

Review Article

Medical Progress

CREUTZFELDT-JAKOB DISEASE AND RELATED TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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UNTIL 30 years ago, Creutzfeldt-Jakob disease was an obscure form of dementia unknown to most physicians. The name is now familiar to the medical community as the major transmissible spongiform encephalopathy (or prion disease) in humans and to research scientists because of its strange causative agent with novel modes of replication and transmission. Furthermore, the term "Creutzfeldt-Jakob disease" has caused distress to patients who have been notified that they may have received tainted blood or blood products, and it has been popularized by extensive media coverage of bovine spongiform encephalopathy ("mad cow disease"), with its profound economic impact in Europe and probable transmission to humans in the United Kingdom. To our knowledge, the incidence of Creutzfeldt-Jakob disease has not changed in recent years, but professional and lay interest in the disease has burgeoned.

The nature of prion diseases and their pathogenesis at a cellular level and in transgenic mice have been recently reviewed in the *Journal*¹ and elsewhere.²⁻⁶ Our review will comment briefly on transmissible spongiform encephalopathies in general and then focus on the practical aspects of the epidemiology and diagnosis of Creutzfeldt-Jakob disease, the possible modes of transmission and inheritance, and the risks to recipients of blood transfusions, physicians, and relatives. Finally, the British outbreak of bovine spongiform encephalopathy, the recent evi-

dence that this disorder may have been transmitted to humans, and the resultant apprehension in North America will be addressed.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

A number of transmissible spongiform encephalopathies have been described in animals and humans (Table 1). All have incubation periods of months to years, and all gradually increase in severity and lead to death over a period of months. None evoke an immune response, and all share a common noninflammatory pathologic process restricted to the central nervous system. The only macromolecules thus far associated with infection are isoforms of a host membrane sialoglycoprotein called prion protein (PrP). These transmissible agents appear to have common mechanisms of pathogenesis and possibly a common origin. Some have spread across species barriers (transmissible mink encephalopathy and possibly new-variant Creutzfeldt-Jakob disease); some have reached epidemic proportions by entering the food chain (transmissible mink encephalopathy, bovine spongiform encephalopathy, and kuru); and others have been transmitted by inheritance of mutations in the *PrP* gene (familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia).

Scrapie is a subacute, progressive ataxia of sheep and goats. Animals affected by scrapie have been recognized by shepherds for over 200 years, and for many years the disorder was regarded as an inherited, degenerative disease of the brain and spinal cord. In 1936 scrapie was reported to be transmitted from sheep to sheep with an incubation period in excess of one year⁷; nevertheless, controversy continues to this day about the natural mode of transmission and the relative role of genetic susceptibility.⁸ The disease has been experimentally transmitted to many species, but although amplification occurs in some neural cell lines, no in vitro assay has been developed. Therefore, most of our knowledge of the nature of prions comes from studies of the scrapie agent in mice and hamsters.

Recurrent outbreaks of transmissible spongiform encephalopathy have occurred on mink ranches in Wisconsin, and isolated outbreaks have been reported in Canada, Finland, Germany, and Russia.⁹ Feed contaminated with tissue from scrapie-affected sheep was assumed to be the mode of transmission, but recent observations suggest that tissue from other infected animal species may also have been involved.¹⁰ Chronic wasting disease of deer and elk is a trans-

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TABLE 1. TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (PRION DISEASES).*

Animal diseases
Scrapie (sheep and goats)
Transmissible mink encephalopathy
Wasting disease of deer and elk
Bovine spongiform encephalopathy†
Transmissible spongiform encephalopathy of captive wild ruminants††
Feline spongiform encephalopathy‡§
Human diseases
Kuru
Sporadic Creutzfeldt-Jakob disease
Familial Creutzfeldt-Jakob disease
Gerstmann-Strüssler-Scheinker disease
Fatal familial insomnia
New-variant Creutzfeldt-Jakob disease†

*Within each category (animal or human diseases), diseases are listed in the order in which they were recognized to be transmissible.

†These diseases all appear to have a common source.

‡The wild ruminants affected are the nyala, gemsbok, Arabian oryx, eland, kudu, scimitar-horned oryx, ankole, and bongo.

§The felines affected are the domestic cat, puma, cheetah, ocelot, and tiger.

missible spongiform encephalopathy found in captive animals in the western United States and Canada. The disease has been found in wild deer and elk in northeastern Colorado and southeastern Wyoming, and a prevalence of 2.5 percent has been estimated in deer in that area. The mechanism of transmission remains unknown (Williams E: personal communication).

Kuru was the first human spongiform encephalopathy shown to be transmissible. This subacute, uniformly fatal disease of cerebellar degeneration reached epidemic proportions among the Fore ethnic group in a remote mountainous area of New Guinea.¹¹ The disease was spread by ritual cannibalism and has gradually disappeared over the 40 years since the practice ceased. The similarities in the epidemiology, clinical course, and histopathological features of kuru and scrapie led to the suggestion that transmission of kuru to nonhuman primates should be attempted.¹² Intracerebral inoculation of brain homogenate transmitted the disease to chimpanzees, with incubation periods of 14 months or more,¹³ and serial transmission with limiting dilutions established the occurrence of replication.

