

# **Mad Cows and Rogue Proteins**

by  
Derek Cluck

Dr. Rainer Glaser  
Organic Chemistry 210

"Mad-Cow Disease" is a phrase heard many times in popular news media and referred to often by comedians all over the world. However, behind the catch phrases and jokes lies a truly amazing, unique, and alarming family of diseases. "Mad-Cow Disease", or more properly, Bovine Spongiform Encephalopathy (BSE), is just the most well known of a number of degenerative neurological diseases with one thing in common--they are caused by a single molecule, a prion. These "proteinaceous infectious particles", prions, are a relatively newly discovered phenomena to science. Prions were only first theorized as late as 1982, by neurologist Stanley Prusiner of the University of California at San Francisco, and upset much of the traditional dogma of disease pathology. Most surprisingly, prions are a transmissible disease causing agent, yet have no nucleic acid genome at all; they are simply a single self replicating protein molecule. Also, in addition to being an infectious, transmissible disease, prions are unique in that they can be inherited as a genetic disorder in certain families, and can develop sporadically by a random gene mutation in a previously uninfected individual. Very few diseases are so frustratingly versatile in their cause.

Although the existence of prions has only recently been ascertained, prion-caused diseases have been known and cataloged for decades. Of them, BSE in cattle, scrapie in sheep, transmissible mink encephalopathy, and chronic wasting disease of deer are well known animal diseases caused by neural degeneration from prion infections. Although several prion diseases are known in humans (such as fatal familial insomnia, a rare inherited degeneration of the thalamus causing sleep disorders and dementia, Gertsmann-Straussler-Scheinker disease which damages the cerebellum, disturbing balance and only rarely causes dementia, and kuru, found only in certain tribes in New Guinea), all others are just a tiny percentage of the number of cases attributed to Creutzfeld-Jacob Disease (CJD). CJD, and most other spongiform encephalopathies caused by prions, is characterized by non-inflammatory lesions in the neural tissue of the brain, becoming open, fluid filled vacuoles which expand with the destruction of more neurons. The

vacuoles grow at an increasing rate, with the victim subject to rapidly increasing loss of motor control, dementia, and finally death within a year of first symptoms through subsequent infection and loss of organ control. Creutzfeld-Jacob Disease is still relatively rare, and occurs in only 1 or 2 people per million on the average, and is even rarer in those under 45 years of age (about 5 per billion). However, due to problems with diagnosis of a disease in which the cause almost impossible to detect, and which mimics the effects of many other diseases associated with aging, Alzheimer's disease, for instance, the actual rate of CJD infection may be much higher, even above 1 in 10,000 at the time of the person's death. (Heaphy, 1996)

If prion diseases have been infecting so many people and animals for so long, why the sudden panic over BSE, only one of many types of prion infection? The "Mad-Cow Disease" scare has a valid root in science and should be cause for alarm. Bovine Spongiform Encephalopathy reached epidemic proportions in England in 1986 when up to 1,000 cows a week, and a total of 200,000 so far, were infected and subsequently destroyed to control spread of the disease. (Kluger, 1997) It was found that these animals had become infected, by a then-undetermined agent, through the practice of supplementing animal feeds with rendered waste from food production plants. This waste from cattle, sheep, swine, and other animals was (and still is, in many countries) used as a cheap source of protein on industrialized farms. It was determined that some infective agent had been in one or more of the animals slaughtered for food, their rendered waste reintroduced into the food supply for other animals, thereby infecting them, and the cycle repeated as the newly infected animals were slaughtered, amplifying the disease into an epidemic.

The English government ordered the slaughter of all infected animals, the sale of brains, intestines, and other most likely infected organs banned, and the practice of feeding slaughter waste to animals stopped. (Kluger, 1997) The incidence of disease in British cattle has plummeted, but even such drastic action may have been too little, too

late. The first indication of what may have been happening for years, unseen to the naked eye, was a London Zoo report of cheetahs and other felines turning up with a degenerative neural disease identical to BSE. These felines had been fed from the same beef supply as English consumers, and had been infected with a disease thought to be exclusively bovine. (Marwick, 1996) A highly contagious disease had crossed into another species, and was present in human food supplies. Now the question became, would humans be affected, and if so, how many?

Humans, it turns out, were infected by this outbreak of BSE in England. How many remains to be seen, but recently dozens of people have fallen ill with a new strain of CJD, presumably from eating contaminated beef products. This new form, referred to as variant Creutzfeld-Jacob Disease (vCJD), is even more lethal than the previous strain, and has affected mostly persons under the age of 45, rather than predominantly the elderly, as the original strain had. More and more cases of vCJD have been diagnosed as time goes on, and the total could be horrifically high. Due to the long incubation period of prion diseases (up to 12 years in cross species infectious, such as this), the total number infected may not be known for years to come.

