had scrapie prions. None of the other genotypes tested positive for scrapie (*206*).

Studies with goats indicate that variations at positions 143 and 154 of the prion are important determi-

nants of susceptibility to scrapie. Some goats were found to harbor protease-resistant prions even though they were clinically normal (*55*).

Disease Process. Previous reports have described two clinical presentations of scrapie. In one of these, sheep present initially with signs of intense pruritis with scraping against posts to relieve the itching; in other cases, sheep have difficulty coordinating muscles (ataxia) and weakness particularly in the hind limbs. A study of 129 Irish sheep cataloged early and late symptoms of scrapie and attempted to find correlations with prion genotypes. Pruritis and teeth grinding were associated with a positive nibble reflex and this in turn was associated more often with certain genotypes (*172*). Clinical signs in sheep infected with scrapie overlapped significantly with those observed in sheep infected with BSE. However, pruritis was the first recorded symptom in 79% of scrapie sheep but only in 42% of the BSE sheep. Ataxia was more frequently the presenting sign in BSE sheep (32%) than in scrapie sheep (9%) (*193*). In mice and hamsters infected with scrapie, some behavioral changes are evident before clinical signs appear (*34;115*).

Recent experiments demonstrated that infectious prions are present at low but consistent levels in muscles of some naturally and experimentally scrapie-infected sheep. Relative prion concentrations indicate that infectivity is about 5000-fold lower in muscles that in brain tissue. Abnormal prions were detectable in muscles several months before clinical signs of scrapie appeared (*14*).

Following an oral scrapie infection of lambs, scrapie prions were detected in tonsils, lymph nodes and spleen after only five weeks (*175*). Peripheral lymphoid tissues appear to be the location for prion replication. A substantial network of nerve fibers has been detected in lymphoid tissue, and these are likely to mediate invasion of the enteric nervous system by disease prions (*174*). Follicular dendritic cells in the spleen of scrapie-infected hamsters were found to have high titers of infectivity and appear to function in propagating the infection to the central nervous system (*25*). Other studies with hamsters identified the vagus and splanchnic nerves as routes carrying infectious prions from the gastrointestinal tract to the brain and spinal cord (*261*). In vitro cultures of mouse brain cells from a scrapie-infected mouse were able to transfer scrapie prions to other noninfected cells when the two cell types were cultured together (*216*).

Scrapie prions were detected in the central and peripheral nervous systems, lymphoid tissues, and gastrointestinal tract of a goat with natural scrapie (*380*). Accumulation of scrapie prions in different cell types has been investigated by several research groups (*12;107;137*). One report describes the widespread presence of scrapie prions in skeletal muscles of infected hamsters (*371*).

Scrapie Prion Strains. Several different scrapie strains have been detected in naturally infected sheep. At least two prion strains have been identified in Italian sheep with iatrogenic scrapie (*407*). Strain differences may affect the accumulation of protease-resistant prions in different areas of the brain and infectivity for mice. Prion strains from Japanese sheep were found to induce disease in mice after incubation periods ranging from 133 to >400 days. Co-infection of mice with two of these strains showed that neither strain interfered with the replication of the other (*187;189*). A new strain of scrapie was associated with five unusual cases of scrapie in Norway. These occurred in sheep with genotypes rarely associated with scrapie and the prominent neurological symptom was ataxia. Molecular analysis indicated a distinct form of the scrapie prion which also differed from the BSE prion (*47*).

Meat and bone meal from BSE-infected cattle was fed to sheep during the BSE epidemic in the UK, raising questions whether scrapie and BSE can be distinguished in sheep and whether UK sheep are infected with BSE (*198*). Experimental feeding of sheep with BSE-infected bovine brain does induce spongiform encephalopathy in susceptible animals (*209*). Histochemical analysis of brain and lymphoreticular tissue in scrapie- and BSE-infected sheep demonstrated that there were differences in the pattern of accumulation of prions in phagocytic and brain cells (*208*). Other molecular analyses of prions from BSE- and scrapie-infected sheep using different monoclonal antibodies demonstrated differences in the mass of the non-glycosolated form of the prion in the brains affected by the two diseases. This glycoform analysis could be used in distinguishing the two diseases (*282;356*).

Transmission/Epidemiology. Transmission of scrapie among sheep is believed to be horizontal, from one animal to another in a flock, and possibly also verti-

cal, from mother to offspring. Placental tissue from some scrapie-infected ewes is known to be infective but it is not known whether the developing fetus can acquire scrapie through the placenta. Analyses of placental tissue and genotyping of ewes and their offspring demonstrated that the placenta was infective only if the mother harbored scrapie prions and if both mother and lamb were of a scrapie-susceptible genotype. Despite the presence of scrapie prions in the placenta, no scrapie prions were detected in tissues from lambs 8 days before lambing, at birth, or 10 days after birth. Three weeks after birth, disease prions were detected in the intestinal tract of some lambs in the flock even if their mothers were not carrying scrapie. This suggests that lambs become infected after birth (*13*). Another study, using naïve sheep from New Zealand introduced into a flock with known scrapie, demonstrated that the introduced sheep acquired scrapie by lateral transmission (*321*).

Prevalence of scrapie in Great Britain, based on an abattoir survey, is estimated at 0.22% (*162*). A potentially useful method for active surveillance of scrapie is sampling and analysis of tissue from the third eyelid of target sheep (*286*). Studies of the population dynamics of scrapie in sheep flocks indicate that the disease is more common in ewes than rams and that older animals are less susceptible to the disease. Sheep with different susceptible genotypes appear to have different incubation periods. Modeling of the dynamics of a scrapie outbreak indicates that the mean incubation period is less than 1.5 years (*164;259;313*).

Intracerebral inoculation of the brain tissue from scrapie-infected sheep into raccoons (*168*) and elk (*167*) successfully induced a spongiform encephalopathy in these animals. It is not known whether they would be susceptible to an oral challenge.

Chronic Wasting Disease (CWD)

Reviews. Chronic wasting disease has been found in both captive and wild deer and elk (cervids) in the U.S. and Canada. A review in 2002 described the epidemiology and prevalence of this disease, clinical signs, diagnostic methods, and control strategies (*398*). Reviews in 2003 summarized pathogenesis of CWD and discussed possible origins of this disease, its transmission, species susceptibility, surveillance systems, and control strategies (*328;397*). Scrapie and chronic wasting disease share several characteristics: lateral transmission among animals, presence of infectious prions in lymphoid tissues early in the disease, pathogenesis, and clinical presentations. Differences and similarities between these diseases were recently reviewed (*396*). Evidence related to the potential for transmission of CWD to humans consuming infected deer tissues has been reviewed. Conversion of the human prion protein by CWD-associated prions has been observed in vitro but investigations of 12 cases of apparent TSEs in persons known to consume deer meat concluded that none was related to CWD. Further monitoring and epidemiological studies should be continued to address this question (*45*).

Prevalence. CWD was first detected in deer and elk in Colorado and Wyoming. Since then the disease has also been detected in captive deer and/or elk in Kansas, Minnesota, Montana, Nebraska, Oklahoma, South Dakota and Wisconsin, and in Alberta and Saskatchewan in Canada. It is also present in wild deer and/or elk in Illinois, Nebraska, New Mexico, South Dakota, Utah, Wisconsin, and Saskatchewan (*18;205;213;280;392*). In addition, one case of CWD was reported in Korea in 2002 in an elk imported from Saskatchewan in 1997. Several other cases of CWD occurred in the farm of origin after the elk was sent to Korea (*347*).

Epidemiology. Mechanisms for the spread of CWD among wild and captive cervids appear to be primarily lateral (directly from one animal to another) rather than maternal (from mother directly to offspring). In other words, CWD behaves like an infectious disease. Observations of cohorts of deer at a research facility in Colorado indicated that maternal transmission contributed little, if any, to the occurrence of CWD. Offspring of uninfected does were just as likely to contract the disease as offspring from infected females (*267*). This is in agreement with the fact that CWD prions are found in lymphatic tissues associated with the gastrointestinal system but are not detectable in the placenta of infected deer and elk.

Infectious prions may be deposited in the environment where they can bind to clay particles in the soil and may be consumed when deer and elk feed (*294*). A recent series of experiments with mule deer demonstrated that animals could become infected when they were introduced into paddocks in which CWD-infected deer carcasses had decomposed in situ and into paddocks in which infected deer had last resided

over two years previously. Infectious prions persisted in the environment of these paddocks for at least two years. Deer also became infected when introduced into a herd containing known infected animals (*268*).

The rationale and data that support models for spread of CWD have been critically examined (*329*). The dire predictions of some models are based on the theory that CWD is spread strictly by frequency-dependent transmission, where transmission is strictly a function of the proportion of infectious individuals in a population regardless of population density in an area. According to these analysts, data so far do not support a strict frequency-dependent model; rather transmission of CWD is likely also a function of the density of animals and distribution of social groups in an area. An understanding of mechanisms of transmission is important because different modes of transmission have different implications for management strategies.

In humans and sheep, some genetically determined prion protein sequences have been shown to be more susceptible to conversion to the disease-associated form than others. Recent analyses of prion protein genes in 26 CWD-positive and 100 CWD-negative white tailed deer in Wisconsin identified four different alleles of the prion gene. Although one allele was overrepresented in infected animals, at least 86–96% of deer tested had prion protein combinations that could support CWD. This suggests that the deer herd in south-central Wisconsin does not have a significant natural resistance to CWD (*211*). Another investigation has documented some variation in prion genes in mule deer, but whether these differences are related to susceptibility to CWD is not known yet (*64*).

Reports of three cases of CJD in the U.S. in unusually young people (28–30 yr.) who regularly consumed deer or elk meat have raised concerns about the possible transmission of CWD to humans. The deer and elk consumed by the patients were not known to have come from endemic areas although some of the meat did originate in Wyoming. No strong evidence for a causal link between CWD and these cases was found but neither was it ruled out (*43*).

Prion diseases are usually restricted to one or a few closely related species. A survey was conducted to determine whether beef cattle grazing for at least four years in a CWD-endemic area of northeastern Colorado showed any signs of TSE-infection. Although the cattle had likely been exposed to cervids with CWD and to an environment that presumably contained the infectious agent, none of the 262 animals tested showed any indication of disease. This is consistent with other data indicating that cattle have a very low susceptibility to CWD (*157*).

So far CWD has not been detected in studies of caribou in northern Quebec (*239*), white-tailed deer in Missouri (*50*), white-tailed deer in the wild in Minnesota (*269*), or in wild cervids in Montana (*280*). Attempts to infect raccoons intracerebrally with brain tissue from CWD-infected mule deer did not produce a TSE after three years (*168*).

Disease Characteristics. Examination of the brains of diseased mule deer and elk revealed a pattern of widespread spongiform changes characteristic of all TSEs, with intracytoplasmic vacuolation of neurons, neuronal degeneration and amyloid plaques (*355*). These amyloid plaques react immunologically with antibodies raised against scrapie amyloid. A substantial proportion of plaques were described as florid (*245*). Behavioral and physiological changes in a Rocky Mountain elk with CWD were described (*32*).

Recent evidence indicates that abnormal prions are present in the tonsils, pituitary gland, adrenal glands, and pancreas of CWD-infected deer (*341*). Both deer and elk with CWD accumulate disease-specific prions in lymphoid tissue, including tonsils and some tissue associated with the intestines. Recent research demonstrates that these prions are present in the palatine tonsil before they can be detected immunohistochemically in the brain and before clinical signs of disease appear (*354*). Analyses of tonsil tissue from deer and elk have been used to test for CWD (*180;266;287;353;393;401*).

A close examination of lymphoid tissue revealed that the CWD prions accumulate primarily extracellularly and are associated with follicular dendritic cells and possibly with B cells. Prion aggregates were also observed in macrophages. These cell types may be involved in prion replication but their specific roles are not yet known (*339*).

Transmissible Mink Encephalopathy (TME)

A review in 2003 briefly summarized available information on the origins, transmission, and risk factors of TME. No recent cases of TME have been reported (*397*).

In hamsters, TME causes two different sets of symptoms: "drowsy" (DY) with a very long incubation period and "hyper" (HY) with a shorter incubation period (*52*). Experiments have demonstrated that DY TME prions can interfere with the replication of the pathogenic HY TME prions in hamsters under some circumstances. Intraperitoneal inoculation of hamsters with DY TME prions 60 days prior to inoculation with HY prions significantly extended the incubation period normally observed for HY prions. It appears that there is some competition between these two agents at some cellular replication site prior to invasion of the nervous or lymphoreticular system (*37*).

Inoculation of the sciatic nerve of hamsters with HY prions demonstrated that these agents were capable of retrograde travel (opposite to the direction of travel of nerve impulses) along the nerve to the spinal cord and then to the brain. Prions appeared to ascend the spinal cord at a rate of 3.3 mm/day (*38*). Inoculation of HY prions into the tongue of hamsters resulted in TME symptoms after an average of 79 days. This compares with an average incubation period of 68 days following inoculation of the sciatic nerve and of 191 days following oral ingestion of HY prions. Topical application of TME to the tongue following a superficial wound also resulted in a higher incidence of disease and a shorter incubation period. This may have implications for an increased susceptibility of livestock and humans with tongue abrasions who are exposed orally to prions (*39*).

Feline Spongiform Encephalopathy (FSE)

Three cases of feline spongiform encephalopathy were reported for two captive zoo animals and a house cat in the past two years. A six-year-old Burmese house cat became the first diagnosed case of FSE in Switzerland. The cat was brought to the veterinarian after it had seizures and trouble walking (*116*). A ten-year-old German zoo-borne Asiatic golden cat that lived in German and Dutch zoos for six years before importation to an Australian zoo was diagnosed after death with a preclinical case of FSE. The animal died of other causes, but histopathological examination of the brain after death revealed some spongiform changes and FSE prions (*406*). An immunohistological analysis of 27 different tissues from a cheetah with FSE, born in France, revealed that FSE prions were present in the brain, spinal cord, retina, peripheral nerves, lymphoid tissue, adrenal glands, and kidney. These results suggest the possibility that disease prions could be present in urine (*244*). All of these cats were believed to be infected by consumption of feed containing meat and bone meal from BSE-infected animals.

Spongiform Encephalopathies in Humans

Human spongiform encephalopathies have been the subject of several recent reviews (*94;386;395*). These diseases are relatively rare and at present there is no effective treatment. Therefore, it is important to understand how they are acquired so that transmission can be prevented. The Centers for Disease Control in the U.S. has a program for surveillance monitoring of various forms of CJD in the U.S. (*44*).

One human TSE, fatal familial insomnia, is associated with a dominant mutation of the prion protein. In a preliminary experiment to determine whether SIDS (sudden infant death syndrome) might be associated with abnormal prions, researchers examined brain tissue from two SIDS victims and from an adult dying of carbon monoxide poisoning. Protease-resistant prion fragments were detected in some brain areas of the two SIDS victims but not in the control brain tissue. Sequencing of the DNA coding for a region of prion protein from one SIDS child revealed some differences from DNA coding for normal prions. Further research on samples from more SIDS cases is needed (*49*).

Creutzfeldt-Jakob Disease (CJD)

Most cases of CJD are sporadic, having no known source of infectious agent and occurring in people 50–70 years of age. Approximately 10–15% of cases of CJD appear to result from genetic transmission. In addition, there have been a number of cases of inadvertent iatrogenic transmission of CJD during medical procedures. The BSE epidemic has been linked to cases of variant CJD (vCJD), which generally occur in younger people. Several clinical, physiological, and prion structural features can be used to differentiate among these forms of CJD, but the differences are not always straightforward (*204;255*).

Over 250 cases of iatrogenic CJD have been documented. Most of these cases were acquired by injec-

tions of contaminated growth hormone from the pituitary gland or transplants of contaminated dura mater from the brain from cadavers with sporadic CJD. Some other cases occurred after corneal transplants, gonadotropin injections, and use of contaminated surgical instruments or encephalographic needles (*315*). A case of iatrogenic CJD was described in a 58-year-old person who had received a dura mater transplant 20 years prior to death. Florid plaques, similar to those seen in vCJD, were present in the brain but glycoform analysis of prions from this case differed significantly from those observed for vCJD (*230*).

Sporadic CJD

Clinical, pathological, and molecular studies of 89 cases of sporadic CJD revealed the presence of 4 types of protease-resistant prions that differed in molecular structure and in the relative amounts of di-, mono-, and unglycosylated prions. These prion types were different from those detected in cases of vCJD. Some prion types were associated with certain amino acids at codon 129 (methionine or valine) and there were some differences in average onset of clinical illness and its duration. As with kuru and vCJD, CJD cases occurred most often (57 of the 89 cases) in persons with methionine-methionine at codon 129 (*183*). Analyses of samples from a population in Crete demonstrated that a relatively high incidence (57%) of methione-methionine genotypes was associated with a relatively high incidence of sporadic CJD (*298*).

Although sporadic CJD is generally considered to be distinct from vCJD and unrelated to BSE, some recent data cast doubt on these assumptions. Sporadic CJD cases in Switzerland nearly doubled in 2001 as compared to the previous five years, and preliminary results for 2002 indicated that CJD cases were continuing to increase. Glycoform analysis of prions from the 2001–2002 cases indicated that these were not vCJD cases. This sharp increase in sporadic CJD cases has not been explained as yet, but the authors note that Switzerland had the highest incidence of BSE in continental Europe during 1995–1998 (*153*).

Transgenic mice containing the human prion protein gene with methionine at codon 129 were inoculated intracerebrally with BSE prions, vCJD prions (pathogenic prion type 4), and prions from cases of sporadic CJD (prion types 1, 2, and 3). BSE and vCJD inocula produced a similar neurological and pathogenic phenotype, providing further evidence that vCJD is derived from BSE. Interestingly, in some mice the BSE inoculum produced disease symptoms that were indistinguishable from type 2 sporadic CJD. This suggests that some sporadic CJD cases may result from BSE exposure. Some of the mice inoculated with BSE or vCJD developed a subclinical infection that was only diagnosed after death. This raises concerns that there may be a significant number of people who were exposed to BSE who are now carrying a subclinical infection and some of their tissues may be infective to others (*23*).