Creutzfeldt-Jakob disease occurs as both a sporadic and a familial disease. Its epidemiologic and clinical patterns are different from those of scrapie and kuru, but it produces similar spongiform changes in the nervous system. These similarities prompted studies of transmission from humans to nonhuman primates. Successful transmission has occurred with samples obtained from more than 300 pa-

tients.^{14,15} Transmission can occur by peripheral routes of inoculation, but larger doses are required than for transmission by intracerebral inoculation. In some cases, oral transmission has been possible, with even larger dosages of inoculum.¹⁶

Species barriers exist against all transmissible spongiform encephalopathies, but these barriers are more impenetrable in some species than in others. In all affected species, infectivity is greatest in brain tissue, is present in some peripheral tissues, but generally has been absent from all body fluids except cerebrospinal fluid.

Nature of Prions

Studies of the scrapie agent and more limited studies of prions of human origin indicate that the agents are resistant to treatments that inactivate nucleic acids and viruses (alcohol, formalin, ionizing radiation, proteases, and nucleases) but that they are inactivated by treatments that disrupt proteins (autoclaving, phenol, detergents, and extremes of pH). Prusiner showed that infectivity colocalized with a protein and proposed the term prion "to denote a small proteinaceous infectious particle which is resistant to most procedures that modify nucleic acids."¹⁷ The protease-resistant protein associated with disease (designated PrP^{res} or PRP^{Sc}) proved to be an isoform of a protease-sensitive normal host cellular protein (designated PrP^{sen} or PrPC).¹⁸ In the pathologic process, PrP^{sen} undergoes a post-translational conformational change to PrP^{res}; this conformational change converts the protein from a predominantly alpha-helical structure into one with a large beta-sheet content. This protease-resistant isoform accumulates in neural cells, disrupting function and leading to vacuolization and cell death.

Studies in transgenic mice indicate that mutations in the gene coding for PrP or overexpression of the gene can lead to spongiform changes.¹⁹ Mice lacking the PrP gene cannot be infected with scrapie, an observation unequivocally showing that PrP is necessary for the disease.²⁰ Whether PrP is sufficient is unclear. A chaperone protein that binds PrP and facilitates conversion may be necessary,² and cogent arguments continue to question the "protein-only" theory.²¹

Pathogenesis

In lambs exposed to scrapie-infected flocks, infectivity is first found at about one year of age in the lymphatic tissues and intestines, suggesting transmission by way of the alimentary tract. Infectivity in the brain is found at about two years of age, and infectivity slowly increases in the brain, with resultant spongiform changes and clinical disease during the subsequent year.²²

In mice experimentally infected by subcutaneous inoculation, infectivity is found first in the lymphat-

ics and spleen and later in the brain.²³ Infectivity has been found in the blood of experimentally infected rodents,²⁴ and the presence of differentiated B cells appears important for invasion of the nervous system of the mouse after intraperitoneal inoculation.²⁵ After inoculation into the limbs, however, the scrapie agent travels along the nerves to the central nervous system,²⁶ so multiple routes of invasion of the nervous system may be possible. The route of entry of the pathogen, infectivity of blood and tissue at different stages of the infection, and variations between species and between the routes of inoculation of the nervous system are central to many of the questions about the risk of infection that are discussed below.

SPORADIC CREUTZFELDT-JAKOB DISEASE

Early Recognition and Epidemiology

Creutzfeldt-Jakob disease was first described clinically and pathologically in the 1920s. However, the patient described by Creutzfeldt and three of the five patients described by Jakob would not fulfill present-day criteria for the diagnosis.²⁷ Subsequently, an array of eponyms was applied to similar diseases with differing clinical presentations or topographic distributions of pathological findings. The experimental transmission of Creutzfeldt-Jakob disease to chimpanzees in 1968¹⁴ led to a rational delineation of the syndrome and acceptance of the flawed designation of Creutzfeldt-Jakob disease.

The disease occurs worldwide with an incidence of 0.5 to 1.5 cases per million population per year. There is no seasonal distribution, no evidence of changing incidence over the years, and no convincing geographic clustering, except for areas with large numbers of familial cases.

Many series and case-control studies have searched for risk factors. By analogy to other spongiform encephalopathies, dietary factors, exposure to animals, and occupational exposures have been scrutinized. The incidence in Australia and New Zealand, which are free of scrapie, is similar to that in the United Kingdom, where scrapie in sheep is endemic. Neither consumption of brains and offal nor lifetime vegetarianism alters the risks. Surgeons, pathologists, butchers, abattoir workers, and cooks exposed to blood and uncooked animal products do not have increased risks.^{28,29} Only a single conjugal pair of cases has been verified,^{30,31} and there is no evidence of transplacental infection. This is not an epidemiologic picture that would suggest a transmissible disease, and the documented absence of communicability should reassure medical personnel and family members who care for patients with this disease.

Clinical Disease

Eighty percent of sporadic cases of Creutzfeldt-Jakob disease are diagnosed in persons between 50

TABLE 2. MAJOR CLINICAL SIGNS IN SPORADIC CREUTZFELDT-JAKOB DISEASE.

