Chapter 5
Bovine Spongiform Encephalopathy: Comparison Between the "Prion" Hypothesis and the Autoimmune Theory

5.1 Bovine Spongiform Encephalopathy as an Environmental and Nutritional Problem Involving Cattle

The first cases of bovine spongiform encephalopathy (BSE), a neurological disorder, were identified in the late 1980s in cattle raised to produce beef for human consumption and in dairy herds especially in southern England (Kimberlin 1993).

Changes in the composition of cattle supplementary feeds especially the use of "green offal" were suggested to be involved in the origin of this disease (Anderson et al. 1996).

"Green offal" in abattoirs, consisting of intestines and their contents, as well brain and spinal cord were then extensively used to produce these supplementary cattle feeds as "meat-and-bonemeal" (MBM).

The occurrence of BSE appeared to be associated with the consumption of feeds which may have contained scrapie "infected" products leading to the "recycling" of the infection (Crawford et al. 1991).

The possibility of infection was further enhanced by changes in the rendering process whereby the heating processes were significantly reduced because of financial considerations, thereby possibly increasing the quantity of the "scrapie" infectant in the feeds.

It has also been proposed that BSE-affected cattle might enter the human food chain and cause Creutzfeldt-Jakob disease (CJD) or a related neurological disorder in humans (Anderson et al. 1996).

Other groups suggested that organo-phosphate might be involved. Anticholinesterase "organophosphates" (Ops) (Phosmet) are used primarily as pesticides in cattle and sheep are often added to grains and nuts which go into cattle feeds. The organophosphates are mutagenic compounds and were thought to induce

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mutations in the cellular "prion protein" (PrP), resulting in the formation of the infectious "prion agent" (Purdey 1994). Cattle which have been exposed to high organophosphate concentrations appeared to exhibit classic BSE symptoms such as hind leg weakness, stumbling and a staggering gait. However geographical studies on the use of organo-phosphates did not correlate with the distribution of BSE in England.

5.2 Features of Bovine Spongiform Encephalopathy

The clinical features of bovine spongiform encephalopathy (BSE) vary daily during the early stages and it can take 1–6 months for the disease to develop and the diagnosis to be made.

Behavioral changes that frequently occur include a change in the milking order and a reluctance to pass through narrow passages.

Affected cattle become disorientated and are often found staring for long periods of time.

They avoid other cows in open areas and can attack humans or other cows especially when located in confined spaces.

The most noticeable characteristic features are changes on movements.

Shortened strides, swaying and difficulty in moving around corners occur early but later there is progressive weakness and ataxia with weight loss.

There is a characteristic hind quarters weakness and ataxia with affected cows readily falling down whilst still standing on their fore-quarters.

The inability of affected cattle to coordinate movements implies that irreversible nervous damage has occurred.

Histopathological examination of the brain and medulla in affected animals shows the presence of spongiform changes owing to the presence of vacuoles of varying sizes. Vacuolation of brain tissue is frequently found as the main feature of BSE.

Vacuoles are formed as a result of degeneration of myelinated axons and swelling of dendritic cells (Jeffrey et al. 1992).

The most prominent changes affect the solitary tract nucleus, the spinal tract nucleus of the trigeminal nerve and the vestibular nuclei.

As the disease progresses the vacuoles increase in size and number, often coalescing together thereby giving rise to the characteristic spongiform appearance.

It has been suggested that vacuolation may be due to the presence of prion (PrP) molecules. The site of most intense vacuolation is also the site of highest prion concentration and this observation has been used to support the "prion hypothesis" which implies that the prions are the "infectious agents" in BSE (Prusiner et al. 1993).

5.3 The Prion Agent as the Cause of BSE

One of the main features observed in BSE is the accumulation of prions (PrP's) in brain tissues.

Prions have unique biological properties: they have been defined as small proteinaceous particles that resist inactivation by procedures which modify or destroy nucleic acids. They have a molecular weight of 27–30 kDa and exist in different polymorphic forms which include rods with a diameter of 10–20 nm and 100–200 nm in length (Prusiner 1994).

There are two forms of prions: the host cellular form PrP^{c} and the infectious form PrP^{sc} also known as the scrapie infectious agent. It has been proposed that prions are the "transmissible pathogens" causing degenerative diseases which affect the central nervous system in both humans (CJD, kuru and Gerstmann-Sträussler-Scheinker syndrome) and animals (scrapie, BSE, transmissible mink encephalopathy).