Protease-resistant prions are present in the brain and spinal cord of sporadic CJD patients (*171*) and have more recently been detected in peripheral nerves (*138*), spleen and skeletal muscle (*152;227*), and olfactory epithelium (*360;408*) of some sporadic CJD patients.

Variant Creutzfeldt-Jakob Disease (vCJD)

A new type of CJD appeared in the UK in 1994–1996. This disease differed from the familiar sporadic CJD in several ways. It affected much younger people, with an average age of 26 years as compared to 64 years for classical CJD, and the clinical phase of the illness lasted about 10 months longer. As of the end of April 2004, 146 probable and confirmed cases of vCJD have been diagnosed in Great Britain (*117*) and there have been 10 cases identified in other countries: 6 in France, 1 each in Ireland, Italy, Canada, and the U.S. (*235;387*). The case patients from Ireland, Canada, and the U.S. spent significant periods of time in the U.K. and are believed to have acquired the infection there.

Although the total number of cases of vCJD identified so far is relatively small, there has been a steady increase in the number of cases during the past several years. Attempts to predict the future extent of the vCJD epidemic have produced very different estimates, ranging from hundreds to thousands of cases. One recent analysis of incidence trends indicates that the underlying incidence (based on date of symptom onset) is increasing by about 18% per year (*15*). The extent of exposure, incubation period, and susceptibility of people with different prion genotypes and at different ages are uncertain and so it is difficult to predict the magnitude or length of the outbreak. Data on patients indicate that persons aged 10 to 20 years are at highest risk of infection, and some analysts

predict that three-fifths of new cases will be male. Incubation periods appear to range from 11 to 17 years on average. The epidemic is expected to continue for some time (*96;101;150;382*).

At present all genetically analyzed vCJD patients have been found to have only methionine at position 129 in the prion protein. It is not yet known whether people with other genotypes are also susceptible to this disease but have longer incubation periods. If so, there may be another surge of cases at some time in the future (*387*). Among UK blood donors, the methionine-methionine genotype was present in 42% of samples, as compared to 34% of Irish blood samples, and 49% of Finnish samples (*284*).

Several review articles have discussed the origin, pathology and epidemiology of vCJD (*73;201;202; 345;374;387*). A great deal of evidence indicates that vCJD originated when susceptible people consumed central nervous tissue from BSE-affected cattle. Neuropathological examination of the brains of inbred strains of mice revealed no significant differences between those infected with BSE from cattle and those challenged with vCJD from human cases (*65*).

In early 2004, a research paper provided evidence that human vCJD may be transmitted by blood transfusions. Comparison of data on blood donors and recipients and on cases of vCJD in the UK revealed that 48 individuals received a blood transfusion from a person who later developed vCJD. One of these individuals developed symptoms of vCJD 6.5 years after a transfusion of red blood cells at 68 years of age (significantly older than than the usual vCJD cases). The blood donor in this case did not exhibit symptoms of vCJD until 3.5 years after the blood donation. It may be that both the donor and recipient contracted vCJD by eating contaminated beef, but the researchers estimate that the probability of observing a case of vCJD in a transfusion recipient in the absence of transmission by transfusion is about 1/15,000 to 1/30,000. Seventeen people (currently aged 29–88 years) who received blood products (from persons later diagnosed with vCJD) one to nine years ago remain alive and do not as yet have symptoms indicative of vCJD (*247*).

It has been proposed that the number of vCJD cases is too small for a foodborne epidemic with such a broad population exposure and that vCJD is merely a rare clinical form of sporadic CJD that may have a common etiology with BSE (*240*). Another proposal was that humans acquired a rodent form of a TSE from ingestion of rodent parts or droppings (*95*).

Variant CJD has many similarities with other human spongiform encephalopathies, but there are pathological differences and laboratory tests that can be used to diagnose vCJD (*203;412*). Examination of the cerebrum and cerebellum of the brains of the victims of vCJD revealed an extensive distribution of florid plaques — large rounded amyloid deposits with a dense central core surrounded by peripheral amyloid fibrils and a rim of spongiform change. The pattern of these lesions was examined in brain tissue from 11 vCJD patients to determine whether it was consistent with transport of abnormal prions by blood. Florid plaques were spatially related to blood vessels and pathological lesions of vCJD (*21*).

Brain lesions cause numerous physical and psychological symptoms. Neuropsychological profiles of 24 vCJD patients indicated an overall pattern of impaired performance particularly on tests of memory, executive function, speed of attention, and visuoperceptual reasoning (*218*).

Disease-specific prions are found in tissues outside of the brain and spinal cord in vCJD patients. These prions have been detected by immunohistochemical staining in neurons of the sympathetic nerve ganglia (*165*) and in tonsils, spleen, lymph nodes, and appendix (*171;312;323*) of vCJD patients. Abnormal prions are not always detectable in the appendix (*212*) but were found in 19 of 20 appendixes removed at autopsy from patients with vCJD and in 2 of 3 appendixes that were removed from patients that later developed vCJD symptoms. Screening of 8318 samples of appendixes and tonsils from asymptomatic people in the UK found only one positive sample (*186*). Further testing of tonsil or appendix tissue from 12,674 persons in the UK revealed three appendectomy samples positive for vCJD (*185*).

Kuru

Kuru is a human spongiform encephalopathy that was spread by ritual cannibalism among the Fore people in New Guinea (*192;258*). In mid-1950s there were more than 200 new cases reported per year. The incidence of kuru declined thereafter as cannibalism ceased but a few cases were reported annually into the early 1990s. An analysis of available data on the kuru epidemic, based on the assumptions that the epidemic

arose from a single case of sporadic CJD that occurred around 1900 and that the number of new kuru cases was proportional to the number of cases dying each year, estimated that the mean incubation period was 10 to 13 years from infection to death. However a small number of cases occurring in the early 1990s indicates that the incubation period can exceed 40 years. Incubation periods for adult females were nearly half that for adult males, presumably reflecting their greater exposure to infectious material (*100*). Persons who were methionine-valine heterozygotes at prion codon 129 had significantly longer incubation periods than persons homozygous for methionine or valine. Prion diseases may have exerted a significant selective pressure favoring persons heterozygous at codon 129. Among older Fore women, 23 of 30 women tested were heterozygotes; among younger unexposed women, the proportion of heterozygotes was lower (*263*).

Prions as Causative Agents of TSEs

TSEs and some other neurodegenerative diseases, such as Alzheimer's and Parkinson's, are characterized by neuronal loss and fibrillar protein aggregates. These aggregates appear to be responsible for cell death. Some recent reviews have summarized data from experimental studies aimed at identifying, characterizing and comparing neurotoxic protein aggregates associated with TSEs and with these other diseases (*9;81*). A comprehensive review on prion biology discussed prion structure, replication and propagation and pathogenesis caused by misfolded prions in TSEs (*111*).

Prion strains and structure

Variation in prion strains has been observed in several animal species, and these variations in conjunction with host genetic factors influence the neuropathology and species specificity of TSE diseases. Implications of these differences in prion strains were reviewed (*71*). It is generally accepted that it is the prion protein that causes the neurodegeneration typical of TSEs. Yeast prion preparations composed only of protein have successfully infected other yeast cells, indicating that no DNA or RNA is required for disease transmission (*220*). However, some researchers contend that a nucleic acid is also part of the infectious agent and have discussed data supporting this hypothesis (*278;351*). It may be that RNA is not part of the infectious prion particle but is an important cofactor in the *in vivo* propagation of disease prions (*114*).

Experiments with yeast prions recently demonstrated that the same prion can adopt different, stably propagating conformations depending on the temperature during aggregation to form amyloids. Each different prion strain induces production of prions like themselves when introduced into yeast cells (*362*). Mutations in the yeast prion gene affect amyloid fibril conformation and infectivity (*89*).

Differences have been noted in the DNA coding for prion proteins and in the amino acids present at some locations in prions. TSE diseases in humans have been categorized according to symptoms and, alternatively, according to the mutations present in the prion proteins of affected individuals. A comparison of clinical, neuropathological, and molecular data on over 500 patients with different types of TSE diseases demonstrated that there is some overlap of clinical symptoms among patients with different mutations but that some mutations are associated with characteristic clinical or pathological features, such as onset and duration of illness and pattern of amyloid deposits in brain tissue (*228*). People with methionine at codon 129 are more susceptible to kuru and vCJD. Prion proteins with methionine at this position more readily fold into the pathogenic β-sheet form and have an increased ability to aggregate into fibrils as compared to prions containing valine at codon 129 (*296*).

Three isoforms of the prion have been identified in cases of sporadic CJD (*409*). Analyses of prion proteins in a number of cases of sporadic or familial CJD and fatal familial insomnia and in their relatives indicated that mutations causing these TSEs arose independently in each case (*102*).

Variations in prion protein genes have been reported for cattle (*173;184;277;361*), sheep (*155; 173;184*), deer (*64;173*), and chimpanzees (*349*). Some genetic differences have been associated with species specificity (*36*), differences in cell types or brain regions affected by disease prions (*156;207*), and infectivity (*90*). Prion protein gene sequence of Canada's first non-imported case of BSE has also been reported (*98*).

Prion strains may also vary in the number and type of oligosaccharides or glycans attached to the

prion protein (glycosylation). Recent reviews describe glycosylation patterns and functions and discuss possible pathological implications of different glycoforms (*136;318;319*). Normal prion proteins exist in several glycoforms, and it has been suggested that the distribution of these different glycoforms in the mouse brain is related to the pattern of neural degeneration observed in TSE-infected mice (*51*). Patterns of abnormal prion glycoforms are not significantly different in deer with CWD, sheep with scrapie, and cattle with BSE (*310*). Patterns of glycosylation may be related to aggregation and fibril formation (*59*) and to species barriers of TSE diseases (*305*).

Three recent reports presented evidence that more than one strain of BSE exists in cattle. Two old cattle (11 and 15 years) tested as part of routine surveillance in Italy were found to have an atypical distribution of protease-resistant prions in the brain and widespread prion-containing amyloid plaques unlike typical BSE. Prions isolated from these cases differed from the usual BSE prions in the predominance of the low molecular mass glycoform. This disease appears similar to one type of sporadic CJD (*78*). Three old cows (8–15 years) in France were found to contain relatively low levels of a new form of a protease-resistant prion in brain tissue. These prions were characterized by lower levels of the diglycosylated molecular form of the prion, higher molecular mass of the unglycosylated form, and stronger labeling by P4 monoclonal antibody as compared to typical BSE prions (*54*). Atypical protease-resistant prions were detected in two Holstein steers, 21 and 23 months old, slaughtered in Japan in 2003. These strains were characterized by faster migration of the nonglycosylated form during electrophoresis, and a lower resistance to protease digestion (*404*). These results are not entirely unexpected because several prion strains are associated with scrapie and with human CJD. But it raises questions about the origins and interrelatedness of all these prion strains.

Normal functions of prions

Prions are highly expressed in neurons and parts of the prion molecule have been highly conserved during evolution, indicating that they are likely to have an important function. The exact function(s) of normal prion proteins remains uncertain but evidence indicates that they exert a neuroprotective effect through cell surface signalling, acting as antioxidants, and preventing programmed cell death (apoptosis). Prions bind copper and may function in metal homeostasis and may also aid in regulating activity at nerve synapses. Recent reviews discuss these suggested functions (*10;242;256;265;317;383*). Synthesis, turnover, and membrane attachment of normal prions has also been reviewed (*170*).

Early studies of mice with mutations that prevent expression of prions suggested that the absence of normal prions did not have a significant effect on development or survival. A recent evaluation of such mice reveals that there are several biochemical differences between normal mice and those lacking prions. These include altered levels of antioxidants such as melatonin and superoxide dismutase enzymes and changes in the expression of signalling molecules and antiapoptotic proteins. These changes indicate that mice without prions are more sensitive to stress and compensate by altering levels of stress response proteins and antioxidants (*67*). Brain activity of sleep-deprived, prion protein-deficient mice also differs from that of normal mice (*196*). Short- and long-term memory of 9-month-old mice and rats lacking prion protein appears to be impaired (*93*).

Another characteristic of prion-deficient cells is their resistance to infection with *Brucella abortus* (*388*). Apparently a virulence protein of *B. abortus* interacts with the normal prion on the cell surface in order to allow the bacteria to invade the cell.

Copper and zinc bind to the amino end of normal prion proteins and this suggests that prions are involved in maintaining steady state concentrations of these metals in neurons (*327;389*). Prions may also function to transport these metals into cells and may take on antioxidant properties when they combine with these metals (*243*). Protease-resistant, disease-associated prions do not bind strongly to copper, and this difference has been used to separate the two prion forms on a copper-loaded resin (*337*).

An investigation into the role of copper binding by normal prions compared brain copper levels in four strains of mice: wild type, prion knockout mice (no prions produced), mice overexpressing prion protein, and mice producing a mutant prion protein that lacks the copper binding site. Knockout mice and those producing the mutant protein had significantly lower levels of copper in the whole brain and in synaptosomal fractions from the brain than wild type mice. The normal increase in brain copper content as

mice age was not observed in the knockout or mutant mice (*66*).

Altered forms as causes of disease

Misfolding of the prion protein and aggregation of misfolded proteins are involved in pathogenic changes observed in TSEs and in some other neurological diseases. Mechanisms of prion protein conversions, potential cofactors and inhibitors of conversion, strain specificity, and membrane associations have been reviewed (*80*). Data on misfolding of prions and central nervous system pathogenesis have also been reviewed (*63;72;179*).

Conversion of normal prions to disease-specific forms. In vitro experiments utilizing both cell-free systems and cell cultures have been used to investigate mechanisms of prion conversion to the pathogenic, protease-resistant form (*60*). Several factors appear to play a role in this process, including pH (*40*) and prion strain, as demonstrated by the drowsy and hyper forms of the TME prion (*274*). Experiments with yeast prions (PSI) demonstrated that the same prion can adopt different, stably-propagating conformations depending on the temperature during aggregation to form amyloids. Each different prion strain induces production of prions like themselves when introduced into yeast cells (*362*). Infectious yeast prions, produced *in vitro*, were found to contain only protein and were infectious to other yeast cells (*220*). Other experiments with fragments of the human prion protein demonstrated that the pattern and degree of glycosylation of the prion protein affect aggregation and fibril formation (*59*).

Other cellular factors, possibly including nucleic acids, may participate in conversion of normal prions to the pathogenic form (*161*). Addition of TSE-infected hamster brain cells to a homogenate of normal brain tissue causes amplification of the disease-specific prion. However, when single stranded RNA is removed from the homogenates, no amplification occurred. Addition of single-stranded RNA from infected animals greatly increased amplification of the TSE prions (*114*). A small highly structured RNA has been found to bind with high affinity to a human recombinant prion protein. The RNA in these complexes was resistant to degradation by ribonuclease A and the prion became resistant to proteinase K (*6*).

Propagation of prions within the body. Pathogenic prions that enter the body peripherally, for example through the gastrointestinal tract, replicate rapidly in lymphoid organs by converting normal prions to disease-associated forms. These prions then invade the nervous system and cause progressive neurological degeneration (*11;24*). Prion proteins are expressed in many peripheral tissues of adult mice. Highest levels have been detected in afferent nerves in the skin, lining of the aerodigestive tract, sympathetic nerves, dendritic cells and some lymphocyte populations. Other peripheral nerves and associated cells also express prions. This suggests that there may be a variety of alternative routes for propagation and transport of prion infections (*143*). Normal prion proteins are present in the junctions between intestinal enterocyte cells (*271*) and in endothelial cells of blood vessels (*357*). Experiments with cell cultures have provided some insights into the intercellular transfer of the prion protein (*246;348*).

Follicular dendritic cells and enterocytes are believed to aid in transmission of disease prions from lymphatic tissue to peripheral nerves but their presence is not always required (*302–304;338*). Transmission of prions along nerves to the brain occurs in retrograde fashion and may involve Schwann cells associated with the nerves (*142;273*).

Mechanisms of pathogenesis. TSE-associated prions are always present in the central nervous system of affected animals. Depending on the species, route of infection, and the particular TSE disease, these pathogenic prions may also be present in a number of other tissues, including blood vessels (*224*), skeletal muscles (*14;371*), and kidney and adrenal gland (*244*). Some experimental evidence indicates that normal prion proteins, under the influence of pathogenic prions, may aggregate and aid in causing neuronal death (*350*).

Mechanisms of nerve cell death during TSE diseases are not fully understood. However, perturbations in brain metal levels, particularly copper and zinc, and their effects on oxidative stress are believed to play a role (*41;369;402*).

TSEs are uniformly fatal once clinical signs have developed, but some data indicate that under certain circumstances animals may develop subclinical infections (*182*). Subclinical infections have been reported after interspecies transfer of infective tissue (hamster-

adapted scrapie to mice) (*309*) and after administration of relatively low doses of infectious material (*30;367;368*). Animals and humans with subclinical TSEs may be sources of infection for others.

In nature, animals may be exposed to repeated doses of low levels of infectious material and the question arises as to whether these doses have a cumulative effect. Experiments in which rodents were fed multiple low doses of scrapie indicated that it was not the total dose that determined incubation period of the disease (effects were not cumulative). Neither was each exposure an independent event. Statistical analysis demonstrated that there was some interaction between succesive doses that tended to increase risk of infection (*160*).

Stability of prions

Prions are notoriously heat resistant. Inactivation curves generated from data on heating present tails of residual infectivity. Statistical models have been used to interpret these inactivation curves, and these models may be useful in comparing different thermal treatments for inactivation of prions (*295*). Experiments investigating the thermal sensitivity of three scrapie strains found that there was little inactivation at low temperatures but substantial inactivation occurred at higher temperatures. The strains varied somewhat in temperature sensitivity, with temperatures at which substantial inactivation first occurred recorded as 70, 84, and 97ºC (*352*).