SIGN	FREQUENCY*
	%
Cognitive deficits (dementia), including psychiatric and behavioral abnormalities	100
Myoclonus	>80
Pyramidal tract signs	>50
Cerebellar signs	>50
Extrapyramidal signs	>50
Cortical visual deficits	>20
Abnormal extraocular movements	>20
Lower-motor-neuron signs	<20
Vestibular dysfunction	<20
Seizures	<20
Sensory deficits	<20
Autonomic abnormalities	<20

*Data are from the United States,¹⁴ the United Kingdom,³¹ and France.³⁴

and 70 years of age. About one third of patients initially express vague feelings of fatigue, disordered sleep, or decreased appetite. Another third initially have neurologic symptoms, such as memory loss, confusion, or uncharacteristic behavior. The final third initially have focal signs, such as ataxia, aphasia, visual loss, hemiparesis, or amyotrophy. The last group includes the patients who pose the most difficult diagnostic problems, since the insidious onset of muscle wasting in the spinal cord form of the disease may simulate motor neuron disease and the rapid evolution of aphasia or hemianopia may be mistaken for the results of a cerebrovascular event.³²

A diagnosis of Creutzfeldt-Jakob disease is suggested by the typical clinical course of inexorable progression, with the dissolution of cognitive abilities from week to week or even day to day, and the development of myoclonic jerking, particularly startle myoclonus, in response to sounds or touch. An array of other neurologic symptoms and signs may develop (Table 2). When the clinical progression is dominated by ataxia, choreoathetosis, or lower-motor-neuron signs, the diagnosis is often delayed.

During the late stages of the disease, the patient becomes mute and akinetic, and even the myoclonic jerking subsides. The mean survival time is only five months, and 80 percent of patients with sporadic disease die within one year.^{15,33}

Laboratory Findings

Clinical laboratory studies show no evidence of inflammation, no consistent abnormalities of liver or renal function, and no antibodies that neutralize the disease agent. The cerebrospinal fluid has a normal pressure, does not have an increase in cells or abnor-

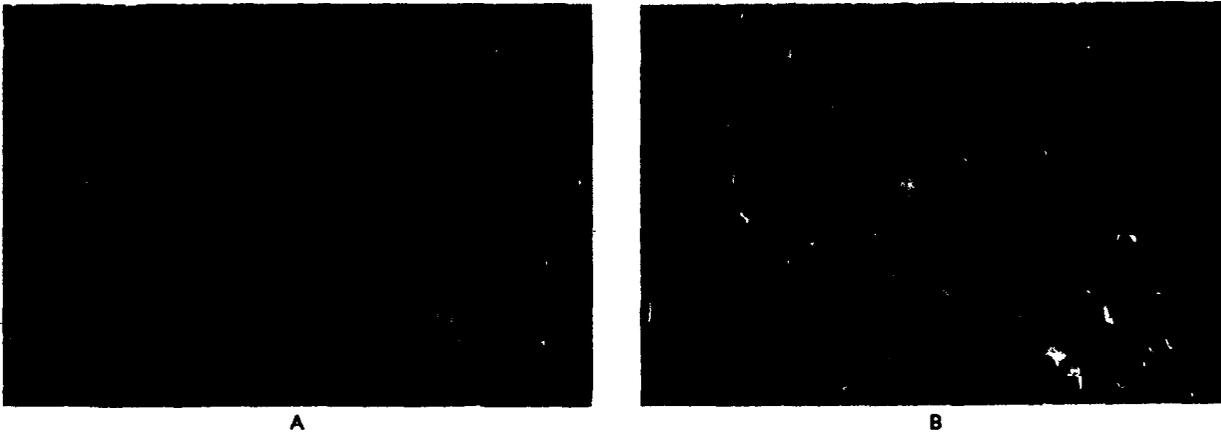


Figure 1. Histopathological Changes in Creutzfeldt-Jakob Disease.

Panel A shows a plastic-embedded section of a brain-biopsy specimen from a patient with sporadic Creutzfeldt-Jakob disease, showing intracytoplasmic vacuoles containing fragments and loops of membranes (arrow). (Cresyl violet, $\times 300$.)

Panel B shows a paraffin-embedded section of a brain-biopsy specimen from a 28-year-old woman with new-variant Creutzfeldt-Jakob disease, showing a large fibrillary amyloid plaque surrounded by patchy spongiform changes (arrows). (Hematoxylin and eosin, $\times 100$; provided by Dr. James Ironside, Edinburgh, Scotland.)

mal levels of immunoglobulin, and has a normal or mildly elevated protein content (only rare instances of more than 100 mg of protein per deciliter have been reported).

Early in the course of the disease the electroencephalogram may be normal or show nonspecific slowing. Later in the disease, periodic, biphasic or triphasic, synchronous, sharp-wave complexes are superimposed on the slow background rhythm in most patients, but these characteristic complexes may disappear as myoclonus subsides in the terminal phase of the disease. Periodic complexes have shown a sensitivity and specificity of 67 percent and 86 percent, respectively, for detection of Creutzfeldt-Jakob disease,³⁵ but if repeated recordings are obtained, more than 90 percent of patients may show periodic abnormalities.³⁶

The results of brain imaging are usually normal in the early stages of the disease. As the disease progresses, computed tomography (CT) may show progressive generalized atrophy. Magnetic resonance imaging (MRI) may show hyperintense signals in the basal ganglion on T_2 -weighted images; a retrospective study detected these changes in 79 percent of patients.³⁷ New pulse sequences may increase the sensitivity and prospective value of MRI.

Abnormal protein patterns in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease were originally found with two-dimensional electrophoresis,³⁸ but the method was not practical for routine use. Partial sequencing of these proteins showed that they matched a normal brain protein known as 14-3-3, and a rapid immunoassay for the protein has proved useful in the diagnosis.³⁹ Elevated levels of

this protein are found in the cerebrospinal fluid of patients with viral encephalitis and during the first month after cerebrovascular accidents. In patients with progressive dementia without pleocytosis who are subsequently confirmed to have sporadic Creutzfeldt-Jakob disease, the sensitivity is 96 percent and the specificity is 99 percent. Assays of neuron-specific enolase and S100 protein have not shown similar sensitivity or specificity.

