CME CREDIT

TAKE-HOME POINTS FROM EDUCATIONAL PRESENTATIONS BY CLEVELAND CLINIC FACULTY AND VISITING PROFESSORS

Kuru and mad cow disease: Understanding the prion theory

G. RICHARD OLDS, MD

Chairman, Department of Medicine, MetroHealth Medical Center, Cleveland; Charles H. Rammelkamp Jr. Professor of Medicine, Case Western Reserve University College of Medicine; expert in geographic medicine and tropical disease. Chaired a number of committees for the World Health Organization.

ABSTRACT

A preponderance of evidence indicates that several neurodegenerative disorders are caused by prions: abnormally folded proteins that can induce abnormal folding in other normal protein molecules. Further, these "infections" can cross some species barriers.

FOR DECADES, the causative agents of different spongiform encephalopathies in man and animals, such as kuru, scrapie, and Creutzfeldt-Jakob disease, were a mystery. The diseases were transmissible, but the victims showed no clinical or pathological signs of infection. Cultures of victims' brains revealed nothing. Further, usual sterilization procedures did not destroy the agents' infectivity.

The implication of these findings seemed preposterous: the agent consisted of protein alone, with no nucleic acid at all. Such a theory would violate all known concepts of infection. Yet it was precisely this theory that Dr. Stanley Prusiner proposed in 1982,[1] and for which he was awarded the Nobel Prize in 1997.

With the recent controversy in England over bovine spongiform encephalopathy (BSE), better known as mad cow disease, and its apparent jump to humans, there is a growing understanding that prions are not just a medical curiosity, but a potentially grave public health threat.

This brief article presents a short history of the development of the prion theory of disease, an overview of that theory, and some of the lessons that our understanding of kuru and mad cow disease holds for contemporary society.

NEW GUINEA, 1957: A NEW DISEASE AMONG THE CANNIBALS

Shortly after World War II, stories began to circulate among colonial authorities about a strange neurologic disease affecting the Fore (pronounced *four-ay*) people of the remote highlands of Papua, New Guinea, one of the most isolated and primitive areas in the world. In 1957, Dr. Carleton Gajdusek began work among the Fore, which would occupy him for many years and eventually win him the Nobel Prize in 1976.[2]

The disease that Gajdusek found was like no other he had ever seen. Victims were predominately women and children. The tribespeople called the disease kuru (trembling, shaking), because of one of its prominent signs. Other signs were psychiatric changes, inappropriate affect (including inappropriate laughter), and cerebellar ataxia. Dementia occurred only late in the disease, if at all. Once the signs appeared, the disease invariably progressed within months to complete debilitation and death.[3]

The brains of the victims, preserved in formaldehyde and sent to the NIH for pathologic analysis, showed changes similar to those produced by scrapie, a disease of sheep. Specifically, the cerebellums contained florid amyloid plaques and spongiform changes—literally, spongelike holes where neurons used to be.[4]

Studies in chimpanzees confirmed what epidemiologic studies suggested: the disease was transmissible.[5] Extracts from the brains of human victims, injected into chimps, resulted in a disease very much like the human disease, and extracts from the infected chimps' brains could in turn induce the disease in other chimps. Yet, as with scrapie, workers could find

Prions have forced us to rethink our ideas about infectious agents

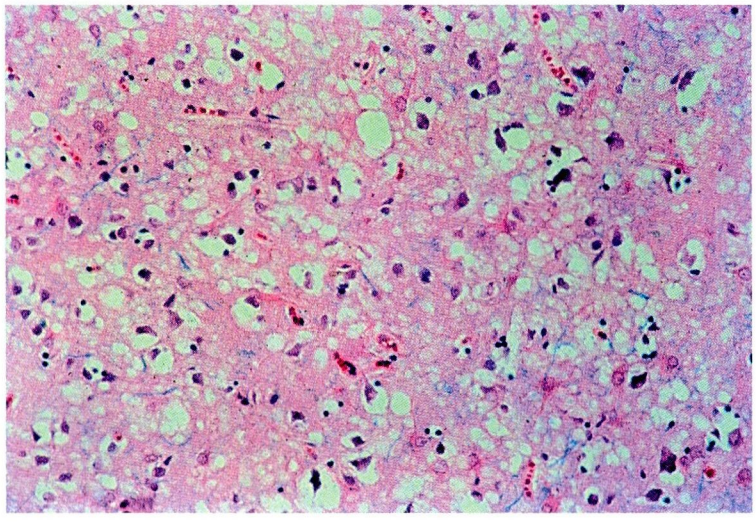

FIGURE 1. Brain tissue from a patient with Creutzfeldt-Jakob disease, showing the characteristic spongiform changes.