Thermal processing combined with ultra-high pressure treatment can decrease infectivity in processed meat spiked with scrapie-infected hamster brain. Several short pulses of high pressure (690–122 MPa) at temperatures of 121–137ºC reduced infectivity by 10^3 to 10^6 mean lethal doses (LD_{50}) per gram of tissue (*70*). Treatment of scrapie-infected hamster brain homogenates with ≥500 MPa pressure at 60ºC converted the scrapie prions to a proteinase sensitive form. Infectivity was not completely destroyed but onset of disease was greatly delayed when treated brain homogenates were inoculated into other hamsters (*149*).

Routes of infection

Horizontal and vertical transfer in animals. Mechanisms for the spread of CWD among wild and captive cervids and of scrapie among sheep in a flock appear to be primarily lateral (directly from one animal to another) rather than maternal (from mother directly to offspring). Observations of cohorts of deer at a research facility in Colorado indicated that maternal transmission contributed little, if any, to the occurrence of CWD. Offspring of uninfected does were just as likely to contract the disease as offspring from infected females (*267*). CWD prions are found in lymphatic tissues associated with the gastrointestinal system but are not detectable in the placenta of infected deer and elk.

Infectious prions may be deposited in the environment where they can bind to clay particles in the soil and may be consumed when deer and elk feed (*294*). A recent series of experiments with mule deer demonstrated that animals could become infected when they were introduced into paddocks in which CWD-infected deer carcasses had decomposed in situ and into paddocks in which infected deer had last resided over two years previously. Infectious prions persisted in the environment of these paddocks for at least two years. Deer also became infected when introduced into a herd containing known infected animals (*268*).

Analyses of placental tissue and genotyping of ewes and their offspring demonstrated that the placenta was infective only if the mother harbored scrapie prions and if both mother and lamb were of a scrapie-susceptible genotype. Despite the presence of scrapie prions in the placenta, no scrapie prions were detected in tissues from lambs 8 days before lambing, at birth or 10 days after birth. Three weeks after birth, disease prions were detected in the intestinal tract of some lambs in the flock even if their mothers were not carrying scrapie. This suggests that lambs become infected after birth (*13*).

Consumption of contaminated brain or nervous tissue. Inoculation of HY prions into the tongue of hamsters resulted in TME symptoms after an average of 79 days. This compares with an average incubation period of 68 days following inoculation of the sciatic nerve and of 191 days following oral ingestion of HY prions. Topical application of TME to the tongue following a superficial wound also resulted in a higher incidence of disease and a shorter incubation period. This suggests that livestock and humans with tongue abrasions may have an increased susceptibility when exposed orally to prions (*39*).

Blood transfusions. In early 2004, two research papers provided evidence that human vCJD and macaque-

adapted BSE, which produces a disease similar to vCJD in monkeys, may be transmitted by blood transfusions. Comparison of data on blood donors and recipients and on cases of vCJD in the UK revealed that 48 individuals received a blood transfusion from a person who later developed vCJD. One of these individuals developed symptoms of vCJD 6.5 years after a transfusion of red blood cells at 68 years of age (significantly older than than the usual vCJD cases). The blood donor in this case did not exhibit symptoms of vCJD until 3.5 years after the blood donation. It may be that both the donor and recipient contracted vCJD by eating contaminated beef, but the researchers estimate that the probability of observing a case of vCJD in a transfusion recipient in the absence of transmission by transfusion is about 1/15,000 to 1/30,000 (*247*).

Brain homogenates from cynomolgus macaques that had been infected with BSE were used to infect other macaques either orally with 5 g of material or by intravenous injection of 0.4, 4, or 40 mg of material. Survival times were 14–26 months shorter for macaques infected intravenously. Disease-specific prions were detected in the spleen, tonsils, intestine, and sciatic nerve in all animals regardless of route of infection but much higher concentrations were present in spleen and tonsils of animals infected intravenously. This demonstrates that the intravenous route is a highly efficient means of transmission (*178*).

Attempts to detect TSE infectivity associated with blood of animals and humans have yielded mixed results. Infective prions are generally not detectable in blood from cases with naturally occurring infections by current methodology. However, when rodents were inoculated with several types of TSEs, infectivity could be detected in blood or its components during both the incubation and clinical phases of disease (*68;86;118;120*). Blood transfusions from sheep infected with scrapie or BSE to other susceptible sheep resulted in disease in 17–19% of recipient sheep within 538 to 737 days after transfusion. Both whole blood and buffy coat fractions from both preclinical and clinical cases in sheep were infective. The donor sheep with scrapie were naturally, not experimentally, infected (*199*).

Other tissues/animal products. CJD from sporadic cases has been transmitted to other humans in over 250 cases. Most of these resulted from injections of contaminated growth hormone from the pituitary gland or transplants of contaminated dura mater from the brains of cadavers. Some other cases occurred after corneal transplants and gonadotropin injections (*315*).

Although most researchers have not detected infective prions in skeletal muscle tissue (that was not contaminated with central nervous system tissue), a recent report indicated that infective prions injected into muscles of mice were propagated and accumulated in the muscles. Prion titers were measured in different muscles and the authors suggested that some of the previous negative results might have occurred because the "wrong" muscles were analyzed. It is not known at present whether muscles from animals that have naturally (not experimentally) acquired a TSE, contain significant levels of abnormal prions (*58*).

Beef tallow, processed at high temperature and pressure by catalytic fat hydrogenation and hydrolytic fat splitting, is considered safe because these processes have been shown to efficiently destroy prion proteins (*19*).

Medical devices. Use of contaminated surgical instruments and encephalographic needles has been responsible for some human cases of sporadic CJD (*315*).

Insects and mites. Flies and mites commonly affect farm animals and have been shown to express prion proteins. Data on the possible role of these pests in the transmission of TSEs have been reviewed (*252*). Fly larvae that have fed on scrapie-infected brain were able to transmit scrapie to hamsters who consumed the larvae. Mites gathered from scrapie-infected flocks were able to induce scrapie in mice after intracerebral injection of homogenized mites. Neither of these experiments exactly replicates what would happen in the field but do suggest the possibility that these arthropods may act as vectors and perhaps as reservoirs of TSE diseases. Of particular concern are fly infestations of the eye because corneal tissues have been shown to contain abnormal prions and some humans have developed CJD following corneal transplants.

Other Causes Proposed for BSE

Autoimmune response induced by bacteria. It has been hypothesized that TSEs could be caused by an autoimmune response triggered by consumption of *Acinetobacter calcoaceticus* from soil during grazing

or from feed. This bacterium contains molecular sequences similar to those found in myelin basic protein normally present in brain tissue. Preliminary experiments with a small number of animals indicated that those with TSEs had elevated levels of serum antibodies to *A. calcoaceticus*. However, recent analyses of serum samples from 344 cattle, 243 sheep, and 3021 elk, some of which were known to have TSEs and others that were healthy, demonstrated that levels of antibody to *A. calcoaceticus* in the healthy and affected animals overlapped considerably thus casting doubt on the hypothesis that *A. calcoaceticus* causes TSEs (*281*).

Other experiments demonstrated that there is an amino acid sequence in the bovine prion protein that is homologous to an amino acid sequence in an enzyme, uridine-diphosphate-N-acetyl glucosamine-1-carboxy-vinyl-transferase, produced by *A. calcoaceticus*. Analyses of sera from 189 cases of BSE and 214 cattle without BSE demonstrated significantly higher average levels of IgG, IgA, and IgM antibodies against this common sequence in the BSE animals. It is suggested that these antibodies may have a role in the pathogenesis of BSE (*27;133;399*).

Malnutrition and mycotoxins. According to this theory, TSEs are caused by high levels of intracellular calcium. The author postulates that in cattle this results from consuming mycotoxin-contaminated feed, while in humans it results from a diet deficient in protein and selenium (*358*).

Eradication and Control of TSEs

According to EU regulations, fallen stock, clinically sick animals, and cattle >30 months of age must be tested for BSE using rapid tests. In a review of the standardization of these procedures and the experiences gathered during implementation of large scale testing, officially approved methodologies, the organization of numerous laboratories involved, and quality control measures currently in place were described (*74*).

Risk management strategies for TSEs have been reviewed for North America (*219*), Asia (*292*), Europe (*176*), Germany (*332*), and Italy (*75*). Surveillance and control strategies were critically reviewed for some European countries. BSE control measures have been most stringent in Europe because more cases occurred in this region. The most important strategy to prevent new cases of BSE is the ban of meat and bone meal in ruminant feed. In many countries in Asia, TSEs are not notifiable diseases. Japan is an exception and has conducted more surveillance and testing for scrapie and BSE. In North America, management of CWD is an important issue, along with surveillance for scrapie and BSE.

The Office Internationale des Epizooties has developed standards to prevent spread of BSE between and within countries (*272*). However, these recommendations are not always followed (*234*). European and German regulations concerned with controlling TSE diseases have been summarized (*146*).

A systems dynamics simulation model has been developed to model the risk of BSE in different cattle populations and evaluate the potential for various control measures to mitigate this risk (*163*). Effects of control measures, particularly with respect to cattle feed, were calculated by estimating the importance of different transmission routes and the number of new infections induced by one initial infection. Meat and bone meal from rendered, BSE-infected cattle were a major route of transmission but probably not the only route (*109*).

Strategies to reduce or control incidence of TSEs in animals

Surveillance. Surveillance of potentially affected animal populations cannot only identify sick animals but may also provide estimates of the extent of an epidemic and possible modes of transmission. Surveillance strategies may be passive (making the disease notifiable), active (testing slaughterhouse material or animals killed during a hunt), or targeted (testing fallen stock or animals of a certain age or living in a certain area). Targeted screening and passive surveillance for BSE in cattle in Switzerland in 1999–2000 demonstrated that the odds of detecting BSE were 49 times greater in downer cattle and 58 times greater in emergency-slaughtered cattle than in cattle subjected to passive surveillance. A total of 30 cases were identified from passive surveillance (104 clinically suspect cattle tested from total herd of 900,000) and 20 cases were found by targeted testing of all 11,376 emergency-slaughtered and fallen stock (*121*).

A statistical model was devised to evaluate 81 different surveillance strategies for scrapie. Both sur-

veillance of fallen stock and abattoir surveillance had a low efficiency for detecting scrapie-infected flocks although fallen stock analyses were several orders of magnitude better than analyses of slaughterhouse material when comparable numbers of animals were tested. Genotypic differences at prion codons 136, 154, and 171 among the sheep were significantly related to incidence of scrapie (*188*).

Disease-specific prions have been detected in the third eyelid (which contains lymphoid tissue) of scrapie-infected sheep. A survey of 690 sheep in Wyoming by immunohistochemical staining of third eyelid biopsy specimens found 10 positive sheep in the passive surveillance program (sheep with potential contact with an infected sheep at a lambing event, regardless of genetic susceptibility) and 3 cases were identified among sheep in a targeted active surveillance program (genetically susceptible sheep with no record of contact with an infected animal during a lambing event). Suitable eyelid samples were obtained from approximately 80% of sheep (*286*).

A statewide surveillance strategy for detecting CWD in deer in Missouri by analyses of deer heads from hunter-killed deer was described (*50*). Collection and analyses of tonsil samples from hunter-killed deer also appear to be an effective means of surveying prevalence of CWD in wild deer populations (*401*).

The rationale for management of deer and elk herds for spread of CWD has been critically examined (*329*). The dire predictions of some models are based on the theory that spread of CWD is strictly a function of the proportion of infectious individuals in a population regardless of population density in an area. However, transmission of CWD is likely also a function of the density of animals and distribution of social groups in an area. An understanding of mechanisms of transmission is important for developing effective management strategies.

CWD in cervids and potentially contaminated feed are also a concern of zoo directors and veterinarians. Several cases of spongiform encephalopathy occurred in ruminants and wild cats in zoos in the UK during the BSE epidemic and were believed to be caused by contaminated feed. Strategies to prevent TSE diseases in captive zoo animals were reviewed (*373*).

Breeding and reproductive strategies. In sheep and humans, some genetically determined prion protein sequences have been shown to be more susceptible to conversion to the disease-associated form than others. Scrapie control plans that focus on culling scrapie-susceptible sheep and introducing more resistant sheep have been proposed for the UK and EU (*1;130*). Data from several sheep surveys indicate that there are 5 common alleles (gene variations) coding for the normal prion protein. The ARR allele is associated with resistance to scrapie while the VRQ allele is linked to the highest susceptibility. One complication noted during genotyping of sheep in the UK is that a small percentage of sheep (approximately 0.08%) carry three or more alleles coding for the prion protein instead of the usual two alleles. The significance of these complex genotypes is not fully understood (*108*).

A case of scrapie in an important breed of Italian sheep (Massese) prompted a plan for disease control by eliminating sheep with susceptible genotypes. According to the strategy developed, all rams would be eliminated except for those with the ARR/ARR genotype. The only ewes maintained would be those that carried at least one ARR allele and no VRQ alleles. It is hoped that this strategy will eradicate scrapie from the flock (*276*).

Recent analyses of prion protein genes in white-tailed deer indicated that although one allele was overrepresented in infected animals, at least 86–96% of deer tested had prion protein combinations that could support CWD (*211*).

Studies of embryo transfer from cattle clinically affected with BSE indicated that embryos are unlikely to carry BSE infectivity even if they are collected near the end stage of maternal disease. A total of 587 viable embryos, all from cows with clinical BSE and some with sires that also had BSE, were transplanted into recipient heifers. Of the 266 live offspring produced, none developed BSE during 7 years' followup. Extracts from 1020 non-viable embryos were injected intracerebrally into susceptible mice and none produced disease in the mice during two years' followup (*403*).

Feed. Epidemiological studies of the BSE epidemic in the UK indicate that the use of rendered carcasses of animals with BSE (and possibly scrapie) as cattle feed supplements played a major role in the spread of BSE. Changes in rendering processes may have allowed more disease-specific prions to survive but it appears that none of the rendering procedures used would completely destroy all infective prions (*364*). Some analyses suggest that certain rendered products used in milk replacers, such as mixed animal fats,

may have been particularly important pathways for infection (*215*). Epidemiological studies of BSE in Germany indicate that contaminated feed supplements made from infected German cattle may have been a factor in the epidemic of cases in this country. But a risk assessment indicated that this means of transmission was of relatively low efficiency in Germany (*411*).

It is now illegal to use meat and bone meal (MBM) from ruminants as feed supplements for other ruminants. Other uses for MBM, following treatment by vacuum pyrolysis, have been proposed, such as for energy production and improving the soil (*87*). A recently released FDA report indicated that 1664 of 11,375 renderers, feed mills, feed mixers, and other mills were handling materials prohibited from use in ruminant feed. Of these establishments, 6 were cited for substantial objectionable conditions and practices and 171 firms had some objectionable practices that should be corrected (*3*).

Although soil is not a specific constituent of animal feed, grazing animals most likely consume soil as they feed on grasses and other forage plants. A recent series of experiments with mule deer demonstrated that animals could become infected when they were introduced into paddocks in which CWD-infected deer carcasses had decomposed in situ and into paddocks in which infected deer had last resided over two years previously. Infectious prions persisted in the environment of these paddocks for at least two years (*268*). Experiments have demonstrated that scrapie-specific prions readily adsorb to clay particles in soil while sand particles adsorb fewer prions. This suggests that soil may be an environmental reservoir for TSE diseases and care should be taken in the landfill disposal of contaminated animals and equipment (*294*).

Strategies to minimize or eliminate human exposure

Strategies employed in the UK to prevent the introduction of BSE-contaminated materials into the human food supply were recently reviewed (*62*). These include the banning of captive bolt stunning of cattle prior to slaughter, exclusion of mechanically recovered meat, specified risk materials, fallen cattle, and cattle older than 30 months of age from the human food chain.

Slaughter methods. To prevent the possibility of foodborne transmission of TSEs, central nervous system tissue from cattle is not permitted in human food. However, some stun and slaughter methods may spread small pieces of brain or spinal cord to other parts of the carcass. Assays of meat and blood after captive bolt stunning and carcass splitting using a band saw have demonstrated the presence of central nervous system tissue in blood and skeletal muscle as well as on the hide, equipment, and personnel (*16;17;177;190;251;300*). This contamination was still detectable after the carcass had been washed or steam-vacuum cleaned. Potential dispersal of central nervous system tissue containing abnormal prions during commercial stunning and dressing was also investigated by use of an antibiotic-resistant *Pseudomonas fluorescens* as a marker. This organism became widely dispersed throughout the slaughter-dressing environment and within the carcass of test animals (*105;301*).

Some alternative methods in which the spinal cord is not opened during carcass splitting (*177;375*) or when stunning is accomplished by an electrical or a non-penetrating stunner (*17*) resulted in little or no contamination of blood or skeletal muscle with nervous tissue.

Some data suggest that nervous tissue outside of but close to the spinal cord (autonomic nervous system) may also harbor infectious prions. Such tissue should also be evaluated for infectivity (*147*). Observations at 27 slaughtering facilities in Germany revealed that workers did not consistently sort cuts of meat and bone containing the sympathetic nerve ganglia along the spinal cord. In some cases, they were sorted into boxes destined for nonfood uses and in other cases they were put in boxes for meat or fat for consumption. Better cutting instructions were recommended to prevent these tissues from entering human food (*134*).

Treatment of meat. If TSE-infected animals are slaughtered for food, some meat products may contain TSE prions, particularly if tissue from the brain or spinal cord of the infected animals is present in the food. This is believed to be the cause of the vCJD epidemic in humans that closely followed the BSE epidemic in cattle in the UK. Lymphatic tissues, including tonsils, spleen, and lymph nodes, from sheep with scrapie and deer with chronic wasting disease may also contain infectious prions. Some research indicates that TSE prions are present in skeletal muscles of infected rodents (*58*) and sheep

(*14*). However, other analyses have not detected infective prions in striated muscles (*166*).

Unlike other foodborne infectious agents, TSE prions cannot be reliably destroyed by heating. The Weibull frequency distribution model was found to provide the best description of the non-linear prion survival curves observed during heating. This model may be useful in assessing the effectiveness of heat combined with other treatments for the destruction of infective prions (*295*). Experiments investigating the thermal sensitivity of three scrapie strains found that there was little inactivation at low temperatures but substantial inactivation occurred at higher temperatures. The strains varied somewhat in temperature sensitivity, with temperatures at which substantial inactivation first occurred recorded as 70, 84, and 97ºC (*352*).