Since familial Creutzfeldt-Jakob disease is dominantly inherited, sequence analysis of the gene for PrP is not indicated unless there is a history of dementia in a first-degree relative.

Pathological Findings

Histologic examination of the brain and immunostaining for PrP^{sc} are the gold standards for diagnosis. The crucial features are spongiform change accompanied by neuronal loss and gliosis; amyloid plaques are found in 10 percent of brains in the sporadic form of the disease. In contrast, plaques are common in kuru, some familial spongiform encephalopathies, and new-variant Creutzfeldt-Jakob disease (Fig. 1). In all cases, immunocytochemical staining for PrP^{sc} shows diffuse synaptic and perivacuolar staining, with striking staining of plaques when they are present.⁴⁰

Possible pathological diagnosis from peripheral tissue has been suggested by the report of PrP^{sc} staining of cells in the germinal centers of tonsillar tissue from the autopsy of a patient with new-variant Creutzfeldt-Jakob disease.⁴¹ Tonsillar biopsy has not been shown to be an effective diagnostic method early in the disease or in sporadic or familial cases.

Differential Diagnosis

Rapidly progressive dementia with myoclonus and various other neurologic findings suggests the diagnosis of Creutzfeldt-Jakob disease, but it is essential to examine the cerebrospinal fluid to rule out infections such as tertiary syphilis or subacute sclerosing panencephalitis. Toxins such as bismuth, bromides, and lithium must be excluded. In Creutzfeldt-Jakob disease, in contrast to toxic and metabolic disorders, myoclonus is rarely present at the onset, and seizures, when they occur, are late in the course of the disease. Alzheimer's disease, particularly familial cases with myoclonus, poses the most difficult problem in the differential diagnosis.

With a typical presentation and myoclonus, a rapid course, an electroencephalogram showing periodic complexes, and the presence of 14-3-3 protein in the cerebrospinal fluid, the diagnosis is reasonably assured. In many patients dementia is not the initial prominent feature, however, and myoclonus may develop late or be obscured by other movements. Because electroencephalographic and cerebrospinal fluid tests are not 100 percent specific, the diagnosis is often uncertain, requiring either watchful waiting or brain biopsy.

Risks of Infection from Patients and Tissues

Consistent experimental transmission of infectivity has been possible with homogenates of brain, spinal cord, and eye tissue. Transmission occurs in less than half of the attempts with preparations of lung, liver, kidney, spleen, lymph node, and cerebrospinal fluid. Transmission to primates has never occurred with any body fluid other than cerebrospinal fluid. Universal precautions should be applied when caring for patients with Creutzfeldt-Jakob disease. In view of the lack of communicability and the lack of any excess risk for caretakers, added isolation precautions, such as gowns or masks, are unnecessary. Isolation precautions during hospitalization simply terrorize medical personnel and family members and impede future placement in a nursing home. Cerebrospinal fluid should be obtained carefully, with the use of double gloves and protective glasses, and specimens should be marked as infectious.

Biopsies and autopsies require similar precautions. There is no justification for refusing to perform an autopsy or biopsy. The risk is less than that of a biopsy or autopsy on a patient who is seropositive for hepatitis B virus or the human immunodeficiency virus. Indeed, the risk is theoretical and has not been demonstrated. Safety gloves, disposable aprons, and eye and mouth coverings should be used in handling tissues. Instruments should be disposable or should be decontaminated by soaking in 1N sodium hydroxide (40 g per liter) or undiluted sodium hypochlorite for one hour and then autoclaving at 134°C for one hour. Tissue should be blocked thinly for histologic analysis and soaked in concentrated

formic acid for 1 hour and then in 4 percent formaldehyde solution for at least 48 hours.⁴² Archival formalin-fixed tissue embedded in paraffin and stored at room temperature may be infectious for years and should be handled with caution.

The demonstrated hazard is not to physicians but from physicians, who have transmitted the disease through transplantation procedures, contaminated instruments, and drugs derived from affected human tissues.

IATROGENIC CREUTZFELDT-JAKOB DISEASE

The human-to-human transmission of a spongiform encephalopathy was tragically demonstrated among the Fore people with kuru. Whether that unique epidemic originated with an index case of sporadic Creutzfeldt-Jakob disease or some other source will probably never be known.

Surgical Transmission

Physicians have inadvertently transmitted sporadic cases of Creutzfeldt-Jakob disease in the course of a variety of procedures (Table 3). The first suspected human transmission was reported in 1974 when rapidly progressive disease developed in a woman 18 months after she received a corneal transplant. The donor had died of undiagnosed Creutzfeldt-Jakob disease.⁴⁵ Human transmission was more dramatically and convincingly demonstrated when Creutzfeldt-Jakob disease developed in two young patients 16 and 20 months after they underwent surgery to excise epileptic foci. Stereotactic electroencephalographic exploration was undertaken at the time of surgery with silver electrodes that had previously been implanted in a patient with known Creutzfeldt-Jakob disease.⁴⁶ The electrodes had been "sterilized" with 70 percent alcohol and formaldehyde vapor, yet two years later these electrodes were retrieved and implanted into a chimpanzee in which the disease subsequently developed. Contaminated neurosurgical instruments have been suspected as modes of transmission in other patients.⁴⁷ For example, an early paper on spongiform encephalopathy described three patients who had been operated on by the same neurosurgeon in the same neurosurgical unit during an eight-month period.⁴⁸

Over the past 10 years, more than 80 cases of Creutzfeldt-Jakob disease have been recognized 16 months to 17 years after neurosurgical placement of grafts of human cadaveric dura mater. Since almost all the patients received dura mater from a single surgical-supply company, most of the outbreak had a common source.⁴⁹ Two other cases developed in patients who had extraneural inoculation of lyophilized cadaveric dura mater, to embolize the intercostal arteries in one patient and to treat a nasopharyngeal angiofibroma in the other.^{50,51}

TABLE 3. TRANSMISSION OF PRION DISEASES FROM HUMAN TO HUMAN.