Prions are resistant to many processes that would denature or inactivate viruses. Prions isolated from CJD and kuru-infected humans were found to be protease resistant and this was also a characteristic of prions obtained from scrapie infected sheep.

The native prion (PrP) is located on the cell surface and could possibly be involved in processes of cell interaction.

Prion proteins do not contain nucleic acids and therefore are unable to undergo self-replication in a normal host. They are encoded in the host genome and are found in both cattle and humans.

Both native and "infectious" forms are believed to be produced by the host cells but the difference in structure is obtained by some form of post-transcriptional modification.

The prion agent appears to have the following features (Prusiner 1993):

1. PrP and the scrapie agent co-purify in detergent extractions.
   Limited proteolysis leads to the aggregation of prions into amyloid rods.
2. PrP^{sc} is absent in normal uninfected animals.
3. Separation by immunoaffinity techniques using PrP antibodies coupled to protein-A Sepharose results in the co-purification of PrP and the infectious agent suggesting similar biochemical composition.
4. Purification involving hydrolysis or selective modification decreases the prion titre probably through denaturation.
5. The prion agent is resistant to heating at 100 °C as are in general all proteins.

It has been suggested that the prion agent appears to be a novel type of "infectious particle" not containing nucleic acids and is different from parasites, bacteria or viruses which all require nucleic acids for their replication.

Although the prion protein is somehow involved in these neurological disorders there could be another mechanism by which it could cause pathological damage to explain its great ability to cross species barrier and induce neuronal disease (Lasmezas et al. 1997).

5.4 Difficulties Associated with the Prion Hypothesis

There are several problems associated with the concept that prions are the causative agents of BSE:

1. The prion hypothesis has not been able to account for the different types of PrP^{sc} which have been detected in scrapie brains. Since PrPs do not contain any nucleic acids, genetic mutations cannot explain the occurrence of these different forms of prions which have different incubation times and also various types of rod shapes.
2. The mode of replication proposed by the prion hypothesis does not conform to current concepts of molecular biology. Replication of this proteinaceous molecule is somehow dependent on cell division. However it is difficult to explain how it leads to exponential increase in the numbers of PrP^{sc} molecules without some form of ribosomal processing.
3. The two isoforms PrP^{c} and PrP^{sc} are believed to have the same amino acid sequence. Current knowledge of protein structure suggests that identical amino acid sequences would have similar secondary and tertiary structures that exist in the most stable energy state.
4. It has been suggested that replication occurs, first, by the "infectious prion" forming a homotypic $PrP^{c} - PrP^{sc}$ complex. The "infectious prion" then transforms the cellular isoform PrP^{c} into the "infectious form" PrP^{sc}. Such a mode of replication requires the protein molecules to overcome a high activation energy to transform the dominant alpha helix PrP^{c} to the beta pleated sheet PrP^{sc} structure. The mechanism of how this occurs independently without the involvement of an energy source is not clear.
5. The "infectious protein" PrP^{sc} has not been individually isolated, there is no immunological evidence of its presence in affected animals and there are no microbial methods of culturing the agent.
6. The histological evidence of damaged brain tissues is not evidence of the presence of a replicating "infectious agent".
7. Immunodeficient mice such as SCID mice, do not develop scrapie following inoculation with affected brain tissue. If "infectious prions" (PrP^{sc}) were the cause of spongiform encephalopathies, SCID mice should be extremely susceptible to the development of scrapie following exposure to the "infectious prion", since such animals readily succumb to infection by viruses and bacteria. Another possible explanation is that the immune system itself is responsible for the scrapie lesions and in animals devoid of an adequate immune system, scrapie does not arise.

These serious molecular and microbiological problems associated with the prion concept, indicates that another hypothesis is required to explain the BSE phenomena.

In the absence of a clear virological theory to explain BSE and scrapie or a chemical theory such as the exposure to organo-phosphates there is another possible

mechanism involving immunological pathways leading to the development of autoimmune diseases.

Autoimmune diseases have been clearly described over the last 50 years and could provide an explanation for the origin of BSE.

5.5 General Theory of Autoimmune Diseases

Autoimmune diseases occur when the host mounts an immune response against antigens found in external agents or the environment which contain sequences resembling self tissues.

In 2009, at the "Autoimmunity Symposium" held in Dresden, Professor Yehuda Shoenfeld, from Israel proposed his famous "Conjecture" that "All autoimmune diseases are produced by external agents, unless proved otherwise".

The molecular mimicry theory states that the self antigen has a sequence which is biochemically and immunologically similar to agents or microorganisms present in the environment (Ebringer 1978).