SOURCE: COURTESY OF RICHARD A. PRAYSON, MD.

no trace of an agent, either bacterium or virus. Further, victims showed no signs of infection, such as fever or adenopathy. Workers at that time speculated that kuru, scrapie, and similar diseases were caused by "slow viruses," although the actual agent eluded them.

Gajdusek and others eventually linked kuru to ritual cannibalism. Women and children comprised most of the kuru victims because they, and not men, consumed the brains of their family members who had died. The women shared this protein meal with the small children that always accompanied them. Adult men, who ate the flesh, were largely unaffected. After cannibalism was eliminated in the 1960s, the incidence of kuru steadily declined.

In prion diseases, the normal protein shape mutates

UNITED STATES, 1974: IATROGENIC TRANSMISSION OF CREUTZFELDT-JAKOB DISEASE

Creutzfeldt-Jakob disease is a rare, degenerative neurologic disease, also characterized by spongiform changes in the brain (FIGURE 1), that usually occurs sporadically in persons in their 60s, although there is sometimes a hereditary linkage. In 1974, a case occurred in a middle-aged woman who had received a corneal transplant from a donor with Creutzfeldt-Jakob disease.[6] Other cases were linked to the use of brain electrodes previously used in patients with Creutzfeldt-Jakob disease, and to growth hormone extracted from human cadavers.[7]

UNITED STATES, 1982: THE PRION THEORY PROPOSED

Noting that the infectious agents in kuru, scrapie, and Creutzfeldt-Jakob disease were impervious to normal sterilization procedures, and were not destroyed by electron radiation or ultraviolet light at a wavelength specifically absorbed by nucleic acids, scientists began to develop an alternate theory, that proteins alone were responsible for these diseases.[1]

In this theory, a naturally occurring protein is transformed into a mutant form—a "prion"—that is then able to convert proteins with normal three-dimensional structures to the mutant form, giving an infectious property to this inanimate protein.

Normal prion protein

Within the neurons of most mammals is a protein, called prion protein (PrP). This prion protein, which is naturally produced in the cytoplasm, has a phospholipid anchor and appears to bind to the cell membrane.

The function of normal prion protein is unclear. Genetically engineered mice that cannot produce prion protein grow and reproduce normally. These "knockout" mice exhibit only subtle abnormalities, such as altered circadian rhythms, progressive but mild ataxia, and decreased gamma-aminobutyric acid (GABA)-mediated synaptic inhibition. This latter effect suggests that prion proteins are involved in regulating GABA activity.

However, these knockout mice cannot get spongiform encephalopathies when injected with infectious prions, providing the best evidence to date in support of the prion.

Structure of normal and abnormal prion protein

The backbone of normal prion protein is twisted into spiral shapes known as "alpha helices" (FIGURE 2). In prion diseases, the normal shape of the protein is transformed, structure, with the spiral helices stretched out into a flat configuration, known as beta sheets.

Normal prion protein survives in the neurons for only a few hours, before it is digested

TABLE 1

Known prion diseases

Diseases in animals

- Scrapie
- Bovine spongiform encephalopathy
- Transmissible mink encephalopathy
- Feline spongiform encephalopathy
- Exotic ungulate encephalopathy

Diseases in man

- Kuru
- "Spontaneous" Creutzfeldt-Jakob disease
- "Hereditary" Creutzfeldt-Jakob disease
- Gerstmann-Sträussler-Scheinker disease
- Fatal familial insomnia
- "New variant" Creutzfeldt-Jakob disease (probably human bovine spongiform encephalopathy)

by proteases. However, the mutant, beta-sheet formation, or "prion" cannot be degraded and instead accumulates in the neurons. It is this accumulation of mutant protein that is believed to cause neuron destruction and the clinical manifestations of prion disease.

Prions are not destroyed by normal sterilization techniques

The development of mutant prion protein and prion diseases

Prion diseases appear to arise in three ways, as described below.

Inheritance of a grossly mutated prion protein gene produces an unstable form of prion protein that folds spontaneously into the beta-sheet conformation. This is often an autosomal dominant condition and leads to 100% penetrance of the disease.

Point mutations, ie, substitution of a single amino acid for another in the protein's primary structure, may make the protein more susceptible to abnormal folding. Point mutations of this type increase the risk of spontaneous transformation to the beta-sheet conformation but do not confer the disease with 100% penetrance. These mutational defects may account for most cases of "spontaneous" Creutzfeldt-Jakob disease (estimated at 1 case per million population per year).