MODE OF TRANSMISSION	EXAMPLE (NO. OF CASES REPORTED)	INCUBATION PERIOD
		yr
Intracranial transplantation or inoculation	Dural grafts (>80)	1.3-17
	Inadequately sterilized instruments (?)	0.6-2.2
	Stereotactic electrodes (2)	1.3-1.8
Extracranial transplantation	Corneal grafts (2)	1.3-1.5
Extracranial inoculation of neural tissue	Human growth hormone and gonadotropins (>100)	4-19*
	Arterial embolization with lyophilized dura mater (2)	3.5-7.5
Extracranial inoculation or oral exposure	Possible exposure to bovine spongiform encephalopathy (23)†	?
	Transmission of kuru by ritual cannibalism (several thousand)	4-40 or more

*Numbers represent minimal incubation periods, since hormones were given over periods of years (mean time from midpoint of treatment to onset, 12 years).

†There were 22 cases in the United Kingdom and 1 in France. The 26-year-old French bodybuilder with new-variant Creutzfeldt-Jakob disease may have received injections of bovine pituitary somatotropin.^{43,44}

Transmission by Pituitary Hormones

In 1985 Creutzfeldt-Jakob disease developed in four patients who had received human growth hormone, all of them under 40 years of age. Injection of the hormone, which was derived from pooled cadaveric human pituitary glands, had been discontinued 4 to 15 years before the onset of disease.⁵² Recombinant growth hormone was licensed promptly, but between 1963 and 1985, about 8000 children and adolescents in the United States received human growth hormone to treat short stature.

More than 100 cases of Creutzfeldt-Jakob disease worldwide have been related to growth hormone and gonadotropic hormones. The largest number has been in France, where the disease has developed in about 2.5 percent of recipients, with a mean incubation period of eight years. In the United Kingdom about 1 percent of recipients have been affected, with a mean incubation period of 12 years. In the United States the disease has developed in 0.2 percent of recipients (16 patients), with a mean incubation period of 18 years.⁵³ The differences in the frequency of transmission and the length of incubation probably reflect variable contamination resulting from different protocols for hormone extraction. Nevertheless, in all affected countries, growth hormone-related disease begins with cerebellar ataxia and movement disorders, with dementia developing late. Pathological changes in the cerebellum and basal ganglia are prominent on autopsy. These findings are reminiscent of kuru, suggesting that age at expo-

sure or the route of inoculation influences the clinicopathological features.

Risks Associated with Blood Products

Although the hazards of injection or transplantation of affected human tissues are obvious, the possible hazards of transmission through human blood products are debatable. Several sorts of evidence have failed to demonstrate a role of human blood products. No epidemiologic evidence has incriminated a history of blood transfusion. The disease has not been found in patients with hemophilia. Intravenous drug use does not increase the risk. Tracking of blood donated by those in whom Creutzfeldt-Jakob disease subsequently developed has not uncovered the disease in recipients.⁵⁴ Finally, the transfusion of full units of blood from patients with Creutzfeldt-Jakob disease into chimpanzees, the most susceptible hosts, has failed to induce the disease.⁵⁵

However, anecdotal reports of disease after the administration of blood products to humans,^{56,57} and several reports of disease in mice inoculated intracerebrally with blood from patients with Creutzfeldt-Jakob disease,^{58,59} have led to concern. The Food and Drug Administration has mandated that products containing blood from persons in whom the disease subsequently develops be withdrawn from the market, and the agency has left the option of notification of recipients to the supplier. Persons who receive such notice cannot do anything to prevent disease, and as their advisors, physicians can only give strong reassurance that the risk is theoretical and not documented.

FAMILIAL CREUTZFELDT-JAKOB DISEASE

Between 10 and 15 percent of persons with Creutzfeldt-Jakob disease have a family history consistent with an autosomal dominant inheritance of the disease. In most of these kindreds, point mutations, deletions, or insertions are found in the coding sequence of the gene for PrP on the short arm of chromosome 20. More than 20 mutations in this gene have been described that are associated with phenotypes mimicking typical Creutzfeldt-Jakob disease or that induce distinctive progressive diseases with spongiform changes in the nervous system.

In general, familial Creutzfeldt-Jakob disease has an earlier age of onset and a more protracted course than sporadic disease. The typical electroencephalographic changes are often missing, and the 14-3-3 protein is not detected in cerebrospinal fluid in about half of cases.⁶⁰ The neuropathological changes may vary in topographic distribution and in the prevalence of amyloid plaques, but the essential changes of vacuolization of neural cells with gliosis and neuronal loss are generally present.