The question arises whether spongiform encephalopathies could be explained by an autoimmune mechanism.

The autoimmune theory has provided aetiopathological models which demonstrate how *Streptococcus, Klebsiella* and *Proteus* bacteria are involved in the development of rheumatic fever, rheumatoid arthritis (Wilson et al. 1995) and ankylosing spondylitis (Fielder et al. 1995):

1. Rheumatic fever is the classical prototype of an autoimmune disease. A bacterial infection of the tonsils by group A *Streptococci* evokes antibodies which bind to heart tissue resulting in acute rheumatic fever because there is molecular mimicry between cardiac tissues and *streptococcal* antigens.
2. Rheumatoid arthritis is a systemic disorder which affects preferentially the peripheral joints. The majority of rheumatoid arthritis patients carry either HLA-DR1 or HLA-DR4 antigens.

   Injection of HLA-DR4 lymphocytes into rabbits produced antibodies to *Proteus* bacteria, a common urinary microbe. A urinary tract infection readily explains the increased frequency of rheumatoid arthritis in women. Furthermore *Proteus* haemolysin shows molecular mimicry or crossreacts with antigens present in HLA-DR1 and HLA-DR4 lymphocytes. Antibodies to *Proteus* bacteria have been found in rheumatoid arthritis patients from 17 different countries.
3. Ankylosing spondylitis is an arthritic disorder of the spine and 96 % of patients with the disease possess the HLA-B27 antigen whilst it is only present in 8 % of the general population in the UK or the USA. Injection of HLA-B27 lymphocytes into rabbits produces antibodies against the bowel microbe *Klebsiella.* The hexamer amino acid sequence QTDRED was found to be present in both the HLA-B27 molecule and *Klebsiella pneumoniae* nitrogenase reductase enzyme (Schwimmbeck et al. 1987). Ankylosing spondylitis patients from 16

different countries have elevated levels of antibodies against the bowel microbe *Klebsiella.*

If molecular mimicry operates in these three human diseases it is possible that a similar mechanism might operate in BSE.

A possible model to explain BSE in terms of molecular mimicry is provided by the immunological responses observed following immunization of human subjects with extracts of rabies viruses.

5.6 Experimental Allergic Encephalomyelitis as a Model of BSE

Pasteur and his colleagues, in 1890s, developed a therapeutic model to stop the paralytic and lethal effects of rabies in individuals who had been bitten by rabietic dogs or wolves. They injected saline extracts of brain tissues from animals infected by the rabies virus into humans and the majority of patients survived and recovered. However a small proportion of such injected patients developed a neurological disease involving ataxia, lower limb paralysis, inability to swallow liquids, hence the term "hydrophobia" and ultimately death.

It was only in the 1930s that it was discovered that saline injection of normal brain tissues leads to a condition which was called "experimental allergic encephalomyelitis (EAE).

EAE is an inflammatory autoimmune condition following immunization with brain tissues.

It has been used as an animal model for studying demyelinating diseases occurring in humans such as multiple sclerosis. This experimental disease affects the central nervous system and leads to the formation of large plaques of demyelinated gliotic scar tissue traversed by axons which also eventually become destroyed.

The pathogenesis of EAE is mediated by immune responses mounted against self antigens present in the myelin tissues.

Demyelination leads to the formation of plaques which coalesce to produce vacuoles and then a characteristic spongiform appearance.

The question arises whether BSE could be a form of EAE?

5.7 Growth Hormone Injections as a Cause of CJD or EAE

Growth hormone replacement therapy has resulted in some patients developing a form of Creutzfeldt-Jakob Disease (CJD) which is a progressive spongiform encephalopathy, resembling BSE and manifested by presenile dementia, ataxia and myoclonus tremors, leading eventually to a fatal outcome (Richard et al. 1994).

Prior to 1985, cadaveric growth hormone extracts were used in the treatment of children having a small height. This practice has been discontinued as this treat-

ment resulted in some patients developing Creutzfeldt-Jakob disease (CJD) (Ellis et al. 1992).

Since then recombinant DNA has been used to produce growth hormones which do not contain the putative "infectious agent", namely "denatured normal brain" tissue.

The administration of growth hormone preparations probably contaminated with nervous tissue antigens led to the development of pathological brain features similar to those observed in CJD or animals with EAE. The growth hormone preparations which contained such denatured brain tissue antigens may have been responsible for the development of this fatal spongiform condition in the injected children.

5.8 The Autoimmune Theory of BSE

The hypothesis is proposed that BSE is caused by an autoimmune response similar to that which occurs in EAE or in patients treated with growth hormone preparations.