"Infection." Perhaps the most startling element of the prion theory of disease was the insight that mutant, beta-sheet prion protein can transform the spiral structure of normal prion protein, even though both normal and mutant protein configurations have the same amino acid sequence. There are two theories on how this transformation might occur.

In the dimerization theory, it is thought that a mutant prion protein molecule links with a normal prion protein molecule, transforming the latter's helical structure into the beta-sheet structure. Then the two mutant molecules disassociate and move on to transform more normal prion protein molecules.

In the second theory, is a single beta-sheet molecule in a neuron acts as a seed to transform the normal protein molecules into a crystalline aggregation of abnormal protein.

In either case, the presence of the mutant, beta-sheet protein or prion initiates a cascade of conversion to the abnormal form.

Thus, prions act like infectious agents. Prions appear to be able to cross species barriers, but often after a marked lag time. Because prions are proteins, they are unaffected by sterilization techniques designed to kill most bacteria and viruses. Cooking meat, for example, does not inactivate prions, nor do most hospital sterilization procedures used on reusable medical equipment. Clearly, tissue or organ transplants can transmit prion diseases.

Today there are at least five known prion diseases of animals and six of humans (TABLE 1). The pathology of each includes spongiform encephalopathy, amyloid plaques, and prion-associated fibrils in the brain.

ENGLAND, 1986: AN EPIDEMIC OF "MAD COW DISEASE"

Prions might have remained largely a medical curiosity were it not for recent events in England. Scrapie has occurred in England for several hundred years. Exactly how sheep acquire this disease from other sheep is unknown (sheep are vegetarians), but it is clearly infectious and probably transmitted through fecal-oral contamination.

In 1986, British veterinarians first noticed a scrapie-like illness in cattle.[8] A review of animal husbandry practices revealed that cattle were often fed the remains (mostly ground bones) of dead cows and sheep to enhance growth and milk production. In a sense, cattle had been turned