Several mutations are of special note. The most common mutation leading to the typical clinical and

pathological findings of Creutzfeldt-Jakob disease is at codon 200. Clusters of disease among Libyan Jews in Israel, in a region of Slovakia, and in Chile are all explained by this mutation.⁶¹

Distinctive Syndromes

Several mutations lead to phenotypes that have been regarded as different diseases. Gerstmann-Sträussler-Scheinker disease is an autosomal dominant illness characterized by severe cerebellar ataxia and often spastic paraparesis. In some families myoclonus is not prominent, and dementia may develop late in the course of illness. The disease has a prolonged course of 5 to 11 years, yet the mean age at death is only 48 years. The neuropathological findings are distinct, with many PrP^{Sc}-positive amyloid plaques throughout the brain.⁶² In two different mutations in kindreds from Indiana and Sweden, neurofibrillary tangles were found in the cerebellum and neocortex — pathological features akin to those of Alzheimer's disease. The most frequent mutation associated with Gerstmann-Sträussler-Scheinker disease is at codon 102, but the syndrome has also been associated with mutations at other sites.

Fatal familial insomnia is an even stranger phenotype, with a mutation in the gene for PrP. The illness is characterized by progressive insomnia, dysautonomia, and dementia, leading to death in 7 to 15 months. At autopsy, selective atrophy of the ventral and mediodorsal thalamic nuclei is evident.⁶³ In some patients, spongiform changes are found in the thalamic nuclei, and immunocytochemical staining for PrP^{Sc} is positive. These findings led to sequence analysis of the gene for PrP, which found a mutation at codon 178.⁶⁴ However, this mutation had also been found in kindreds with typical familial Creutzfeldt-Jakob disease. The association of two distinct phenotypes with the same mutation is explained by a polymorphism at codon 129. This polymorphism, involving methionine and valine, is important in sporadic and iatrogenic cases of Creutzfeldt-Jakob disease, since homozygosity is disproportionately associated with disease (Table 4). In the mutations at codon 178, the methionine allele segregates with fatal familial insomnia and the valine with familial Creutzfeldt-Jakob disease. Presumably this site is important in determining the tertiary structure of PrP^{Sc}, and different amino acids at this site can alter the conformation of the PrP^{Sc} present in these two diseases.⁶⁷

Genetic Counseling

Sequence analysis of the gene for PrP can determine the presence of familial prion disease, but pre-symptomatic testing of relatives raises the same difficult issues as the testing of family members at risk for Huntington's disease.⁶⁸ Both are autosomal dominant diseases with high penetrance and uniformly

TABLE 4. POLYMORPHISMS AT CODON 129 THAT MAY INFLUENCE SUSCEPTIBILITY TO OR PHENOTYPE OF DISEASE.*

DISEASE	NO. OF SUBJECTS TESTED	percent of subjects			HOMOZYGOUS
		MET/MET	MET/VAL	VAL/VAL	
Healthy controls	261	37	52	11	48
Sporadic Creutzfeldt-Jakob disease	73	78	12	10	88
Iatrogenic Creutzfeldt-Jakob disease	63	60	11	29	89
New-variant Creutzfeldt-Jakob disease	22	100	0	0	100

*Data are from Brown,⁶⁹ Palmer et al.,⁶⁴ and Brown et al.⁶⁴

fatal outcomes. Both diseases evolve after the child-bearing years, so that knowledge of risk may be of value in estate planning, but no advice can be given to delay the onset or modify the relentless course of the disease. Since the relatives have usually witnessed the frightening, rapid dissolution of cognition in a family member, professional counseling before testing is essential to help them weigh the value and risks of foreknowledge.

BOVINE SPONGIFORM ENCEPHALOPATHY AND NEW-VARIANT CREUTZFELDT-JAKOB DISEASE

The Mad-Cow Outbreak

In the spring of 1985, several dairy cows in the United Kingdom were noted to have become apprehensive, developed aggressive behavior, and showed ataxia leading to falling. The pathological findings included spongiform lesions with gliosis and neuronal loss that resembled scrapie.⁶⁹ Over the subsequent years, the number of affected cows rose sharply from 16 in 1986, to 7000 in 1989, and to 36,000 in the peak year of 1992. Over 170,000 cases of bovine spongiform encephalopathy have been confirmed in more than 34,000 herds, with no evidence of lateral transmission. The uniformity of the disease and its lesions, the explosiveness of the epidemic, and the wide distribution of cases throughout the United Kingdom all pointed to an epidemic with a common source. Supplementation of the diets of calves and dairy cattle with meat and bone meal produced by commercial rendering plants appeared to be the source of the disease. It is assumed that the epidemic started when cows were fed diets containing material from scrapie-infected sheep. After the disease had been transmitted to cows, it was spread further by the addition of material from infected cows to cattle feed. The onset of the epidemic followed changes in the rendering process in the 1970s.