Demyelination and neuronal damage resulting in spongiform formation are the main characteristics of BSE affected cattle.

When applied to BSE, the autoimmune theory proposes that cross-reactive auto-antibodies target bovine self antigens, following exposure to food borne microbiological material and in this it could resemble other autoimmune diseases such as rheumatic fever, ankylosing spondylitis and rheumatoid arthritis.

The autoimmune response could arise as a result of molecular mimicry between biological agents present in the winter feedstuffs, the meat-and-bonemeal (MBM) materials and myelin proteins.

Proteonomic analysis is required to assess whether encephalitogenic peptides producing EAE and environmental bacteria, possess sequences showing molecular mimicry to myelin and other brain tissues.

5.9 Comparisons Between the Prion Hypothesis and the Autoimmune Theory

Scientific theories cannot be proved, since they cannot overcome David Hume's paradox that it is impossible to examine all instances of the problem. However they can readily be disproved and this was first pointed out by the philosopher of science Karl Popper (Popper 1963).

Popper stated that "All good scientific theories should predict possible facts that are incompatible with the proposed scientific theory or hypothesis".

If such a fact is found to occur, the proposed theory has been disproved or is no longer tenable and has to be replaced by a new hypothesis or theory to explain the problem.

The theory or generalization that "All tigers are carnivorous" is incompatible with the observation of a vegetarian tiger.

Table 5.1 Comparison of the experimental and clinical predictions of the prion hypothesis and the "autoimmune theory"

Prion hypothesis	Autoimmune theory
Biological activity of agent retained at 100 °C	Biological activity of bacterial myelin peptides retained at 100 °C
Increased antibody levels to cross-reacting bacteria in sera of affected animals not present	Increased antibody levels to cross-reacting bacteria in sera of affected animals present
Prion proteins are 'infectious particles'	Prion proteins are not 'infectious particles' but breakdown products of damaged nervous tissue
Autonomous 'infectious prions' exist in the environment	Autonomous 'infectious prions' do not exist in the environment
Brain and muscle tissue arc infected by 'prions' in affected animals	Brain and muscle tissues are not infected, but crossreacting autoantibodies binding to nervous tissue are present and cause neurological damage
The agent causing BSE is in the brain and spinal cord of offal material	The agent causing BSE is not in the brain and spinal cord, but in the bacteria present in the 'green offal' material
Consumption of BSE-affected meat is dangerous	Consumption of BSE affected meat is not dangerous
CJD epidemic is expected in the human population	No CJD epidermic is expected since humans do not consume 'green offal' material
'Growth hormone preparations' were contaminated with prions which caused CJD in some patients	'Growth hormone preparations' were contaminated by denatured human brain tissue which caused an EAE-like syndrome in some patients
The 'prion hypothesis' is not compatible with current concepts of molecular biology and postulates the existence of novel particles which cause neurological damage	The 'autoimmune hypothesis' is compatible with current concepts of molecular biology and proposes that BSE/scrapie arc produced by a mechanism involving molecular mimicry between common bacteria and nervous tissue

The "autoimmune theory" provides a new model to explain BSE and scrapie. Furthermore it makes experimental or observational predictions that distinguish it from the "prion hypothesis".

The predictions made by the two theories in relation to BSE are compared and experiments should be carried out to distinguish between these two models (Table 5.1):

1. The two theories have significantly different economic implications.

   The "prion hypothesis" implies that the only method of eliminating BSE or scrapie is by culling all affected animals The "autoimmune theory", however proposes that the removal of the cross-reactive bacteria present, if any, in the supplementary "meat-and-bonemeal" preparations would prevent the development of these neurological diseases and therefore the culling of cattle is and was unnecessary.
2. The "autoimmune theory" predicts increased levels of antibodies against common bacteria which exhibit molecular mimicry with myelin and other nervous tissues.

   Experiments should therefore be carried out to determine if elevated levels of antibodies to these bacteria exist in bovine BSE serum.

3. Moreover, sporadic cases of scrapie infected sheep should have increased levels of antibodies to some of these microbes (Ebringer et al. 1998).
4. BSE is probably an autoimmune disease caused by bacteria which carry antigens resembling or showing molecular mimicry with brain tissues.
5. The BSE epidemic occurred as a result of producing animal feeds containing high concentrations of bacteria showing molecular mimicry with myelin and other brain antigens.
6. Experiments are required to determine if immune responses to these bacteria have occurred in BSE or scrapie affected animals.

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