Thermal processing combined with ultra-high pressure treatment can decrease infectivity in processed meat spiked with scrapie-infected hamster brain. Several short pulses of high pressure (690–122 MPa) at temperatures of 121–137ºC reduced infectivity by 10^3 to 10^6 mean lethal doses (LD_{50}) per gram of tissue (*70*). Treatment of scrapie infected hamster brain homogenates with ≥500 MPa pressure at 60ºC converted the scrapie prions to a proteinase-sensitive form. Infectivity was not completely destroyed but onset of disease was greatly delayed when treated brain homogenates were inoculated into other hamsters (*149*).

Disinfection. Prion proteins are very resistant to standard methods of disinfection and may adhere to equipment and surfaces such as equipment, counters, and floors. This presents a significant challenge to medical and food-processing establishments. Several decontamination methods were tested for cleaning stainless steel wires having infectious brain material dried on the surface. Some of the treatments (enzymatic cleaning and treatment with sodium hydroxide followed by autoclaving) reduced but did not eradicate infectivity. The most effective treatment involved treatment with an alkaline detergent followed by sterilization in a hydrogen peroxide gas plasma sterilizer (*405*). Routine cleaning of laryngeal mask devices with an enzyme solution and disinfectant followed by autoclaving does not completely remove protein deposits (*92*). Some methods, such as autoclaving in sodium hydroxide solutions, can eradicate infective prions but are too corrosive for equipment and produce noxious fumes. Recommendations for the most feasible decontamination methods to date have been reviewed (*262;363*).

Infection control guidelines relative to spongiform encephalopathy diseases have been developed and reviewed for Danish hospitals (*210*). Although the risk of contamination in hospitals is considered minimal, procedures are recommended for quarantines and for decontamination and disposal of potentially contaminated equipment. When available, it may be advisable to employ single-use instruments.

Prions are known to be sensitive to basic solutions but the exposure necessary to effectively decontaminate surfaces is unknown. Experiments with scrapie prions bound to powdered iron showed that protease resistance decreased by ≥4 $\log_{10}$ after exposure to 0.1 M NaOH for 15 minutes at room temperature. Infectivity of the treated material was not tested but the increase in protease sensitivity suggests that this procedure may be useful in treating lightly contaminated equipment (*214*). Two disinfectants with different constituents were tested for their efficacy in destroying scrapie infectivity in hamster brain homogenates. Environ LpH was at least 10^4-fold more effective than Environ LpH-SE in destroying infectivity after 16 hours of contact (*311*). Pretreatment of cattle and sheep brain tissue containing BSE and scrapie prions with a detergent and heat was found to allow extensive degradation of prions by several protease enzymes. Following treatment, no prion proteins were detectable by immunoassay (*238*).

Prevention of transmission by blood and transplanted tissues. CJD from sporadic cases has been transmitted to other humans in over 250 cases. Most of these resulted from injections of contaminated growth hormone from the pituitary gland or transplants of dura mater from the brains of cadavers. Careful screening of donors of tissues and blood should be implemented to prevent iatrogenic cases of CJD and vCJD (*315*).

In early 2004, two research papers provided evidence that human vCJD and macaque-adapted BSE, which produces a disease similar to vCJD in monkeys, may be transmitted by blood transfusions. Comparison of data on blood donors and recipients with that on cases of vCJD in the UK revealed that one individual developed symptoms of vCJD 6.5 years after a transfusion of red blood cells from a blood donor who was later confirmed to have vCJD (*247*). Small

amounts of brain homogenate from cynomolgus macaques with BSE that were injected intravenously into other macaques caused BSE, and the monkeys died 14–26 months earlier than macaques given oral doses of the infected brain. This demonstrates that the intravenous route is a highly efficient means of transmission of BSE/vCJD (*178*). Blood transfused from sheep infected with scrapie or BSE to other susceptible sheep resulted in disease in 17–19% of recipient sheep within 538 to 737 days after transfusion (*199*).

Research indicating that vCJD (as well as BSE and scrapie) may be transmissible by blood transfusions has generated discussion on procedures to ensure the safety of the supply of human blood for transfusions (*68;86;118;120*). Experimental results indicated that a dose of 50 kGy could inactivate 1.5 log of scrapie infectivity in a human albumin solution spiked with infected hamster brain. This irradiation dose had moderate effects on the fragmentation and aggregation of the albumin itself (*264*).

Treatment of TSEs

The advent of vCJD and the realization that some other degenerative diseases may result from protein misfolding has stimulated research to identify drugs and other treatments that would slow or reverse the progression of these neurological diseases. Four recent reviews summarized information on therapeutic agents currently under investigation and explained their probable mechanisms of action. All of these agents have a beneficial effect in some in vitro systems and some have been shown to prolong incubation periods or alleviate some symptoms in laboratory animals. As yet, none have been very effective in treating human TSEs and all have some toxic or undesirable side effects when used to treat or prevent TSEs in animals. Some treatments are undergoing preliminary tests in humans (*69;127;226;316*).

These agents include: (i) compounds that react with cell membranes and may prevent attachment or entry of abnormal prions, such as polyanions; (ii) drugs that intercalate with the β-sheet structures characteristic of disease-specific prions and may facilitate clearance, such as tetracyclines, iododoxorubcin, Congo Red, and peptide β-sheet breakers (*144;314;320*); (iii) other molecules that bind to prions such as heparan sulfate proteoglycans (*5;306*) and copper (*181;342*); (iv) agents that stimulate breakdown of prions, such as polyamines; (v) antifungal compounds that bind to cholesterol in membranes near where prions are believed to reside, such as amphotericin B; (vi) compounds that prevent conversion of normal prions to the abnormal form, such as chlorpromazine and quinacrine (*35;225;275;322;377*); and (vii) antibodies to prion proteins (*344;391*). Other compounds with reported anti-prion activity include curcumin (*82*), dimethyl sulfoxide (*336*), cyclo-oxygenase inhibitors (*41*), and some other drugs and natural compounds including polyphenols (*223*).

Follicular dendritic cells (FDCs) in lymphoid tissues appear to be an important site of prion replication prior to neuroinvasion. Mice that lack mature FDCs are less susceptible to infection with TSEs, and compounds that interfere with the integrity or function of FDCs extend survival time in experimental animals by blocking prion replication and spread to the brain (*253*).

Prion diseases are accompanied by disturbances in the antioxidant defense systems. A case report of a CJD patient treated with antioxidants demonstrated that this therapy might delay severe symptoms and death by more than a year. The patient eventually died but lived for about 1.5 years longer than expected (*128*).

Some scientists are investigating the possibility of developing a vaccine to prevent infection or slow the development of TSEs. Normally, animals do not appear to mount an effective immune response to prion infection. However, prions do induce some cell-mediated immunity (*31;359*) and some prion-related compounds stimulate antibody production (*20*). Mice vaccinated with a genetically engineered prion protein prior to exposure to scrapie-infected brain tissue developed disease symptoms several weeks later than unvaccinated mice. The vaccine did not completely prevent disease and had some toxic effects but it demonstrates the possible utility of this approach (*333;343*). Injection of mice with CpG oligodeoxynucleotides, compounds known to stimulate innate immunity, delayed or prevented development of scrapie after intracerebral infection (*335*).

Another approach to preventing TSE diseases is the depletion of normal prion protein levels. It has previously been observed that mutant mice that do not produce normal prions are resistant to prion diseases. Inhibition of prion protein synthesis has counteracted

disease symptoms. Normal prion levels have been reduced in transgenic mice (*254*) and in neuroblastoma cells treated with small interfering RNAs (*106*).

Several in vitro assay systems have been developed to screen potential therapeutic compounds for anti-prion activity. These include: (i) a yeast-based assay (*28*); (ii) protease-resistant prions from scrapie-infected hamsters (*77*); (iii) a neuroblastoma cell line persistently infected with a mouse-adapted scrapie strain (*299*); and (iv) a solid-phase assay utilizing protease-sensitive prions from hamsters (*260*).

Diagnostics

Reviews and general methodology

Classical diagnostic methods to detect TSE diseases include post-mortem examination of central nervous system tissue by histopathology and immunochemistry to detect abnormal prions and clinical diagnosis by detection of disease-specific proteins in the cerebrospinal fluid and neuro-imaging technologies. More recent molecular diagnostic methods were reviewed with respect to their specificity and sensitivity (*231*). Another recent review discussed the importance of prion protein conformation and aggregation in devising specific tests to distinguish abnormal, disease-specific prions from normal prions in healthy animals. Current diagnostic methods and possible future developments were discussed (*48*).

Many of the currently used immunological methods for the detection of abnormal prions require a complex sample preparation to remove normal prions by protease degradation and a denaturation step to allow the protease-resistant abnormal prion to react with antibodies. Two research groups have recently reported the isolation of antibodies specific to the disease-associated form of the prion. Disease-specific prions have a tyrosine-tyrosine-arginine region exposed by their abnormal folding and normal prions do not, so one group has isolated antibodies for this region of the prion and this allows specific detection of abnormal prions (*293*). A monoclonal antibody has also been raised against a peptide from the C-terminal end of the prion protein. When used in a sandwich ELISA to assay brain tissue from normal and CJD-affected humans, without pretreatment to remove normal prions, this antibody reacted only with prions from CJD brains (*334*).

Another research group has identified some polymeric compounds that attach only to abnormal prions. The polymers also bind strongly to certain solid phase materials and can be used to coat immunoassay wells. Test material, for example brain homogenate, can be added directly to the wells, abnormal prions bind to the polymer and then, after washing away the normal prions and other proteins, the attached abnormal prions can be detected by immunoassay (*236*). Protease-resistant, disease-associated prions do not bind strongly to copper whereas normal prions do bind to copper, and this difference has been used to separate the two prion forms on a copper-loaded resin (*337*). BSE, scrapie, and normal prions were distinguished by monoclonal antibodies after refolding in the presence of copper (*370*). Still another strategy involves the use of both an anti-DNA antibody, OCD4, and gene 5 protein, which combine preferentially with disease-specific prions from brains of both humans and animals (*413*).

One difficulty in determining the presence of abnormal prions in living animals is that very low concentrations of these prions are usually present in the extraneural tissues, such as blood and urine, that could be sampled easily. An RNA molecule that binds specifically to prions (both normal and abnormal forms) has been isolated and attached to beads in a column. This column can then be used to concentrate prions present in low concentrations in body fluids and was found to increase the sensitivity of a Western blotting technique by 1000-fold (*410*).

Determination of disease in animal/human tissues

Brain and spinal cord tissue. BSE/TSE prions are readily detected in central nervous system tissue of cattle and other animals by a variety of rapid immunological assays (*283*) even if the tissue has undergone some autolysis (*112*). A commercial version of a sandwich immunoassay using two monoclonal antibodies, used to test BSE brain tissue, was found to be more sensitive than the mouse bioassay in detecting infectivity (*158*). A conformation-dependent immunoassay that employs high-affinity recombinant antibody fragments was also found to effectively determine prion titers and was able to distinguish between BSE and CWD prions (*326*).

Two characteristics of abnormal prions — protease-resistance and aggregation to form polymers

and fibrils — have been exploited in a procedure utilizing protease digestion and filtration of tissue extracts through a slot blot device (0.45 µm pore size). Aggregates retained on the filter were then detected immunologically (*400*).

Scrapie-associated molecular changes in the brains of hamsters are detectable by infrared (IR) microspectroscopy of frozen brain sections. The IR analysis indicates molecular changes in spectral regions that contain contributions from carbohydrates, the phosphate backbone of nucleic acids, and membrane components. These changes were evident in preclinical stages as well as in terminal stages of the disease (*222*).

Transgenic mice, expressing the normal bovine prion gene, have been generated and can be used as indicators of the BSE infectivity of tissue samples. As early as 150 days post-inoculation, infective prions can be detected by immunohistochemistry in the transgenic mice (*79*). Quantification of prion proteins by inoculation of dilutions of nervous tissue into mice can be a slow and costly process. As an alternative, an *in vitro* assay system using susceptible neuroblastoma cells has been developed and found to have a linear dose-response over two logs of prion concentrations (*221*).

Symptoms of vCJD and sporadic CJD are similar and it can be difficult to distinguish the two diseases before death and subsequent examination of brain tissue. Analyses of proteins in the cerebrospinal fluid from cases of vCJD, sporadic CJD, and other neurological diseases revealed that apolipoprotein E levels were higher in cases of vCJD than in cases of sporadic CJD. Assays for this protein, in conjunction with assays for other cerebrospinal fluid proteins, may be useful in the *ante mortem* diagnosis of vCJD (*91*).

Routine and reliable detection of BSE in slaughtered animals would be aided by a standardized procedure for sampling central nervous system tissue from cattle. A technique has been described for extracting sufficient nervous tissue from the brainstem region, known to be rich in infectious material, by use of an appropriate disposable instrument (*384*). A convenient dipstick assay has been devised using a sandwich ELISA specific for prion proteins and crystalline bacterial cell surface layers as an immobilization matrix (*385*). This method may be useful in field determinations of TSEs.

Blood. Some recent research indicates that vCJD may be transmissible by blood transfusions. Blood is a convenient tissue to analyze to determine TSEs in living animals. However, disease-specific prions are either absent from or present at very low concentrations in blood (*68*). vCJD prions added to plasma were detected by a sandwich conformation-dependent assay. Only the abnormal (disease-specific) form of the prion reacted in this assay; the normal prion did not interfere with detection of the vCJD prions (*46*). An immunocompetitive capillary electrophoresis assay has been shown to detect proteinase-resistant prions in the blood of scrapie-infected sheep. However, this assay failed to detect disease-specific prions in blood of humans or chimpanzees with CJD (*85*).

Several assays for other compounds in the blood have been developed to help distinguish TSE-affected animals from animals that are healthy or have other diseases. A new Fourier transform infrared spectroscopy method combined with advanced computer-aided pattern recognition techniques (artificial neural networks) was devised to identify scrapie infection by the presence of characteristic molecular alterations in the blood of infected hamsters. The sensitivity and specificity of these tests were 97% and 100%, respectively, and this test can be fully automated (*330*). A similar assay used to test blood from cattle with and without BSE had a sensitivity and specificity of 96% and 92%, respectively (*241*).

Plasma levels of 20β-dihydrocortisol were found to be an average of five times higher in sheep with scrapie than in healthy sheep, and urinary creatinine levels were about twice as high in sheep with scrapie than in sheep that were healthy or had other diseases. Measurements of these two compounds correctly classified 98% of healthy sheep and 82% of scrapie-infected sheep (*297*).

The expression of erythroid differentiation-related factor (EDRF) is significantly diminished in the blood of sheep and in the lymphatic tissue of rodents and cattle suffering from transmissible spongiform encephalopathies. However, analyses of EDRF mRNA in blood of healthy humans revealed that there is a wide variation (a range of two log units) in normal levels of this factor. Therefore, EDRF expression does not appear to be a good marker for TSE diseases in humans (*154*).

Lymphoid tissue. Disease-specific prions accumulate in lymphoid tissue in sheep with scrapie and in deer and elk with chronic wasting disease (CWD) but not in cattle with BSE. Examination of tonsil tissue from deer appears to be an effective strategy for determining the incidence of CWD (*287;353;401*). To test the reliability of immunohistochemical tests for CWD prions in tonsils and retropharyngeal lymph nodes as indicators of CWD, tissue samples from 1372 mule deer were analyzed. Seventy-four deer had CWD prions present in the medulla oblongata of the brain and all but two of these also had CWD prions in tonsils and/or lymph nodes. Another eight deer had positive lymph nodes and/or tonsils but negative results for brain tissue (*266*). Further immunohistochemical tests of tonsilar and lymph node tissue at six- to nine-month intervals in deer in a CWD-endemic herd revealed that CWD could be detected in tonsils up to fourteen months before clinical signs of disease were observed (*393*). An assessment of three different visualization systems for detection of scrapie prions in lymphoid tissue concluded that the catalyzed signal amplification system was significantly more sensitive than the avidin-biotin-peroxidase system and the Envision system (*270*).

Using immunohistochemical assays as a standard, an ELISA (enzyme linked immunosorbent assay) was evaluated for accuracy in detecting CWD prions in lymph nodes and brain tissue of deer and elk. Overall agreement between the two tests was >95.6%. Therefore, this ELISA appears to be an excellent rapid test for screening large numbers of samples (*180*).

Disease-specific prions have been detected in the third eyelid (which contains lymphoid tissue) of scrapie-infected sheep. A survey of 690 sheep in Wyoming by immunohistochemical staining of third eyelid biopsy specimens found 13 positive sheep. Suitable eyelid samples were obtained from approximately 80% of sheep (*286*).

Determination of nervous tissue or abnormal prions in food

Standard micro-sausages containing defined amounts of BSE-positive bovine brain tissue were produced to test analytical procedures. Using a commercial immunoassay for BSE-associated prions, it was possible to detect BSE-positive brain tissue down to a concentration of 0.25%. Negative results were obtained with this assay for 30 retail meat products (*249*).

Since the highest concentrations of abnormal prions occur in the brain and spinal cord, numerous methods have been developed to detect the presence of central nervous system tissue in foods. Three types of compounds are analyzed:

Long-chain fatty acids, such as nervonic acid and cerebronic acid. Cerebronic acid (C24OH) was found to be the best fatty acid indicator of the presence of bovine central nervous system tissue in experiments using gas chromatography–mass spectrometry (*248*). An on-line liquid chromatography–gas chromatography procedure was found to measure nervonic acid (C24:1) concentrations with excellent linearity and good repeatability (*33*).

Proteins that are specifically present in the central nervous system, such as glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), neurofilament (NF), and myelin basic protein (MBP). Surveys using immunoassays for NSE and GFAP found CNS tissue in 4–18% of samples of different German sausages (*191;250*).