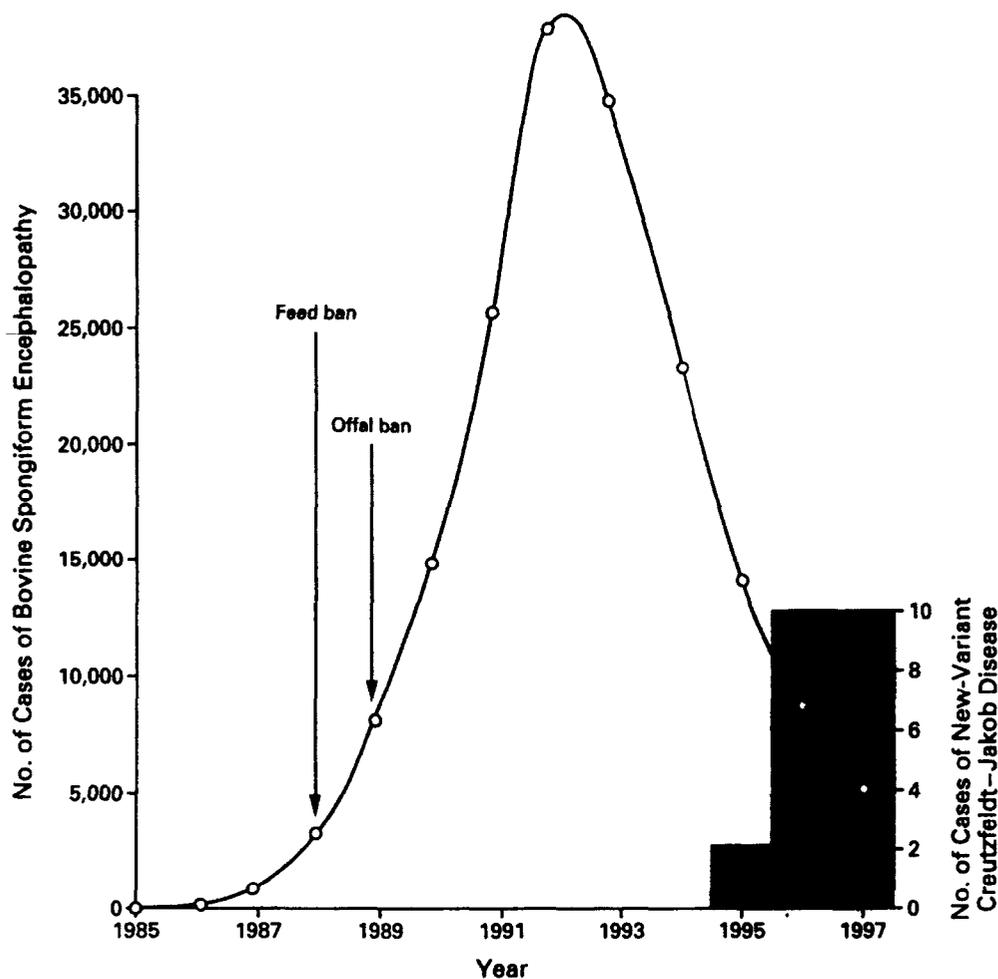


Figure 2. Cases of Bovine Spongiform Encephalopathy (Linear Graph) and New-Variant Creutzfeldt-Jakob Disease (Bar Graph) in the United Kingdom.

The decline in the incidence of bovine spongiform encephalopathy began five years after the imposition of the ban on feeding ruminant-derived protein to ruminants. Cases of new-variant Creutzfeldt-Jakob disease have been reported since February 1994. It has been postulated that these patients were exposed to contaminated meat products before the ban on cattle offal in human food was imposed in 1989. In the absence of knowledge of the duration of the incubation period in humans, this cluster of cases provides little information on the possible future incidence of the disease. Dates represent the years when cases were reported rather than years of onset or death.

At this time continuous heating replaced batch heating to conserve oil during the oil crisis. Also, with the decline in the tallow market due to dietary shifts away from lard, more tallow was left in bone meal and hydrocarbon solvents were removed from the processing, increasing the likelihood of persistent infectivity.

Bovine spongiform encephalopathy has been experimentally transmitted to a variety of species, including laboratory rodents and nonhuman primates. It has been transmitted orally to some species.⁷⁰ Infectivity in cattle has been curiously limited to the brain, spinal cord, retina, and (in experimentally infected cattle) ileum. Infectivity has not been detected in muscle, milk, or blood.⁷¹

A statutory ban on feeding ruminant-derived protein to ruminants was imposed in the United Kingdom in 1988. After four to five years (the incubation period of the disease), the number of cases began to decline and has continued to decline at a rate of about 40 percent per year (Fig. 2). A small number of cases have been reported in other countries, either in cattle imported from the United Kingdom or in cattle given imported feed. Domestic cats, as well as captive exotic ruminants, wild felines, and one rhesus monkey, have died of spongiform encephalopathies, presumably from eating beef or bone meal.^{71,72}

Concern mounted about possible oral transmission across species barriers. In 1989 cattle offal was

banned from human food in the United Kingdom, but by this time an estimated 450,000 infected cattle had entered the food chain.⁷³ In 1997 the sale of meat on the bone was banned in Britain to minimize potential contamination by the dorsal-root ganglia.

New-Variant Creutzfeldt-Jakob Disease

Because of concern about cross-species transmission in the United Kingdom, a national surveillance unit for Creutzfeldt-Jakob disease was established in 1990. No unusual cases were noted during the first four years of monitoring, but between 1994 and 1997, 22 cases of what is now called new-variant Creutzfeldt-Jakob disease were reported (Fig. 2). These patients are younger than those with the more familiar forms of the disease, with prominent early psychiatric and behavioral manifestations and persistent paresthesias and dysesthesias. Cerebellar ataxia uniformly develops, and the course of the disease is prolonged (Table 5). The electroencephalogram fails to show typical periodic complexes. The pathological examination shows prominent and diffuse PrP^{sc} plaques reminiscent of kuru (Fig. 1B). None of the patients with new-variant Creutzfeldt-Jakob disease have had mutations in the gene for PrP, but all have been homozygous for methionine at the polymorphism at codon 129. All patients had eaten meat, although one had become a strict vegetarian in 1991. None had knowingly eaten brains, but before the ban on offal, brain and spinal cord were often included in sausages, hamburger, and other processed meats. The oral intake of meat products contaminated with bovine spongiform encephalopathy before the bans on ruminant-derived feed and on offal has been proposed as the origin of these cases.⁷⁷ Others have argued that the reporting of these variant cases in younger patients is an artifact of intense surveillance.