But what causes such a virulent and infectious disease that it can cross from species to species, and is universally fatal? As noted before, prions are a single protein, which replicates itself and disrupts neuron metabolism, causing the cells to lyse, and nervous system degeneration to result. This protein is not some foreign invader to the body, as is a virus or bacteria. Instead, it is a normal protein called PrP sup C found on the surface membranes of all mammalian neurons in some form. This protein is manufactured in the endoplasmic reticulum of the cell, packaged in the Golgi apparatus, and transported to the cellular membrane, as are most other membrane proteins. Where the difference lies in this protein, and makes it so dangerous, is the existence of two stable conformational isoforms of the protein, PrP sup C, the normal, and PrP sup Sc, the disease causing prion. (Cohen, 1994)

PrP sup Sc, the infectious prion, is formed from the normal protein PrP sup C by interaction of the two, and direct changes in PrP sup C's conformation by PrP sup Sc. PrP sup Sc acts as a kind of chaperone protein (another recently discovered type of molecule which directs construction of normal cellular proteins to certain isoforms) by bonding to the unmodified PrP sup C molecule, via disulfide bonds, at certain points which prevent the PrP sup C from bending into its usual conformation, and instead forcing it into the alternate of PrP sup Sc and thereby forming another prion. (Chernoff, 1995) This process proceeds exponentially and eventually converts all the normal PrP sup C proteins into their prion forms.

The difference between the two isoforms chemically is nonexistent, however, their structures are totally different. The normal, PrP sup C, is a protein composed primarily of alpha-helices (42%) and almost none of the beta-sheet conformation common to cellular proteins. PrP sup Sc is nearly reversed, and instead has a large proportion of beta-sheets (43%) and a much reduced alpha-helix structure. (Cohen, 1994) The consequence of this is twofold. Firstly, the beta-sheet form of PrP sup Sc makes it unusually attractive for bond formation with other, similar proteins which increases the likelihood that it will bond to and convert the normal protein, leading to a fairly high rate of conversion. Secondly, this ease of bond formation leads to "clumps" of PrP sup Sc protein complexes, all bound to one another. These sticky clumps protect the inner PrP sup Sc proteins from proteolytic enzymes normally released by a cell which has detected damaged, or abnormal proteins such as prions. (Choo, 1994) Those protected prions cannot be broken down by the infected cell, and are present to convert more of the cell's own proteins to prions in a continuous cycle. Insoluble PrP sup Sc sheets continue to accumulate until they fill the vacuoles of the infected cell and disrupt it's normal metabolic activity, causing the cell to eventually die and lyse itself, releasing the prion proteins to enter the surrounding neural cells and begin conversion of their normal proteins. Normal cellular defenses against infection are ineffective against a prion infection.

Given that prions are so difficult to detect and destroy, very few options for treatment are open to the medical community. One method for prevention found in mice while testing for prion necessary genes has recently presented itself. The gene coded product PrP sup C is necessary for PrP sup Sc to be replicated, and it was found that mice with the gene for PrP sup C removed were very resistant to prion infections and continued to thrive without the neuron protein. If this protein is not essential for life, it may be possible to remove the gene in embryonic humans or other animals, or somehow prevent production of it in another way, to make individuals immune to prion infection. (Chernoff, 1995) Another option considered has been the creation of a molecule which, when bound to the alpha-helical form of protein PrP, prevents its conversion to the PrP sup Sc beta-sheet form by making the PrP sup C form much more energetically stable. (Cohen, 1994) Both of these possible treatments, and any other innovative possibilities, are years and millions of dollars of research away, and will not have any impact on those with prion diseases now, however.

The prion diseases will likely not have the impact of most communicable diseases which catch the public eye, such as HIV, but are instead a signal of a new type of social disease. Our industrialized social system transports disease and organisms very readily via quick transport of people, food, and other materials. BSE/vCJD, for instance, a century ago would have only been present on a few farms in England, and not transported to the thousands of areas hundreds of miles away from the initial source. Human society has become so decentralized in food production, trade, and travel, that diseases which once were localized and could be diagnosed before they were allowed to spread are being carried quickly around the world. Now, any person or animal infected with a communicable disease can be on a plane, and to another part of the world in a matter of hours, while the disease he may be harboring takes weeks or years to show symptoms, by which time it is too late, and another possibly lethal pathogen has been spread.

### Personal Note:

During the course of researching and writing this paper, I found that a personal friend of mine for two years has been diagnosed with vCJD. He has lived in England his entire life, in the region of Liverpool, and had gone to the hospital when he noticed a vague loss of control and reflexes along his right side. A subsequent MRI scan showed "tiny gnat-like lesions which seemed to be growing" along the left hemisphere of his cerebrum. The diagnosis came back with a 90% probability of CJD. He is under 40, and has been told he has about 12 months to live, perhaps 9 of which he will be able to function reasonably well. "Mad-Cow Disease" is far from a simple joke on the evening news, in my experience.

### **Works Cited and Referenced Materials**

Chernoff, Yury O. et al. "Role of the Chaperone Protein Hsp104 in Propagation of the Yeast Prion-Like Factor [psi sup plus]" Science Vol. 268 (5212) (1995) : pp.880-884

Choo, Vivien. "Clue to Prion Replication." The Lancet Vol. 344 (8920) (1994) : p.468

Cohen, Fred E. et al. "Structural Clues to Prion Replication" Science Vol. 264 (5158) (1994) : pp.530-531

Heaphy, Shaun. "Prion Diseases" Internet. (1996) :  
(<http://www-micro.msb.le.ac.uk/335/Prions.html>)

Kluger, Jeffrey. "Could Mad-Cow Disease Strike Here?" Time January (1997) : pp.52-53

Marwick, Charles. "Views of Reason for Mad Cow Disease Vary Widely." JAMA Vol. 276 (6) (1996) : pp.438-440

Prusiner, Stanley B. "The Prion Diseases." Internet. (1996) :  
(<http://www.nmia.com/~mdibble/prion.html>)