As part of an international collaborative trial, twenty-one laboratories in nine European countries evaluated the performance of two commercial kits for the detection of central nervous system tissue in meat: (i) ScheBo Brainostic test, which detects NSE by Western blotting and (ii) the r-Biopharm Ridascreen Risk Material ELISA, which detects the presence of GFAP. These tests were used to analyze sausages containing 0, 0.5, 1.0 and 2.0% brain tissue. Good results were obtained with both tests, with a detection limit of 0.5% when sausages were raw or only moderately heat treated. For strongly heated sausages, detection limit was 0.5% for the GFAP test and 2% for the NSE test (*7*). Both tests reliably detected the presence of 1% or more of central nervous system tissue according to results from one laboratory (*197*).

Cooking and some processing steps for meat may alter the conformation of the proteins to be tested and decrease the utility of some assays. An immunohistochemical procedure using anti-NF antibodies was found to be the most reliable test for nervous tissue in cooked meats according to one set of experiments (*26*) while another research group reported that assays using anti-MBP were more reliable for cooked meat (*366*).

DNA or RNA sequences that code for these specific proteins. Reverse transcription polymerase

chain reaction (PCR) assays have been developed to detect mRNA for GFAP, which indicates the presence of central nervous system tissue from any slaughter animal, and for myelin basic protein, which indicates the presence of bovine brain or spinal cord (*237*).

Determination of ruminant and other animal protein in feed

Many countries prohibit the use of meat and bone meal derived from ruminants in feed for cattle, sheep, and goats. Since 2000, the European Union has also banned the use of processed proteins from mammals, birds and fish in feed for animals being raised for human food. To enforce such laws, analytical methods have been developed to detect the presence of meat and bone meal supplements in animal feed.

A 2003 article reviewed the history and rationale for banning meat and bone meal in cattle feed. The authors described methods currently available for detecting meat protein in feed and for distinguishing different species of animal meat and pointed out the strengths and weaknesses of these procedures. These methods include: (i) Microscopic analysis based on the presence of bone fragments; (ii) Polymerase chain reaction (PCR) methods to detect DNA sequences specific to certain species of animals; (iii) Enzyme-linked immunosorbent assays to detect species-specific antigens or proteins; (iv) Near infrared microscopy to detect animal constituents in feed, and fish can be distinguished from terrestrial animals; (v) Near infrared spectroscopy for use as a screening method to identify feed containing animal protein. These tests differ in their specificity, interference from milk proteins, limits of detection, and heat stability (*151*).

Five PCR detection methods have been described recently. Identification of bovine materials in animal feed was achieved using a bovine-specific lactoferrin DNA fragment. This method is heat stable and had a limit of detection of 0.02% bovine derived meat and bone meal in feed (*148*). Detection of low levels of bovine, ovine, porcine, and chicken meat and bone meal in feed by use of mitochondrial DNA sequences specific to those 4 species was described in reports from two research groups (*88;229*). Mitochondrial gene sequences from poultry, pork, ruminants, and fish were used in a PCR assay to detect contaminant meat levels as low as 0.002% (*104*). Yet another PCR method was designed to detect meat from cattle, hogs, sheep, goats, rabbits, horses, chickens, trout, and European pilchard (fish) in feed (*61*). This method was able to detect 0.0625% of these meats, even after they were subjected to severe rendering treatments.

Detection of collagen, a major animal protein present in gelatin, in animal feedstuffs has been achieved by mass spectrometry. This may be the basis for a rapid and sensitive method for the detection of contamination of feed by meat and bone meal (*288*).

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FRI BRIEFINGS

Bovine Spongiform Encephalopathy

A Review of the Scientific Literature

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Emergence of BSE

Bovine spongiform encephalopathy (BSE) was first recognized in British cattle in 1986. The yearly peak reported incidence in Great Britain was in 1992, with 36,680 confirmed cases. Cases continue to be diagnosed to this date, with 781 cases detected in 2001 and 642 as of Aug. 2002 (*83*). A total of approximately 179,000 confirmed cases of BSE have occurred in Great Britain.

BSE has also been reported from cattle in several European countries, Israel, and Japan (*203*). Countries reporting BSE cases in 2001 and/or 2002 are listed in the Table 1.

Table 1. Confirmed BSE Cases Reported

Country	2002*	2001
Austria		1
Belgium	21	46
Czech Republic	5	2
Denmark	1	6
Finland		1
France	159	274
Germany	54	125
Greece		1
Ireland	209	246
Israel	1	0
Italy	4	48
Japan	2	3
Netherlands	13	20
Poland	1	0
Portugal	18	110
Slovakia		5
Slovenia	2	1
Spain	84	82
Switzerland	8	42

*These numbers were posted on the Office International des Epizooties web site on Sept. 5, 2002. Actual reporting dates for different countries ranged from January to August, 2002.

The BSE epidemic was almost certainly caused by feeding to cattle the rendered carcasses of animals containing the causative agent of the disease. Whether scrapie-infected sheep or cattle with unrecognized BSE were the original source of the infection is still disputed. Scrapie in sheep sometimes presents with symptoms of tremors and lack of coordination (similar to BSE) and it may have been rendered material from these animals that originally affected the cattle (*174,195*). In the early 1980s, changes in rendering processes eliminated a step of tallow extraction with steam and organic solvents. Although none of the rendering processes used at the time would have completely eliminated the infective BSE agent in meat and bone meal, the elimination of the hot solvent extraction step may have permitted an increase in the survival of infective prions. As animals were fed the infective meat and bone meal supplements and then were themselves recycled through rendering, the concentration of the infective agents increased in meat and bone meal, eventually causing a full-blown epidemic (*45,46,98,258*).

There does not appear to be a difference in susceptibility to BSE among different breeds of cattle, as has been reported for scrapie in sheep (*147,272,274*). Oral dosing of calves with BSE-infected brain tissue is followed, within six months, by detectable infectivity in the small intestine (*284*). BSE has also been transmitted orally to sheep (*102*), mice (*264*), lemurs (*37*), cats, several species of zoo animals in England (*75,232*), and apparently to humans, resulting in vCJD. Inoculation of pigs with BSE by several routes (but not orally) resulted in brain lesions similar to those seen in spongiform encephalopathies (*231*).

Sheep in the UK have also been fed meat and bone meal supplements, and there is some concern that humans may be exposed to some ovine products from BSE-infected sheep. There is no evidence for this to date but investigations are underway to determine whether sheep diagnosed with “scrapie” really have scrapie or BSE (*100*).

An unsolved question is why this epidemic arose to such an extent only in Great Britain. Other countries also fed meat and bone meal to cattle and also had sheep, some of which were infected with scrapie. Great Britain does have a higher sheep to cattle ratio than many countries and therefore may have a higher proportion of sheep carcasses entering the rendering process. The scrapie agents tested to date are distinct from the BSE agent, but it is possible that passage of the scrapie agent through cattle modified its structure to form the prions characteristic of BSE (*57*).

A recent report from a Review Committee in Great Britain pointed out that from about 1970 to 1988 meat and bone meal were introduced into starter rations for calves. This practice was less common in the U.S. and continental Europe than in England. Evidence from some computer-simulation models indicates that the majority of BSE-affected animals were infected as calves. Young calves may be more susceptible to prion infections than adult cattle. All three of these factors —

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changes in rendering processes, meat and bone meal in starter rations, and a relatively high proportion of sheep in rendered material — probably combined to initiate the BSE epidemic in the U.K. (*140*).

In July 1988, the feeding of ruminant-derived protein to ruminants was prohibited in Great Britain. However, the number of confirmed cases continued to rise for several years due to the long incubation period of the disease. The number of confirmed cases in the U.K. in 2000 was only 3.5% of that reported in 1992. Eighteen cases of BSE have been diagnosed in cattle born after Aug. 1, 1996, the date when extra controls on animal feed containing mammalian meat and bone meal were considered to have been fully implemented. It is uncertain whether these BSE cases resulted from maternal transmission or from illegal use of feed containing ruminant meat and bone meal (*83*).

Meat and bone meal and central nervous system tissue, in particular, are known vehicles for transmitting BSE. There is no evidence that milk, catgut, or bone grafts from cattle contain infectious agents (*1,261,287*). The question of infectivity in other bovine products has been considered by the European Commission. Tallow is considered to be an unlikely source of transmissible spongiform encephalopathy (TSE) infectious agents as long as the tallow is produced from animals fit for human consumption, purified, and not contaminated with more than 0.15% insoluble impurities (*14,240*). Gelatin and collagen produced from bovine hides do not present a risk for TSEs provided contamination with potentially infected materials is avoided (*94,96*). Risk of contamination is much higher if bones are used as the source of gelatin. Organic fertilizers should not be produced from animal materials suspected or confirmed of carrying the TSE agent (*93*).

One item of historical interest is a brief report by a French veterinarian of a case of scrapie in a cow (bull?) in 1883 (*235*). The animal had symptoms of intense itching around the base of the tail, followed two weeks later by progressive paralysis of the hind legs and other neurological signs. Finally, the animal fell down and didn't get up and the vet advised destroying it. It was butchered and sold in the butcher shop! Unfortunately (from our vantage point) there's no further information on whether there were sheep with scrapie on or near the farm or whether any other cattle showed symptoms. This was presented as a new observation, so apparently the vet hadn't seen or heard of any other cases before.

Other Spongiform Encephalopathies in Animals

Scrapie

Scrapie is a fatal neurodegenerative disease occurring naturally in sheep and goats. It was first recognized in Europe more than 250 years ago and has been reported from most sheep-raising countries, with the exceptions of Australia and New Zealand. Disease symptoms vary among individual animals and breeds of sheep and goats. Some animals rub and scratch against fixed objects while others display lack of coordination and tremor without scratching. The incubation period is believed to be 2 to 5 years after exposure to the disease agent, and sheep usually die within 6 months of showing symptoms (*75,174*). Several scrapie prion strains have been identified from different sheep with the disease (*57,116*).

Transmission

Spread of scrapie among sheep or goats in a flock can occur when uninfected animals ingest fetal membranes voided by other sheep during lambing. Experimental feeding of scrapie-infected membranes to sheep and goats causes symptoms of scrapie in about 21 months (*205*). Several studies documenting horizontal transmission of scrapie from naturally infected animals to previously unexposed animals have been described. It is not clear how the disease was transmitted, however, because attempts to detect scrapie prions in the materials a lamb or kid might ordinarily ingest have been largely unsuccessful.

Some evidence indicates that vertical transmission (from ewe to lamb) of scrapie can also occur. Lambs from scrapie-infected mothers are at greater risk for contracting scrapie and may be infected during birth or in utero by gulping amniotic fluid (*59*). When lambs from scrapie-infected ewes were isolated from their mothers shortly after birth, they were four times less likely to develop scrapie (*143*). This indicates that exposure may occur after birth. Analyses of uterine tissues from pregnant, scrapie-infected ewes revealed that the tissues contacting the fetus did not contain scrapie prions although some other reproductive tissues did (*273*). Experiments with scrapie-infected monkeys indicated that vertical transmission of scrapie did not occur in this species (*6*).

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It is not known whether, or how long, preclinical animals are infective to others in a flock. However, injection of tissues (spleen, lymph nodes, brain) from 14 of 28 lambs considered clinically and histologically normal induced TSE disease in mice. These lambs were offspring of scrapie-infected dams and were 4 to 23 months old. No tissues from seven lambs younger than 4 months old were infective to mice even though these were also offspring of infected dams (*143*).

An Italian epidemic of scrapie in 1996–1997 affected an unusually high percentage of goats and appeared simultaneously in several age cohorts of at least 20 flocks of sheep and goats. Epidemiological evidence suggested a point source for the outbreak, and it appeared that a vaccine used to immunize the animals against *Mycoplasma agalactiae* could have been the cause. This vaccine was prepared by a single company from formol-treated brain and mammary tissue of sheep infected with *M. agalactiae*. None of the donor sheep showed clinical signs of scrapie, but some may have been harboring the infectious agent (*3,62,274*).

Scrapie is transmissible orally to squirrel monkeys (*112*) and intracerebrally to cynomolgus monkeys (*111*), mink (*128*), and to rats and mice but not to rabbits and guinea pigs (*22,77*).

Attempts to infect cattle with scrapie have not been very successful. Cattle fed raw brains containing a North American strain of scrapie failed to develop neurological disease (*78,79*). Disease was induced in some cattle inoculated intramuscularly, subcutaneously, intracerebrally, and orally. Neurological symptoms were observed in some animals and abnormal prions were detected in the brain, but the cattle differed clinically and histologically from BSE-infected cattle (*65,113,142,227*). These experiments suggest that feeding meat and bone meal supplements containing scrapie-infected tissue is unlikely to produce BSE. However, different strains of scrapie with different properties may produce somewhat different symptoms and so these experiments do not prove or disprove the hypothesis that the scrapie agent was involved in the emergence of BSE.

Scrapie Prions

Several different scrapie strains have been detected in naturally infected sheep, causing different patterns of accumulation of protease-resistant prions in different areas of the brain. Host genotype appears to affect the magnitude of scrapie prion accumulation (*116*). When tissues containing different prion strains were injected into mice, differences were observed in the ensuing incidence of infection, incubation period, and neuropathology. The effects of these variant strains in mice were distinguishable from those of BSE (*57*). Protease-resistant prions isolated from sheep infected with scrapie were distinguishable from those from sheep infected with the BSE agent (*152*).

Scrapie prions appear to persist for years in the environment. Initial attempts to eradicate scrapie in Iceland involved the slaughter of all sheep on affected farms in a defined area. After 2 to 3 years, the farms were restocked with young lambs from a scrapie-free area. Scrapie reappeared in the new sheep on many but not all farms that previously had the disease (*143*). When scrapie-infected hamster brain was mixed with soil and buried in a garden in a temperate climate, 2–3 log units of infectivity (of an initial 4.8 log units) remained after three years (*51*). Apparently the scrapie agent can survive somewhere in the environment for at least three years.

Scrapie Resistance

Numerous studies have shown that some breeds of sheep and goats are more resistant than others to development of scrapie. Susceptibility to this disease is dependent on the genetically determined amino acid sequence of the normal prion proteins (PrP). Variations at prion codons 136, 154, and 171 in sheep and at 143, 154, and 240 in goats appear to be important determinants of susceptibility or resistance to scrapie (*28,35,115,146–149,174*). Some variants are associated with a longer incubation period for this disease, and there is evidence that sheep from some resistant breeds can get scrapie if they live long enough.

Breeding of resistant strains of sheep and goats may be a useful strategy for minimizing scrapie in flocks. However, it is not known whether resistant sheep and goats, which have been exposed to scrapie but are not clinically ill, can be carriers of the disease. Some scrapie-exposed goats that were apparently healthy and had no histopathological lesions in the brain were found to contain protease-resistant prions (*35*). Some asymptomatic sheep from a scrapie-infected flock in Iceland had a subclinical infection with lesions in the brain and/or scrapie-associated

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prions present. However, none of the sheep in this flock with the resistant AHQ genotype had either clinical or subclinical signs of scrapie (*269*). Therefore, some scrapie-resistant animals may not act as carriers while others may be capable of spreading the disease even though they do not appear ill.

Disease Development

Ingested scrapie prions have been monitored by immunohistochemical staining of tissues of sheep and lambs (*8,103,129,130,184,278*). Scrapie prions first accumulate in Peyer's patches in the intestinal wall. Later, prions were successively detected in lymphatic tissue draining the Peyer's patches, the spleen, nervous tissue and finally in the brain stem after about 9 months (*158,279*). Experiments with immunodeficient mice indicated that differentiated B lymphocytes were necessary for neuroinvasive scrapie (*41*). Altered levels of antioxidant activity and of copper, zinc, magnesium, and calcium were detected in brains of scrapie-infected mice (*298*). These are likely to be related to pathogenic changes in the central nervous system during disease development.

Sheep with clinical signs of scrapie have detectable scrapie prions in their spleen, tonsils, central nervous system, peripheral nerve ganglia, and lymphoreticular system (*129,131,249*). Scrapie-associated prions were not detected in peripheral blood leukocytes of infected sheep by an immunohistochemical method (*131*).

Scrapie is detectable in preclinical sheep by histological changes in the brain (*126*), immunohistochemical assay for scrapie prions in brain tissue (*155*), presence of scrapie prions in tonsils (*153,238*), and a monoclonal antibody (MAb)-based immunohistochemical assay of nictitating membrane ("third eyelid")-associated lymphoid tissue (*200*).

TME — Transmissible Mink Encephalopathy

Transmissible mink encephalopathy is a rare disease of ranch-raised mink. It was first recognized in the U.S. in 1947, and there have been five reported outbreaks in the U.S. In addition, outbreaks have been reported in Canada, Finland, East Germany, and Russia. Epidemiological studies indicate that the disease is foodborne since outbreaks include geographically distant ranches that shared a common feed source (*75,177*).

Disease Development

Incubation period for TME in mink has been estimated to range from 7 to 12 months. Early symptoms include behavioral alterations and difficulty in eating. As the disease progresses, animals lose muscle coordination and, at autopsy, the brains of affected animals show typical spongiform degeneration. Most animals die within 7 weeks of the onset of clinical signs (*178,179*). Following subcutaneous inoculation with infective brain tissue, infectivity was first detected in the central nervous system at 20 weeks while the mink still appeared clinically normal. Infectivity was also detected in spleen, liver, kidney, intestine, lymph nodes and salivary glands (*124*).

In hamsters, TME causes two different sets of symptoms: "drowsy," with a long incubation period, and "hyper," with a shorter incubation period. There are also differences in clinical signs, brain lesions and titers of infectious prions. When passaged back to mink, only drowsy was pathogenic (*24,33*). Inoculation of the sciatic nerve of hamsters with TME prions demonstrated that these agents were capable of traveling along the nerve to the spinal cord and then to the brain. Prions appeared to ascend the spinal cord at a rate of 3.3 mm/day (*25*).

A comparison of the effects of the TME agent and the scrapie agent on hamsters demonstrated that they produced similar changes in hamster brains but there were some small differences in clinical signs. These results suggest that TME and scrapie are very closely related (*156,181*).