Recent laboratory studies provide powerful evidence that the causative agents of new-variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy have a common origin. Glycosylation patterns of PrP^{sc} and susceptibility studies in mice show that the patterns in brain tissue of patients with new-variant Creutzfeldt-Jakob disease and animals with bovine spongiform encephalopathy are similar and are distinct from the patterns associated with sporadic and iatrogenic disease.⁷⁸ In inbred mouse strains, different strains of scrapie have distinctive incubation periods and a different topographic distribution of lesions. Preliminary studies of the agents of bovine spongiform encephalopathy, new-variant Creutzfeldt-Jakob disease, and the spongiform encephalopathy of exotic ruminants and cats show similarities in incubation periods and in the distribution of lesions, which are distinct from those of sporadic Creutzfeldt-Jakob disease.⁷⁹ Nevertheless, the common origin of these prions does

TABLE 5. COMPARISON OF NEW-VARIANT AND SPORADIC CREUTZFELDT-JAKOB DISEASE.

CHARACTERISTIC	NEW VARIANT*	SPORADIC
Mean age at onset (yr)	29	60
Mean duration of disease (mo)	14	5
Most consistent and prominent early signs	Psychiatric abnormalities, sensory symptoms	Dementia, myoclonus
Cerebellar signs (% of patients)	100	40
Electroencephalographic periodic complexes (% of patients)	0	94
Pathological changes	Diffuse amyloid plaques	Sparse plaques in 10%

*Data on the new-variant disease are from Will et al.⁷⁴ and Zeidler et al.^{75,76}

not prove that humans were infected by eating bovine nervous tissue or other animal products.

Issues of Concern in North America

A single cow imported from Britain died of bovine spongiform encephalopathy in Canada, but no imported cows are known to have had the disease in the United States. No human disease resembling new-variant Creutzfeldt-Jakob disease has been found in North America, and no increase in the incidence of Creutzfeldt-Jakob disease in persons under 45 years of age has been reported.⁸⁰

The United States has banned the importation of European cattle and sheep and derived products. With few exceptions, the use of materials derived from cattle or sheep from affected countries in cosmetics, pharmaceuticals, and medical products and devices is also banned in the United States. To reduce transmission should the agent of bovine spongiform encephalopathy gain entry, there is also a ban on feeding ruminant-derived food to ruminants. Since American cattle are free of disease, no ban on offal has been imposed.

Concern about an outbreak in the United States focuses on three possible sources: imported cattle, spontaneous disease among indigenous farm animals, and wasting disease of deer and elk. If the disease should arise from any of these sources, its subsequent spread would be dependent on the rendering industry, in particular on the incoming supply of carcasses, the methods of processing, and the distribution of products (tallow and meat and bone meal). Most British cattle imported before the ban have been traced, and the known survivors are under quarantine. Spontaneous spongiform encephalopathy has not been documented in any species other than humans, but an old clinical paper that reported a scrapie-like disease in cows⁸¹ and a recent outbreak of transmissible mink encephalopathy, in which diseased or dead cattle and horses, but not sheep, were

fed to mink,¹⁰ have raised the possibility of low rates of sporadic disease in other species, including cattle. Finally, chronic wasting disease of deer and elk is common in one region of the western United States. ~~Hundreds of thousands of deer and elk are killed by hunters and on highways every year, and a portion of these enter the food chain either directly or through the rendering process.~~

In addition to concern about the food chain, concern has been expressed about the external use of tallow in cosmetics and soap; the use of bone meal in health products, pet food, garden food for roses, and a host of other common products; and the use of bovine serum and gelatin in pharmaceuticals.⁸²

One of the most common questions we have encountered from friends and colleagues who are about to embark on trips to Europe concerns whether or not to eat beef. The risks need to be kept in perspective; both of us have enjoyed British beef in recent years.

CONCLUSIONS

Despite clues provided by iatrogenic and familial cases of Creutzfeldt-Jakob disease and by the possible transmission of bovine spongiform encephalopathy to humans, the cause of the sporadic instances of Creutzfeldt-Jakob disease that make up 85 to 90 percent of cases remains a mystery. Past exposures, dietary eccentricities, occupation, contact with others with the disease, recreational activities, pets, and a myriad of other factors provide no aid in establishing the diagnosis and no clue to causation. The sporadic cases are associated with no abnormalities in the gene for PrP. Somatic mutation has been postulated as the cause, but this seems unlikely, since the phenotype of patients with sporadic disease is more stereotyped than that of patients who have familial disease with mutations in the gene for PrP.

If the prions are composed only of protein and if disease is determined by protein configuration, certain pathogenic isoforms may occur spontaneously or because of mutations in the gene coding for the protein. ~~These pathogenic isoforms would set off a slow cascade of altered conformations by some unknown mechanism. Minor differences in sequence between species in the gene for PrP may determine the risk of spontaneous misconformation, and other sequence differences may determine the likelihood of the disease's crossing species barriers and explain the lack of phylogenetic order in transmission. For example, normal sequence differences could explain why scrapie can be transferred from sheep to mice but not to pigs, why the bovine isoform can cause degenerative disease in cats but not in dogs, and why the disease may be spread to humans by beef products but not mutton.~~

The risk of spread clearly arises from the transfer of cells or extracts to genetically susceptible hosts or

the entry into the food chain of prions that can infect by the oral route and across species barriers. Knowledge of the factors that allow spread could prevent future outbreaks, and knowledge of the replication of the pathogenic isoform could lead to the development of drugs to prevent familial disease and arrest the progression of sporadic cases.

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