Prions: Infectious agents made entirely of protein

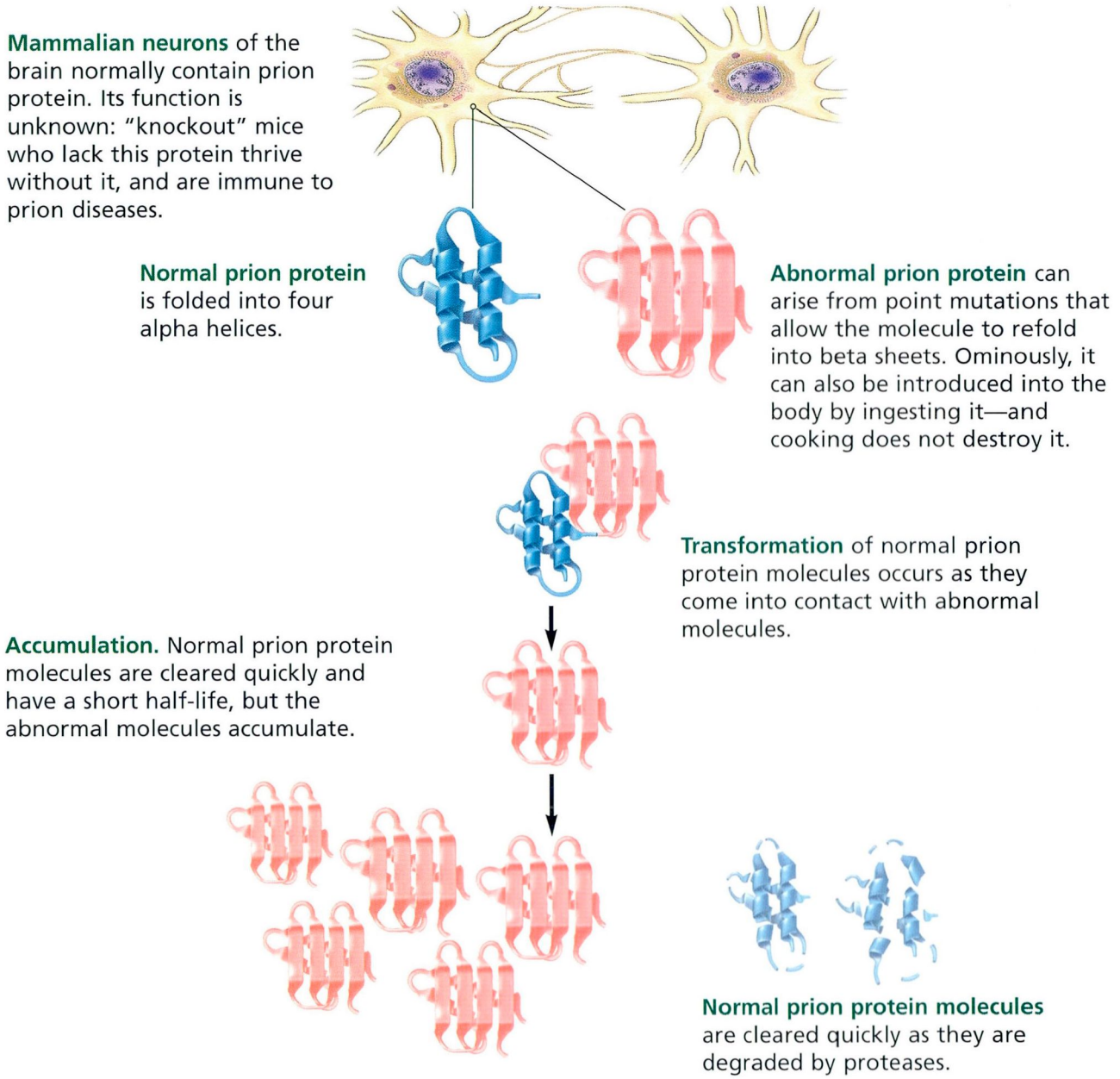

FIGURE 2

into cannibals. By the early 1990s, an epidemic of "mad cow disease" had struck England. The potential infectious nature of this illness prompted many countries to quarantine British beef.

ENGLAND, 1994: A NEW VARIANT OF CREUTZFELDT-JAKOB DISEASE APPEARS

Between February 1994 and August 1997, a "new variant of Creutzfeldt-Jakob disease" developed in 21 British people aged 16 to 39.[9] However, this disease may not be Creutzfeldt-Jakob disease at all, but rather mad cow disease transmitted to humans. Evidence to support this contention:

- The average age of victims is 20 years (compared with 60–70 in Creutzfeldt-Jakob disease).
- The median interval between the onset between symptoms and death is 12 months (compared with 4 months in Creutzfeldt-Jakob disease).
- The early symptoms (psychiatric and

sensory symptoms, absence of electroencephalographic changes) differ from those of Creutzfeldt-Jakob disease (dementia, myoclonus, EEG changes).

- The neuropathologic changes are similar to kuru, rather than traditional Creutzfeldt-Jakob disease.
- Most important, the prion protein extracted from the brains of these patients appears to have a normal primary protein structure and carries the bovine spongiform encephalopathy "fingerprint" when transferred to inbred mice.

In 1996, the Centers for Disease Control and Prevention began active surveillance for Creutzfeldt-Jakob disease in the United States, but did not recommend discontinuing the practice of fortifying the feed of US cattle with slaughterhouse remains. (The federal government finally condemned this practice in 1997.)

Unfortunately, the ban does not apply to *human* food. Bovine offal—the brains, spinal cord, and eyes of cattle—is added to fast-food hamburgers and sausages as a binder, so that the thin patties of meat do not fall apart while cooking. Such food products can carry the designation "all-beef," which would not be true if a vegetable binder were used. Although it was banned in Britain in 1989, the use of bovine offal continues in the United States.

The use of bovine offal in the binders of food should be banned

ACTION NEEDED NOW

A return to the start of this story (Papua, New Guinea) raises some interesting issues about our current situation. Since ritual cannibalism was discontinued, no new cases of kuru have been found in persons born after 1960, but new cases continue to develop in those exposed (ie, who had practiced cannibalism) 20 to 30 years earlier. Experience with AIDS, another illness with a long asymptomatic period, suggests that action should be taken now, even though the apparent number of affected individuals appears small.

For these reasons, and the recent developments in England, we should strongly consider the following actions:

- Continue the ban on feeding animal remains to cattle.
- Screen potential organ donors for their risk of transmitting prion diseases.
- Ban the use of bovine offal as binders in foods such as hamburgers and sausage.

Kuru and other prion diseases have forced us to rethink our traditional ideas about infectious agents and opened the possibility that other human maladies may be caused by alterations in the three-dimensional structure of normally produced molecules. Through our greater understanding of the molecular basis of these diseases, we can better understand the risk profile for their spontaneous development or infectious transfer.

This story should also make us scrutinize the practices of agribusiness, and remind us that we must pay attention to new and emerging diseases even in remote corners of the world, for disease knows no borders.

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