Transmission

When it became apparent that TME was a spongiform encephalopathy, it was first hypothesized that mink acquired the disease by eating tissues from scrapie-infected sheep. However, two lines of evidence argue against this route of transmission (*1*). Efforts to induce TME in mink by feeding them brain tissue from scrapie-infected sheep raised in England and the U.S. were unsuccessful (*177*). It was possible to induce a TME-like illness in mink by injecting brain tissue from scrapie-infected sheep directly into the brain (intracerebral inoculation) of mink (*128*), but no mink became sick after eating brains containing the scrapie agent (*2*). In two of the TME outbreaks investigated, ranchers were certain that they did not feed rendered sheep to their mink. Rather, their primary source of animal protein was "downer" cattle (*178*).

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Mink that were fed or inoculated intracerebrally with BSE-infected bovine brain developed neurological symptoms 15 and 12 months later, respectively. Examination of the mink brains demonstrated spongiform degeneration. This disease was somewhat different from TME in that different areas of the brain were more severely affected and predominant symptoms were different (*224*).

To further investigate the possible connection of TME to disease in cattle, steers were inoculated intracerebrally with brain tissue from TME-infected mink. Within 15 months, all the cattle showed neurological symptoms and were later confirmed to be cases of spongiform encephalopathy (*178,225*). Brain tissue from these cattle fed back to mink induced TME (*180*).

These experiments suggest the possibility that, in rare instances, cattle may be afflicted with a transmissible spongiform encephalopathy that can be transmitted to mink. Central nervous system tissue from one 500 kg cow was estimated to contain enough infectious agents to cause disease in more than 1000 mink if administered in feed. Analyses of data on mink farms and dairy cattle in Wisconsin and using an estimate of 2–3% of adult cattle developing clinical symptoms resulting in non-ambulatory conditions, it has been estimated that only 1 in 975,000 adult cattle would need to be infected with a spongiform encephalopathy if affected cattle were the cause of the relatively rare outbreaks of TME that have been reported (*224,226*).

TME can also induce spongiform degeneration when inoculated intracerebrally into squirrel monkeys and stumptail macaques (*91*), into sheep and goats (*21*), and into skunks, raccoons, and ferrets (*180*).

CWD — Chronic Wasting Disease

Incidence/Prevalence

Chronic wasting disease (CWD) was first observed in captive mule deer (*Odocoileus hemionus hemionus*) in 1967; from 1974–1979 (when accurate records were kept), 80% (54 of 67 deer) that had been held at certain wildlife research facilities in Colorado and Wyoming for more than 2 years showed typical symptoms of CWD. Black-tailed deer (*Odocoileus hemionus columbianus*) (*294*) and Rocky Mountain Elk (*Cervus elaphus nelsoni*) were also diagnosed with CWD (*295*). These captive animals were reported to have had occasional contact along fence lines with other captive wild and domesticated ruminants. Elk at one facility that had close contact with captive deer had a higher incidence of the disease.

Subsequent to the initial outbreak among captive elk in Colorado, all remaining cervids at the facility were killed in 1985, and exhaustive cleanup of the area using calcium hypochlorite was conducted. A new elk herd was started with wild-born calves, and new fencing prevented contact with other wild and domesticated ruminants. During the next 11 years, 4 unrelated elk (17% of animals kept for >15 mo) developed CWD. Lateral transmission appeared to occur but prompt isolation and culling of affected individuals limited the outbreak. It is unknown whether CWD recurred because some contaminants remained in the animal facilities or whether one or more of the wild-born calves was already infected when it arrived at the facility (*189,293*).

CWD was first detected in free-ranging cervids in northcentral Colorado (within 100 km of Fort Collins) in 1981 with confirmed cases in mule deer, elk, and white-tailed deer (*Odocoileus virginianus*). These were obviously sick animals reported by local residents to wildlife officials (*252*). Surveys of brain tissue from wild cervids randomly harvested by hunters in eastern Colorado and Wyoming during 1996–1999, using immunohistochemical detection of protease-resistant prion protein (PrP^{res}), indicated overall prevalences of CWD in mule deer, white-tailed deer, and elk of 4.9%, 2.1%, and 0.5%, respectively (*190*). However, in some areas prevalence of CWD in both species of deer was calculated at approximately 15%.

Harvest-based estimates of disease prevalence may be biased because sick animals may be less alert and therefore more easily killed by hunters or autos, resulting in an overestimate of prevalence. On the other hand, sick animals may die in the back country and never be counted. These potential biases were discussed in a recent paper (*72*).

According to the latest information (available online, not in research papers), CWD has been confirmed in captive elk in Colorado, Kansas, Montana, Nebraska, Oklahoma, South Dakota, Wyoming, Alberta, and Saskatchewan. The disease has also been found in wild deer in Colorado, Nebraska, New Mexico, South Dakota, Wyoming, Saskatchewan, and in 24 white-tailed deer in southern Wisconsin (*12,289*). A National

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CWD Plan has been developed to coordinate and fund programs to prevent the spread and reduce the incidence of CWD, and surveillance studies are now being conducted in numerous states and Canadian provinces (*12,197*).

Disease Characteristics

Symptoms of CWD include excessive salivation, teeth grinding, lowering of the head, drooping ears, and listlessness and depression leading to emaciation. Occasional periods of hyperexcitability were noted in deer but this symptom was more common in elk. Specific motor and sensory deficits were not usually observed. Clinical course of the disease was 2 weeks to 8 months in deer and 1 to 6 months in elk (*294,295*).

Examination of the brains of diseased mule deer and elk revealed a pattern of widespread spongiform changes characteristic of all TSEs, with intracytoplasmic vacuolation of neurons, neuronal degeneration and amyloid plaques (*254*). These amyloid plaques react immunologically with antibodies raised against scrapie amyloid. A substantial proportion of plaques were described as florid (*168*). Distribution and severity of lesions in the brains of CWD animals were most similar to those observed in scrapie and BSE (*20,121–123,296*). Recent evidence indicates that abnormal prions are present in the tonsils, pituitary gland, adrenal glands, and pancreas of CWD-infected deer (*246*).

An immunohistochemical method for determining the presence of the infective protease-resistant prion protein (PrP^{res}) revealed that 10 of 17 captive elk in a South Dakota herd had CWD even though only 2 of them had shown clinical signs of the disease. Such an immunohistochemical test has been used for diagnosing scrapie in sheep (*210*). A monoclonal antibody has been used to successfully detect CWD in the tonsils as well as the brain of infected deer. Of 100 deer with CWD histologically confirmed in brain tissue, 99 were also positive by immunohistochemical staining of tonsil tissue (*253*).

Transmission

Transmission of CWD, like scrapie, is believed to be primarily lateral (i.e. from one animal to another in the field) through bodily secretions or excretions containing the infective agent. Mother to calf (vertical) transmission has not been observed but has not been ruled out (*293*). CWD appears to spread rapidly among white-tailed deer; this may be because they are more social than other cervids. On one game ranch in Nebraska 88 of 168 deer (52.4%) tested had CWD. There was an infection rate of 6.7% for deer within a 5-mile radius of the ranch and 3.5% for deer harvested between 5 and 10 miles of the ranch (*196*).

Oral transmission of the disease has been demonstrated in mule deer fawns fed brain homogenate from a deer with naturally occurring CWD. CWD PrP^{res} were detectable in lymphoid tissues draining the alimentary tract of the fawns within a few weeks after exposure (*247*).

It appears, from analyses of PrP genotypes of healthy and CWD-affected elk, that there may be some genetic predisposition to contract this disease in certain animals (*201*). If so, this is similar to scrapie (*28*) and to human TSE diseases, where some amino acid changes in the PrP protein predispose individuals to some types of TSEs (*165*). PrP genes in mule deer and Rocky Mountain elk are very similar, which may help explain why CWD is easily transmissible between these species (*64*).

Some in vitro studies with PrP^{res} suggest that CWD may not be easily transmissible to other animals because of a species barrier at the molecular level (*222*). Preliminary results from 13 calves inoculated intracerebrally with CWD-infected brain tissue demonstrated the presence of scrapie-associated fibrils and protease-resistant prions in the brains of 3 animals after about two years. The remaining 10 animals were still healthy after three years (*125*). Passage of CWD through ferrets was found to alter the host range of the disease. Attempts to transmit CWD to rodents failed until CWD was passaged through ferrets. Then it was possible to transmit CWD to hamsters (*26*). Therefore, species barriers to transmission of CWD are not absolute.

Reports of three cases of Creutzfeld-Jakob disease (CJD) in the U.S. in unusually young people (28 to 30 yrs) who regularly consumed deer or elk meat have raised concerns about the possible transmission of CWD to humans. The deer and elk consumed by the patients were not known to have come from endemic areas although some of the meat did originate in Wyoming. No strong evidence for a causal link between CWD and these cases was found but neither was it ruled out (*30*).

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Spongiform Encephalopathies in Humans

CJD — Creutzfeldt-Jakob Disease

Creutzfeldt-Jakob Disease was first described in 1920 and remains a relatively rare disease worldwide, with an incidence of 0.5–1.5 cases per million per year. Most cases occur in people 50 to 70 years of age, and in most cases there is no obvious precipitating factor. Approximately 10 to 15% of cases of CJD appear to result from genetic transmission since there is a family history of the disease (*223*). In addition, there have been a number of cases of inadvertent iatrogenic transmission of CJD during medical procedures involving corneal transplants, brain surgery, and injections of human growth hormone and gonadotropic hormones (*127,154*). The period from time of exposure to clinical symptoms ranges from a few years, when the brain was directly exposed, to 5 to 25 years, when a peripheral injection caused infection. Experimental studies with mice indicate that (unlike scrapie) B lymphocytes are not necessary for these prions to travel to the brain (*165*).

Most cases of CJD are sporadic with no known source of infectious agent. An epidemiological study of 405 CJD cases in Europe found no association between the risk for CJD and history of surgery or blood transfusions, consumption of beef, veal, lamb, cheese, or milk, or occupational exposure to animals. Slight increases in risk were associated with consumption of raw meat, frequent exposure to leather, and to fertilizers containing hoof or horn material (*276*).

A clinical and genetic study of 300 sporadic CJD cases demonstrated the existence of six phenotypic variations with somewhat different neurological symptoms and brain pathology. These variants were associated with differences in codon 129 of the prion protein gene (*204*). Other studies have also demonstrated variations in the prion proteins from different cases of sporadic CJD (*17,271*).

A cluster of seven CJD cases occurred in Nassau County, New York, during 1999–2000. Only one of the seven had a family history of CJD. Patients' ages ranged from 57 to 82. There were no known factors to account for this relatively large number of patients from a small geographical area (*2*).

Analysis of deaths from CJD reported in the U.S. from 1979 to 1998 indicates that mortality rates have been fairly constant during this time, with few cases occurring in persons younger than 30 years of age. None of these young victims apparently had the new variant of CJD, which has been associated with BSE (*110*).

vCJD — Variant Creutzfeldt-Jakob Disease

A new type of CJD appeared in the U.K. in 1994–1996. This disease differed from the familiar sporadic CJD in several ways. It affected much younger people, with an average age of 27 years as compared to 64 years for classical CJD, and the clinical phase of the illness lasted about 9 months longer. Examination of the brains of the victims of this new variant of CJD (vCJD) revealed an extensive amyloid plaque formation distributed throughout the cerebrum and cerebellum (*186,292*).

Epidemiological evidence supported a link between BSE and vCJD, and an analysis of the geographical distribution of vCJD demonstrated that more cases occurred in the north of Great Britain as compared to the south (*76*). There is no known reason for this variation but an investigation linked a cluster of vCJD cases in Leicestershire to the local (now illegal) practice of using brain and spinal cord of cattle as part of the meat in meat pies. Central nervous system tissue has also been detected in some German sausages (*171*). Therefore local variations in diet as well as variations in genetic susceptibility may explain uneven distribution of vCJD.

Molecular evidence from prion structure and infectivity studies in mice confirmed the link between BSE and vCJD (*56,68,70,133,241*). In addition to the central nervous system, vCJD prions have been detected in spleen, lymph nodes, retina of the eye, and tonsil tissue (*58,134,135,282*), and a characteristic protein, 14-3-3, has been detected in the cerebrospinal fluid of many patients with vCJD (*60,119,206,292*). Some of these tests may be useful in diagnosing this disease.

As of the end of Aug. 2002, 127 probable and proven cases of vCJD have been diagnosed in Great Britain (*84*) and there have been 8 cases identified in other countries (6 in France, 1 in Ireland, and 1 in Italy), Although the total number of cases of vCJD identified so far is small, there has been a steady increase in the number of cases during the past several years (Figure 1). Attempts to predict the future extent of the vCJD epidemic have produced very different estimates.

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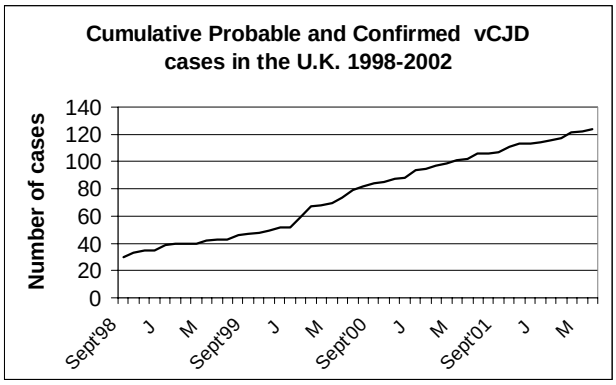
Figure 1



A recent analysis of incidence trends indicates that the underlying incidence (based on date of symptom onset) is increasing by about 18% per year (*9*). Evidence from studies of kuru and vCJD victims indicates that variation in codon 129 of the prion protein affects susceptibility to these diseases (*63*). The extent of exposure, incubation period, and susceptibility of people with different prion genotypes and at different ages are not known at this time and so it is difficult to predict the magnitude or length of the outbreak. The epidemic is expected to continue for some time and may involve hundreds to thousands of cases (*68,80,107,109,275*).

Several reviews summarize and discuss vCJD and the evidence linking it to BSE (*4,45,55,69,74,150,291*).

Kuru

Kuru is a human neurological disorder that occurred in one area in New Guinea. Investigation of the disease and the customs of the affected Fore people revealed that this was a spongiform encephalopathy spread by the ritual cannibalistic consumption of dead relatives as a token of respect. Women and children were the primary victims because they were primarily responsible for preparing the bodies (*5,141,183*). In mid-1950s, there were more than 200 new cases reported per year. The incidence of kuru declined thereafter as cannibalism ceased, but a few cases were reported annually into the early 1990s because of the long incubation period of the disease (*166*).

Analyses of DNA from 80 kuru victims and from 95 of their unaffected neighbors demonstrated that certain people were more susceptible to the disease. Variation at position 129 in the PRNP gene, which codes for prion protein, was associated with disease incidence. Kuru victims were more likely to have the amino acids methionine/methionine at this position, whereas people with valine/valine or valine/methionine at this position were at lower risk for developing kuru (*166*). Patients with methionine/methionine at position 129 had an earlier onset of disease and a shorter duration of illness (*63,166*). This is relevant to current concerns about vCJD because all vCJD patients tested so far have methionine/methionine at position 129.

Kuru has been transmitted to chimpanzees (*106*) and to squirrel monkeys by feeding them tissues from infected chimpanzees. Incubation period was 36 to 39 months (*112*). However, kuru has not been transmitted vertically from infected chimpanzee or rhesus monkey parents to their offspring (*6*). Data from New Guinea indicate that kuru is not transmitted vertically in humans either.

Prions as Causative Agents of TSEs

Normal Functions of Prions

Prions are glycoproteins containing over 200 amino acids with a glycosylphosphatidylinositol anchor that can attach to the surfaces of most types of cells but most commonly attaches to nerve cells. Analyses of various tissues from seven ewes demonstrated that the highest concentration of normal prions was in the brain, followed by the lungs, skeletal muscle, heart, uterus, thymus, and tongue (*192*). Prion structure varies somewhat among different species of animals, but certain parts of the molecule are more highly conserved than others (*300*). Prions also contain two sites where a variety of sugars and oligosaccharides may attach. Variations in the attached oligosaccharides may affect transmission of infectivity across species barriers (*214,229*).

The normal function of prions appears to involve facilitating the transmission of signals and the cellular uptake of ions or molecules (*251*). Since prions are present in high concentrations on neurons, they may facilitate signal transmission across synapses between nerve cells (*44,71,160,193*). There is also some evidence that normal prions function in signal transduction in lymphocytes (*161,167*).

Prions may also act as antioxidants. Prions bind to copper and are thereby changed into a protease-

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resistant form (*221*). The significance of this is not clear. Prions appear to facilitate transport of copper into the cell where it can be incorporated into the antioxidant enzyme superoxide dismutase (*151,159*). Copper–prion complexes also act as antioxidants (*43*). Prions may function to reduce oxidative damage in the body, and some data from mice (wild type and prionless strains) support this hypothesis (*157*). However, other researchers report no differences in brain copper content or the activity of superoxide dismutase in strains of mice with 0-, 1-, and 10 times the normal levels of prion protein (*221*).

Several strains of mice that lack prion proteins have been generated and used to elucidate normal prion function. Generally, these mice grow and develop normally during the first 70 weeks of life. Thereafter, all mice of two of the strains lacking prion proteins develop symptoms of ataxia (muscular incoordination) due to extensive loss of Purkinje neurons in the cerebellum. Some observations indicate that mice without prions have an increased sensitivity to seizures (*182*). Ablation of cellular prions also appears to reduce the number of mitochondria in cells (*187*).

Altered Forms as Causes of Disease

Normal and TSE-associated prions from the same species may contain exactly the same amino acids (primary structure) but they differ in the bending and folding of the molecules (secondary structure). Normal prions have more α-helical structures while TSE-associated prions have more β-pleated sheets and are resistant to attack by protease enzymes. The introduction of TSE-associated prions causes normal prions to change to the abnormal shape and aggregate together to form amyloid fibrils (*66,215,281*). Using monoclonal antibodies specific for the normal prion configuration, it is possible to see a rapid loss in normal prions and increase in scrapie prions in the brain during terminal stages of scrapie in mice (*302*).

More than 20 pathogenic mutations have been described from different inherited human forms of prion diseases, such as CJD (*29,213,228,281*). Some humans and animals have variations in the normal amino acid sequence of prions that render them more susceptible to abnormal folding and development of TSE diseases if they are exposed to TSE-associated prions. Persons with methionine (instead of valine) at position 129 in the prion protein are known to be more susceptible to vCJD and kuru. In vitro studies demonstrated that methionine-containing peptides have a greater propensity to form β-pleated sheets and aggregate to form amyloid-like fibrils (*209*).

The precise mechanism causing these structural changes is not known but may involve the interaction of metal ions (*191,256*) and polymerization through the formation of disulfide bonds (*283*). A denaturing compound, guanidine hydrochloride, also stimulates the conversion of normal to scrapie-type prion structure (*207*).

Infective prions may enter the body in a variety of ways. (*See following section on routes of infection.*) If they enter at a peripheral site, several different routes may be followed to the central nervous system. These often involve the spleen and lymphoreticular system (*48*) and dendritic cells (*16,145*). During the development of TSE diseases, normal prion proteins in the brain change conformationally and aggregate, eventually forming amyloid plaques and fibrils which interfere with brain function. Normal host prions are essential elements in these diseases, as demonstrated in experiments with mutant mouse strains that do not manufacture prions. Even when suspensions of scrapie prions are injected directly into the brains of these mice, they do not develop neurological symptoms because there are no normal prions to be converted into scrapie prions and amyloid fibrils in the brain (*42*).

Initiation and development of TSE diseases depend on both the normal prion structure of an animal and the structure of the abnormal prion being introduced. Normal prions from different species of animals differ slightly in amino acid structure and these differences as well as glycosylation patterns may account for the species specificity of prion diseases and the apparent resistance of some animals to one or another prion disease (*23,212*). Susceptibility or resistance to scrapie in sheep and goats is dependent on the amino acid sequence of the normal prion proteins (PrP) (*28,148*). Analyses of normal PrP genotypes of healthy and CWD-affected elk indicate that there may be a genetic predisposition to contract this disease (*201*), and evidence from studies of kuru and vCJD victims indicates that variation in codon 129 of the prion affects susceptibility to these diseases (*63*). Recent data from studies with inbred strains of mice suggest that there are other genetic factors besides prion variation that can affect incubation period for development of scrapie (*169,176*).

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Some normal prion variants are associated with a longer incubation period before disease symptoms appear (*115*). In fact, the incubation period may exceed the normal life span of the animal. Such asymptomatic animals, which have been exposed to TSE prions, may contain substantial amounts of infective prions and could be "carriers" of the disease (*136*).

PrP genes from 65 different cattle in the U.S. representing 14 breeds have been analyzed, and two distinct types of prion genes have been identified (*185*). However, analysis of PrP genotypes of healthy and BSE-affected cattle in Scotland revealed no significant association between disease and genotype (*149,185*).

Different strains of prion diseases and prions associated with different TSE diseases can cause different behavioral symptoms. For example, some sheep with scrapie rub and scratch against fixed objects while others display lack of coordination and tremor without scratching (*174*). Hamsters infected with TME may become lethargic (drowsy) or hyperexcitable and uncoordinated (hyper) (*33*). Hamsters infected with scrapie prions exhibit different symptoms than those infected with TME prions (*102,181*), and sheep infected with BSE can be distinguished from sheep with scrapie.

Routes of Infection

TSEs, by definition, are transmissible to other animals. A species barrier exists for these diseases, such that BSE is mainly found in cattle, scrapie in sheep and goats, etc. But this barrier is not absolute. Many research groups have demonstrated that a TSE may be transmitted at a very low frequency to another species during the first attempt. If those few infected animals are used to infect others of the same species, many more animals become sick and the incubation period shortens. Therefore, passaging the infective prion through a new species results in adaptation of the prion such that it may become very infective to the new species (*137,164*). Passage of CWD through ferrets was found to alter the host range of the disease. Attempts to transmit CWD to rodents failed until CWD was passaged through ferrets. Then it was possible to transmit CWD to hamsters (*26*).

Horizontal transfer

Animal-to-animal transfer in the field is believed to be the natural route of infection for most TSEs. Infected animals may shed the infective prions in various secretions and excretions, and then other animals could ingest or inhale them (*143*). Spread of scrapie among sheep or goats in a flock can occur when uninfected animals ingest fetal membranes voided by other sheep during lambing. Experimental feeding of scrapie-infected membranes to sheep and goats has been found to cause symptoms of scrapie after about 21 months (*205*).

Scrapie and CWD agents appear to survive in the environment for a long time. Uninfected animals that are introduced into fields or barns previously occupied by infected animals sometimes contract the disease (*143,189*). Experiments involving burial of scrapie-infected hamster brain in a temperate climate soil demonstrated that infectivity survived for 3 years (*51*). This has implications for disposal of infected animals and other material.

Vertical transfer and genetic predisposition

Some forms of CJD are familial (approximately 10–15% of CJD cases), and in most of the families investigated there is a mutation in the gene coding for the normal prion protein. More than 20 such mutations have been described and these act as dominant genes. Therefore a child inheriting this mutation will probably develop CJD later in life (*154,228*).

Evidence from scrapie transmission studies and some data on kuru, CJD, vCJD, and CWD indicates that certain genotypes of animals and humans may be more susceptible to TSE infection. Certain breeds of sheep are more likely than others to develop scrapie when exposed to the infectious agent. This differs from familial CJD in which the affected persons have inherited genes coding for abnormal prions. People and animals who have inherited a predisposition to TSE diseases will only develop the disease if they are exposed to infectious prions. As yet we do not have evidence for familial forms of animal TSEs but they may exist.

Lambs from scrapie-infected mothers were shown to be at greater risk for contracting scrapie. They may have been infected during birth or in utero by gulping amniotic fluid (*59*). However, some experiments demonstrated that lambs from scrapie-infected ewes that were isolated from their mothers shortly after birth were four times less likely to develop scrapie than lambs remaining with infected ewes (*143*). This suggests that the lambs acquire scrapie after birth.

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Some evidence also exists that calves whose mothers had BSE are at greater risk of developing this disease themselves, but it is not known when infection occurs. There appears to be a low level of maternal transmission (*87,97,99,290*).

Embryos from goats infected by intracerebral inoculation with BSE did not develop symptoms of BSE when transferred to other, uninfected females. In another study, BSE did not develop in naturally delivered offspring of BSE-infected female goats. The number of animals involved was small, thereby precluding a definite conclusion about maternal transmission (*101*). A recent review discusses pertinent data on the risk for transmission of TSEs in domesticated ruminants by reproductive technology such as artificial insemination and embryo transfer (*301*).

Maternal transmission of TSEs does not appear to occur in chimpanzees and monkeys infected with kuru, CJD, and scrapie. The sample size in these experiments was too small, however, to rule out the possibility of maternal transmission (*6*).

Consumption of contaminated brain or nervous tissue

All of the TSEs have been transmitted orally to some other animals by consumption of central nervous system tissue. Usually the oral route is a less efficient means of transfer of infectivity compared to direct inoculation into the brain. Data indicate that ingested prions are first taken up by lymphatic tissue in the intestine and then the prions travel to other parts of the body (*8,103,129,130,245,247*).

Kuru is the classic example of oral transmission of a TSE in humans. Spread of this disease occurred by the ritual cannibalistic practices of the Fore people in New Guinea (*5,141,183*). Kuru has also been transmitted orally to non-human primates (*112*).

Scrapie has been transmitted orally to sheep and goats by consumption of fetal membranes (*205*) and to squirrel monkeys (*112*). Attempts to infect cattle orally with scrapie have not been very successful. Cattle fed raw sheep brains containing a North American strain of scrapie failed to develop neurological disease (*78*).

Creutzfeldt-Jakob disease has been transmitted orally to squirrel monkeys (*112*). TME can be transmitted orally to mink (*180*), and CWD can be transmitted orally to fawns (*247*).

BSE has been transmitted orally to sheep (*103*), mice (*264*), lemurs, cats, and several species of zoo animals in England, and, apparently, to humans, resulting in vCJD (*38,75,232*).

Intracerebral injection

This is usually one of the first methods used in the laboratory to test transmissibility of TSEs to various species. Often, but not always, direct inoculation of brain tissue from an infected animal into the brain of another animal produces symptoms of the disease. For example, the BSE agent was injected into macaques, and the monkeys exhibited symptoms 32–38 weeks later (*163*). Scrapie was transmitted intracerebrally to a cynomolgus monkey (*111*), to mink (*128*), and to rats and mice but not to rabbits and guinea pigs (*22,77*).

Human cases of CJD have been caused by brain surgery during which contaminated electrodes were inserted into the brain. These electrodes had been implanted previously in the brain of a person known to have CJD. Other neurosurgical operations using contaminated instruments and transplant material have also been described (*154,215*).

Other injections

There have been a number of inadvertent cases of iatrogenic transmission of CJD during medical procedures involving injections of human growth hormone (more than 120 cases) and gonadotropic hormones (four cases) (*154,215*).

Intravenous injection of vCJD-infected brain homogenate into macaques resulted in disease four months later (*164*).

An Italian epidemic of scrapie in 1996–1997 affecting sheep and goats appeared to have been caused by a vaccine used to immunize the animals against *Mycoplasma agalactiae*. This vaccine was prepared from formol-treated brain tissue of sheep which had been harboring the infectious agent (*3,62,274*).

Blood transfusions

Blood from mice infected with a human TSE contains low levels of abnormal prions. Some blood fractions, particularly those containing red and white blood cells, were infective (*54*). A review of research on attempts to detect infectivity in blood of animals with TSEs reported that infectivity had been detected in blood of sheep, rats, mice and hamsters experimentally infected with scrapie but not in goats or sheep naturally infected with scrapie. Mice and sheep infected with BSE and

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mice and guinea pigs infected with human TSEs had infectious prions in blood. Blood from elk infected with CWD contained abnormal prions (*104*,*236*) but blood from mink with TME was not infectious (*50*). The buffy coat fraction of blood from a BSE-infected primate (*Microcebus murinus*) has also been shown to contain infective prions (*36*).

BSE was transmitted from one sheep to another by transfusion of whole blood from an experimentally infected sheep to an uninfected sheep. The donor sheep was in the preclinical stage of the disease but infective prions were apparently present in the blood (*144*).

To date there is no evidence of transmission of CJD or vCJD in humans by blood transfusion, but the discovery of abnormal prion proteins in the tonsils of people with vCJD suggests the possibility that these prions are also present in blood (*104*,*108*,*135*). Normal prion proteins are present in the platelets of humans and are released from platelets to plasma during cold storage of apheresis platelets (*34*).

Other tissues/animal products

Milk from cows with clinical signs of BSE was fed to and injected into a total of 275 mice. None of the mice developed any neurological disease during more than 600 days of follow up (*261*).

Although most researchers have not detected infective prions in skeletal muscle tissue (not contaminated with central nervous system tissue), a recent report indicated that infective prions injected into muscles of mice were propagated and accumulated in the muscles. Prion titers were measured in different muscles, and the authors suggested that some of the previous negative results might have occurred because the "wrong" muscles were analyzed. It is not known at present whether muscles from animals that have naturally (not experimentally) acquired a TSE contain significant levels of abnormal prions (*39*).

Beef tallow processed at high temperature and pressure by catalytic fat hydrogenation and hydrolytic fat splitting is considered safe because these processes have been shown to efficiently destroy prion proteins (*14*).

Bone grafts derived from bovine tissue have been evaluated as a means of transmitting BSE. Theoretical and experimental data indicate that these materials do not carry a risk for BSE transmission (*287*).

Catgut derived from cattle may potentially be infective because the preparation methods used would not destroy prions. However, there is no evidence that catgut has acted as a vehicle for transmission of BSE (*1*).

Medical devices

Medical devices, including neurosurgical instruments, depth electrodes and other devices used in close proximity to the brain and spinal cord, have been implicated in the iatrogenic spread of CJD (*13*).

Airborne

One potential natural route of infection that has not been investigated is inhalation. Other infectious agents are transmitted this way and one might anticipate that animals living close together could inhale dust particles or airborne secretions containing infective prions.

Stability of Prions — Disinfection and Rendering

Prions are notoriously resistant to heat and other standard decontamination procedures. One report described how electrodes that had been contaminated by insertion into the brain of a person with CJD were then "sterilized" with 70% alcohol and formaldehyde vapor. Two years later these electrodes were still infective when implanted into a chimpanzee (*154*). Scrapie prions bound to a stainless steel surface were not washed off with saline, and they retained significant infectivity after treatment with 10% formaldehyde for one hour (*303*).

Scrapie-infected hamster brain remains infective after autoclaving, and small amounts of infectivity remain even after exposure to dry heat at 360°C for one hour (*52*). Exposure of scrapie-infected hamster brain to 600°C completely ashed the tissue, but when these ashes were reconstituted with saline they were still capable of causing illness in 5 of 35 hamsters tested (*53*). In other experiments, prion rods heated in a lipid or lipid–water mixture were more stable than those heated in water (*15*). Experiments with the BSE prion indicate that its stability is similar to that of the scrapie agent (*26*,*257*,*259*).

Even in nature, subjected to the rise and fall of temperature and varying amounts of precipitation in a temperate climate, scrapie-infected hamster brain

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buried just below the soil surface retained 2–3 log units of infectivity (of an initial 4.8 log units of activity) after 3 years (*51*). This durability has implications for disposal of infected animals and other materials.

The molecular basis of this resistance appears to be the beta-pleated sheet secondary structure of the infectious molecule. In the solid state, the scrapie prion remains infectious after exposure to 132°C for 32 min but is inactivated by treatment with formic acid, trifluoroacetic acid, trifluoroethanol, hexafluro-2-propanol, and sodium dodecyl sulfate. These solvents denatured the protein by decreasing the proportion of beta-pleated sheets and destroying infectivity (*234*).

Two recent reviews summarize experiments with various chemicals and heating procedures to disinfect contaminated equipment (*230,257*). Effective decontamination procedures include exposure to: (a) strong sodium hypochlorite solution and (b) solutions of hot sodium hydroxide (*82,230,257,260,262*); and (c) methanol/sodium hydroxide/methylene chloride extraction and purification followed by steam autoclaving (*86*). Treatment with these chemicals can be corrosive to some instruments. Therefore, some materials cannot be effectively sterilized and must be discarded if they have been contaminated.

High temperatures and pressures used in catalytic fat hydrogenation and in fat hydrolysis reduced protease-resistant prions with degradation factors of 10^3–10^4 and 10^7, respectively (*15*).

Effectiveness of rendering procedures for decontaminating tissues containing infective TSE agents has been investigated and different methods compared. Fifteen rendering procedures were evaluated for their ability to destroy infectivity of the BSE and the scrapie agents. No infectivity was detected in any of the tallow samples but most of the rendering processes did not eliminate infectivity in meat and bone meal. Processes involving exposure to hyperbaric steam were more effective than those that did not (*265,266*). An evaluation of the solvent extraction processes used by British renderers in the past indicated that they could not significantly inactivate the scrapie and BSE agents (*263*).

Other Causes Proposed for BSE

Most researchers in this field consider prions to be the causative agents of transmissible spongiform encephalopathies. However, there are some who contend that there are other causative agents or environmental factors that induce symptoms of TSEs. Brown has discussed several alternatives that have been proposed as causes for BSE (*44*). Among other arguments, he points out that the BSE epidemic in Great Britain only began to subside after the ban on meat and bone meal supplements was introduced in 1988. Some other factors may have influenced the course of the epidemic or the susceptibility of some animals, but the infective agent must have been in the meat and bone meal.

Other factors suggested as etiological agents for BSE include:

Bacterial (*19,90,270*)

Do bacteria induce an autoimmune response or a brain infection? Some data indicate that cattle, sheep and humans with TSEs have autoantibodies to brain neurofilaments. Some of these antibodies cross-react with some bacteria (particularly *Acinetobacter calcoaceticus*), and it has been theorized that affected animals have been exposed to these bacteria and produced antibodies (against the bacteria) which then cross-reacted with neural proteins in the brain and perhaps altered prion structure and activity.

Analyses of brain tissue from 13 cases of CJD and 5 cases of scrapie revealed the presence of 16S rDNA from a bacterium, *Spiroplasma* sp. (*27*) *Spiroplasma* can cause a persistent brain infection with spongiform pathology in rodents. It was hypothesized that this bacterium may play a role in the development of TSEs. No data were presented for analyses of brains from non-TSE victims.

However, no bacteria possess anywhere near the resistance to heat and other treatments that has been observed for the infectious agents of TSEs. Therefore, it is difficult to understand how *Acinetobacter* or *Spiroplasma* could be responsible for BSE

Pesticide exposure (*216–218*)

During the 1980s and early 1990s, cattle in Great Britain were subjected to compulsory treatment with an organo-phthalimido-phosphorus pesticide, phosmet, to

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prevent damage from warble flies. It has been theorized that phosmet may have changed prions in susceptible cattle embryos exposed to phosmet in utero, thereby initiating BSE. Organophosphates are known to have neurotoxic effects. However, some countries, like Japan, that use organophosphorus pesticides extensively have had few or no cases of BSE.

Manganese toxicity/copper deficiency (*31,219,220*)

Manganese (Mn) is known, from some cases of industrial exposure, to have neurotoxic effects. It has been asserted that outbreaks of TSEs cluster in areas where soils and the food chains are relatively rich in Mn. Prion proteins normally bind to copper (Cu), and these complexes may have a protective function in the body by acting as antioxidants (*43,288*). The Mn toxicity theory suggests that Mn interferes with the formation of the antioxidant Cu–prion complex and instead forms Mn–prion complexes that are misfolded and do not act as antioxidants. It has also been proposed that environments with clusters of sporadic cases of TSEs have higher than average intensities of ultraviolet/ozone oxidants. Increased oxidative stress combined with high environmental Mn/low Cu concentrations could cause neurotoxic effects.

However, this theory does not explain the prevalence or occurrence of TSEs in areas that are not manganese-rich. In fact, some of the clusters cited as evidence for this theory have been shown to be caused by familial CJD mutations present in specific populations (*154*).

Selenium deficiency and bacterial toxin (*255*)

It has been suggested that a combination of selenium deficiency and a bacterial toxin, such as the cholera toxin, could cause a deficiency in cellular cyclic GMP levels. This may have deleterious effects on neurons and cause TSEs. No experimental evidence was presented to support this hypothesis.

Eradication/Control/Treatment of BSE

Numbers of BSE-infected cattle in the U.K. have declined steeply during the past decade in response to the ban on use of ruminant-derived meat and bone meal in cattle feed. As detailed in numerous papers, it is not possible to reliably destroy all prions during rendering processes without destroying the nutritional value of the supplements and so this ban must remain in place (*40,188,211,263,265,266*).

Destruction of infected animals and herds containing infected animals has limited the potential horizontal and vertical transmission of BSE. Effects of different culling practices on the eradication of this disease were discussed in an article on transmission dynamics and epidemiology. The authors believe that eventual eradication is possible (*7*).

However, for CWD, a disease more readily spread among animals in a herd, it is likely to take a longer, sustained effort to eradicate the disease (*120*). Efforts in Wisconsin to destroy as many deer as possible in the area where CWD was detected will be monitored to determine if this will prevent spread of the disease to other areas of the state (*289*). Despite efforts to eliminate diseased animals, infective material may persist in the environment and be a source of further cases of CWD (*293*).

Sheep are known to vary in their genetic susceptibility to scrapie, and breeding of resistant strains of sheep and goats may be a useful strategy for minimizing scrapie in flocks (*89,272,274*). However, it is not known whether resistant sheep and goats that have been exposed to scrapie but are not clinically ill can be carriers of the disease (*136*). Some scrapie-exposed goats that were apparently healthy and had no histopathological lesions in the brain were found to contain protease-resistant prions (*35*). Some asymptomatic sheep from a scrapie-infected flock in Iceland had a subclinical infection with lesions in the brain and/or scrapie associated prions present. However, none of the sheep in this flock with the resistant AHQ genotype had either clinical or subclinical signs of scrapie (*269*). Experiments with scrapie-infected mice have shown that a low dose inoculum may induce a chronic, subclinical infection with significant levels of infectivity in the brain (*268*). Therefore, it appears that some TSE-resistant animals may act as carriers and may be capable of spreading the disease even though they do not appear ill.

Some variation exists in PrP genes from different cattle breeds (*185*). However, these differences do not appear to be related to susceptibility or resistance to BSE (*149*). At this point, it does not appear likely that genetic selection for resistant animals could produce BSE-free herds.

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As more is learned about TSE diseases and how prions cause illness, possible therapeutic options are being investigated. Since specific regions of the prion molecules are important for misfolding, it has been suggested that specific short peptides could inhibit misfolding and, therefore, disease progression (*250*). Acridine and phenothiazine derivatives can inhibit the formation of abnormal prion structures and may delay development of BSE and scrapie, but they are not cures (*32,114*). Two recent reviews described the effects of various drugs used in attempts to control development of TSE diseases (*49,105*). No compound completely prevents or reverses the effects of TSEs, but it may eventually be possible to use effective combinations of drugs.

Prion diseases are accompanied by disturbances in the antioxidant defense systems. A case report of a CJD patient treated with antioxidants demonstrated that this therapy might delay severe symptoms and death by more than a year. The patient eventually died but lived for about 1.5 years longer than expected (*88*).

Other scientists are investigating the possibility of developing a vaccine to prevent infection or slow the development of TSEs. Normally, animals do not appear to mount an immune response to prion infection. However, injection of mice with CpG oligodeoxynucleotides, compounds known to stimulate innate immunity, delayed or prevented development of scrapie after intracerebral infection (*242*). Two recent reports described the inhibition of abnormal prion propagation in cell cultures by specific antibodies (*92,208*). Mice vaccinated with a genetically engineered prion protein for several weeks developed disease symptoms several weeks later than unvaccinated mice after injection of scrapie-infected brain tissue. The vaccine did not completely prevent disease and had some toxic effects, but it demonstrates the possible utility of this approach (*248*).

Diagnostics

Relevant Tissues/Animals for Testing

Tissue distribution of the protease-resistant, infective prions causing TSEs varies in different species with the route and stage of infection and with prion or disease type. For example, blood from cattle with BSE does not appear to carry the infective agent, but sheep infected with the BSE agent can transmit this disease to other sheep by a blood transfusion (*144*). Scrapie, vCJD, and CWD prions are detectable in tonsil samples from infected animals/humans and could be used to diagnose these diseases prior to death, but BSE prions are not detectable in tonsils of cattle. Laboratory rodents infected with TSEs by intracerebral infection often exhibit a different tissue distribution of infectivity than that seen in the animals originally infected with the TSE.

Central nervous system tissue (brain and spinal cord) always shows signs of disease — characteristic vacuolation — and TSE-associated prions in clinically affected and many pre-clinical animals. Detection of infective, protease-resistant prions in peripheral tissues or secretions would permit diagnosis and, eventually, treatment of a TSE in pre-clinical animals and humans, and healthy animals could avoid slaughter. Table 2 presents summary results obtained in different labs testing various tissues of TSE-affected animals. References for these data are noted in the sections below.

BSE prions are readily detected in central nervous system tissue of cattle by several test procedures (*117,199*). However, except for reports of small amounts of infectivity in the ileum (*284*) and bone marrow (*285*) and a recent report that protease-resistant prions are present in urine of BSE-infected cattle (*244*), infective prions have not been detected in other bovine tissues.

Scrapie prions are most commonly detected in the brain, spinal cord, lymph nodes, and tonsils. They have also been detected in Peyer's patches in the intestinal wall, lymphatic tissue draining the Peyer's patches, the spleen, nervous tissue, and the nictitating membrane of the eye (*103,129,130,153,238*). Scrapie prions were not detected in peripheral blood leukocytes of infected sheep (*131*) but were present in the buffy coat fraction of blood (*236*). Normal prion proteins have been detected on the surface of peripheral blood mononuclear cells but not of platelets (*132*).

CWD prions have been detected in the tonsils as well as the brain of infected deer using a monoclonal antibody for immunohistochemical staining. Of 100 deer with CWD histologically confirmed in brain tissue, 99 were also positive by immunohistochemical staining of tonsil tissue (*253*). Recent evidence indicates that ab-

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Table 2. Reported Occurrence of Abnormal Prions, Infectivity, or Other Disease-Associated Proteins in Different Tissues*

Tissue	BSE	Scrapie	TME	CWD	vCJD
Adrenal glands				+	+
Blood**	–	– (W); + (B)	– (S)	+ (B)	–
Brain and spinal cord	+	+	+	+	+
Cerebrospinal fluid (Protein 14-3-3)					+
Eye		+		+	+
Kidney			+		
Liver			+		
Lymph nodes		+	+	+	+
Muscles			+		
Nerves, peripheral		+			
Pancreas				+	
Peyer's Patches/ intestine	+	+	+	+	
Spleen	–	+	+	+	+
Tonsils		+		+	+
Urine	+		–		

(+, positive test; –, negative test; blank, not reported)
*These results are for tissues of animals normally infected with these diseases; rodents and other test animals sometimes exhibit infectivity in other tissues.
**Blood: white blood cells (W); serum (S); buffy coat fractions (B)

normal prions are also present in the spleen, pituitary gland, adrenal glands, and pancreas of CWD-infected deer (*246,293*) and in the buffy coat fraction of blood of CWD-infected elk (*236*).

TME: Assays of various tissues of mink with TME for infectivity revealed that the highest titer was present in central nervous system tissue. However, infectivity was widespread throughout the body, with detectable levels in liver, spleen, lymph nodes, kidneys, muscles, and feces (*124,179*).

vCJD prions have been detected in spleen, lymph nodes, retina of the eye, and tonsil tissue (*58,134,135,282*) and a characteristic protein, 14-3-3, has been detected in the cerebrospinal fluid of many patients with vCJD (*60,119,206,292*). These tests may be useful in diagnosing this disease.

FSE: Examination of tissues from cats with feline spongiform encephalopathy (acquired by consumption of BSE-infected tissues) revealed that protease-resistant prions were present in the kidneys of all the cats with FSE that were tested but only a few animals had these prions in the spleen and lymphatic tissue (*232*).

Determination of Disease in Animal/ Human Tissues

Early diagnosis of transmissible spongiform encephalopathies in tissues of animals and humans is an important goal for many reasons. It could identify affected animals and prevent their entry into the food chain or the use of their tissues in medical products or other devices. Healthy animals in a herd could be identified and avoid unnecessary slaughter. Finally, although TSEs are now rapidly fatal diseases, therapies may become available in the future and early diagnosis of these diseases may lead to successful treatment.

Animals with BSE and other TSEs exhibit a number of neurological signs — both behavioral and coordination problems — which may arouse suspicion. But other diseases can cause similar symptoms. The so-called "gold standard" of TSE identification involves histological examination of the brain after death for characteristic vacuolation of neurons (spongiform appearance) and the immunochemical detection of the modified, protease-resistant prions associated with TSEs. However, these tests can take several days and are performed only after the animal is dead.

A mouse bioassay can be used to assess the infectivity of tissues from a suspect animal. Transgenic mouse

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strains expressing the normal bovine prion protein have been constructed to efficiently monitor the presence of BSE prions in tissue samples. The presence of the normal bovine prion eliminates the problem of a species barrier when testing in these mice (*61*).

Additionally, a variety of more rapid, immunological assays have been devised to detect the presence of low levels of TSE-related prions in central nervous system (CNS) tissue and in more easily accessible tissues such as blood and tonsils (*47,199*).

One problem common to all these detection methods is the very low concentration of abnormal prions present in peripheral tissues. Three recently developed procedures may be useful in concentrating the abnormal prions, thereby making them more easily detectable. Human plasma protease plasminogen binds to prions associated with CJD, scrapie, and BSE. Magnetic beads containing immobilized plasminogen can selectively precipitate prions from diseased brain homogenates. These prions can then be detected immunologically (*175*).

A novel procedure that exploits the ability of TSE-associated prions to induce protein misfolding in normal prions has been utilized to detect the presence of abnormal prions in brain homogenates. Hamster brain homogenate (a source of normal prions) was incubated with diluted scrapie brain homogenate and an increase in abnormal prion was noted. After 5 cycles of this amplification procedure, the amount of protease-resistant prions dramatically increased. This method may be useful in detecting TSE prions at very low concentrations (*233*).

Two characteristics of abnormal prions — protease-resistance and aggregation to form polymers and fibrils — have been exploited in a procedure utilizing protease digestion and filtration through a slot blot device (0.45 μm pore size). Aggregates retained on the filter were then detected immunologically (*297*).

Brain and spinal cord tissue

In 1999, the European Union published an evaluation of 4 assays for BSE in bovine CNS tissue (*194*). Coded samples from 1000 animals (some known to be negative, others positive) were tested to determine the sensitivity, specificity, and limit of detection of each assay. Tests A and D could be completed in <24 hours, test B in 7–8 hours, and test C in <4 hours. These times could be reduced by automation. Results are summarized in Table 3.

Further details of Test D have been published (*118*). A commercial version of test D (BioRad), used to test BSE brain tissue, was found to be more sensitive than the mouse bioassay in detecting infectivity (*85,117*). Test B (Prionics) was further described and is currently being used in several European countries as a large-scale screening assay for BSE in cattle (*202*). Since January 2000, it has been included as one of the statutory tests for BSE and scrapie in the U.K. (*73*). A paraffin-embedded-tissue (PET) method with immunodetection has also been developed for detection of abnormal prions in brain tissue (*239*).

Two other assays have been developed recently for detection of TSE prions in CNS tissue. A monoclonal antibody, KG9, revealed widespread granular deposits of abnormal prions in BSE-infected cattle brain but did not react with scrapie-associated prions in ovine brain (*162*). An ELISA based on a monoclonal antibody correctly identified positive and negative samples of bovine brain at 1000–2500-fold dilutions within 6 hours (*277*).

Cerebrospinal fluid (CSF) has been examined for evidence of TSE infection. Protease-resistant prions were not detected in CSF from patients with CJD (*299*). However, the 14-3-3 proteins have been detected in CSF from patients with CJD. They are also present in patients with other types of dementia and are not therefore a specific marker for CJD (*60,206*).

Table 3.

Test	Basis of Method	Specificity	Sensitivity	LOD
A	Noncompetitive immunoassay using two monoclonal antibodies for prion	90%	70%	Undil.
B	Western immunoblotting using monoclonal antibody to detect prion fragment	100%	100%	10^{-1}
C	Chemiluminescent ELISA using polyclonal antibodies	100%	100%	$10^{-1.5}$
D	Sandwich immunoassay using two monoclonal antibodies	100%	100%	$10^{-2.5}$

Sensitivity = % of known infected animals testing positive.
Specificity = % of known uninfected samples testing negative.
LOD = limit of detection; dilutions of tissue in which test detected TSE prions.

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Blood

Attempts to detect TSE-associated prions in blood of animals and humans have yielded mixed results. These proteins are generally not detectable from naturally occurring infections. However, when rodents were inoculated with several types of TSEs, infectivity was detected in blood or its components during both the incubation and clinical phases of disease (*47*,*50*). The highest concentration of infectivity was located in the buffy coat fraction of the blood, which is rich in blood platelets. The transfer of BSE from one sheep to another by transfusion of whole blood indicates that infective prions were present in the blood (*144*).

Immunochemical methods can detect normal and protease-resistant prions in plasma of CJD patients and also in patients with other neurodegenerative diseases (*280*). A capillary electrophoresis immunoassay has been developed to detect scrapie and CWD prions in buffy coat fractions from the blood of sheep and elk, respectively (*236*).

A dramatic decrease in the mRNA coding for erythroid differentiation-related factor has been noted in blood and bone marrow from sheep with scrapie and cattle with BSE as compared to healthy animals. Northern blot analysis of RNA from these tissues may be a useful method for the detection of animals infected with TSEs (*188*).

Urine

A protease-resistant isoform of the prion protein has been detected in urine from scrapie-infected hamsters, BSE-infected cattle, and humans with CJD using an immunoblotting technique (*243*).

Other extraneural tissues

Immunological methods have also been used to detect the presence of TSE prions in: (a) tonsils of preclinical sheep exposed to scrapie (*146*); (b) spleens of scrapie-infected sheep but not of BSE-infected cattle (*249*); and (c) tonsil, spleen, lymph node, and retina of eye of victims of vCJD (*282*).

Determination of Nervous System Tissue in Food

Since prions are present in high concentrations in central nervous system (CNS) tissue, it is important to exclude such tissue from the food chain if there is any possibility that an animal is infected with a TSE. Brain or spinal cord may disperse among other animal tissues during the stunning process or by advanced meat recovery from the backbone. Recent studies have documented the presence of CNS tissues in the lungs, heart, and jugular vein blood of cattle and sheep killed with several types of captive bolt guns and in various tissues of carcasses split by sawing through the vertebral column (*10*,*11*,*138*,*173*,*237*). The European Commission has issued a preliminary scientific opinion on BSE risk relative to different stunning methods (*95*). Potential dispersal of central nervous system tissue containing abnormal prions during commercial stunning and dressing was also investigated by use of an antibiotic-resistant *Pseudomonas fluorescens* as a marker. This organism became widely dispersed throughout the slaughter–dressing environment and within the carcass of test animals (*81*).

Once meat is ground up, it would be necessary to use some analytical technique to detect the presence of central nervous system tissue. Cholesterol levels are higher in CNS tissue than in muscle tissue. Therefore, analyses of meat products for cholesterol concentrations may reveal contamination with brain and spinal cord. A cholesterol test kit, "Enzym.BioAnalysis Cholesterin/R-Biopharm," was found capable of detecting brain and spinal cord at concentrations of 1.0% and 0.5%, respectively, in comminuted meat (*198*). Although this assay is easy to perform, it is not very specific.

Enzyme-linked immunoassays have been developed to detect the presence of proteins found in the central nervous system, such as glial fibrillary acidic protein (GFAP), neurofilament (NF), myelin basic protein (MBP), and neuron-specific enolase (NSE). GFAP was readily detectable in ground beef spiked with brain or spinal cord but was absent in pure ground beef (*237*). Monoclonal antibodies to GFAP and NSE have been used to assay 622 German meat products for the presence of central nervous system tissue. CNS tissue was detected in 9.7% of liver sausage samples and 20.8% of kochmettwürste but was absent in meatballs and some other sausages (*172*,*286*). Other surveys found CNS tissue in 13.7% of 102 Brühwurst samples (*139*) and 4% of 120 liver sausages (*171*).

These immunoassays can successfully detect 1% (or less in some cases) of CNS in raw ground meat samples. However, cooking and some processing steps may alter the conformation of the proteins to be tested and

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decrease the utility of some assays. The anti-NF test was the most reliable for cooked meats according to one set of experiments (*18*) while the anti-MBP assay was most reliable in another set of tests (*267*).

Bovine BSE-infected brain was incorporated at several levels into micro sausages. An immunoassay for BSE prions was capable of detecting BSE-positive samples when CNS concentrations were as low as 0.25% (*170*).

Determination of Ruminant Protein in Feed

Since regulations in many countries prohibit the use of ruminant tissue in feed intended for ruminants, methods are needed for determining the animal species present in meat and bone meal. A species-specific PCR (polymerase chain reaction) assay has been devised for the detection of bovine, ovine, poultry, and porcine meat (*67*). This method may be useful in distinguishing these species but needs some refinement to achieve greater accuracy.

SUMMARY

Bovine spongiform encephalopathy and vCJD are the most recently identified diseases caused by unique infectious proteins known as prions. Normal prions, with several α-helical structures, may have a role in copper transport, nerve conduction, cell signaling, and antioxidant reactions. Pathogenic prions, with more β-sheet structures, are extremely resistant to heat, protease enzymes, and disinfectants. They pervert normal prions by changing their structure and causing them to aggregate and interfere with brain function. In nature, prion diseases are known to be transmitted from animal to animal, and mutant genes may cause disease or an increased susceptibility to abnormal prions. The high concentration of pathogenic prions in central nervous system tissue makes it particularly important to prevent these tissues from entering the food chain. Assays are being developed to detect infected animals from peripheral tissues, but as yet there are no effective treatments for prion diseases.

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