Angela Halpin

The tale behind mad cows

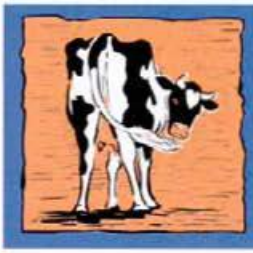
Roger Beckmann introduces prions, the acrobatic proteins whose antics have puzzled biologists and scandalised British beef.

Amid all the media attention about 'mad cow disease', one important subject is rarely mentioned: how the disease is caused. But for many biologists, that's the most exciting part of the whole story; the mysterious pathogen has been described as 'the most bizarre infectious agent ever imagined'. It is neither bacterium nor virus, nor any other recognisable organism, and its existence challenges many basic biological assumptions about the nature of infectious disease.

Dr Harvey Westbury of CSIRO's Australian Animal Health Laboratory (AAHL) at Geelong in Victoria is an expert on rare viral diseases. He says that mad-cow disease is not caused by a virus, but by something completely different. 'You can't even test the animals for the presence of the disease in the normal way that we use for viral diseases. There is no immune response.'

Mad-cow disease belongs to a group of unusual diseases (many with equally unusual names) called spongiform encephalopathies. These can affect a wide range of mammals, including humans. The cattle version, more properly called bovine spongiform encephalopathy (BSE), came to the attention of scientists when the first cases were reported in Britain in 1986. But scrapie, the sheep version, has been around for at least 200 years and occurs in many other countries as well. Fortunately, neither disease occurs in Australia.

In both conditions, the brain degenerates: small holes appear where cells have died, giving brain tissue a spongy appearance under the microscope. Because of the brain damage, affected animals show behavioural changes, including nervousness, unusual posture and uncoordinated movements. Eventually the animals collapse. The diseases are nearly always fatal, but affected animals are usually destroyed before death ensues.



'Clearly, this pathogen was not a virus or any other known organism and was almost certainly not alive in any real sense. Yet it appeared to be able to multiply.'

Mad-cow disease is thought to have begun in Britain after cattle were fed a protein supplement derived from the offal and bone of scrapie-infected sheep. This practice had been going on for some time before BSE appeared, but in the early 1980s, partly in order to save money, the processing of sheep offal changed. The high temperatures and quantities of chemical solvents used to sterilise the material were reduced slightly, and as a result, the scrapie 'bug' survived the procedure and was present in the supplement given to cows. (An alternative view is that BSE was a rare, unidentified condition in cattle that was amplified through the protein-supplement feed.)

Back then, no-one knew exactly what that 'bug' was, although the preferred theory then was that it was some sort of unusual slow-acting virus. It probably spread between sheep in the field when they ate the deposited placenta from a ewe during lambing time. (Many herbivores will eat placentas when they get the chance, as a way of gaining extra protein that is readily digestible.)

Dr Harvey Westbury of CSIRO's Australian Animal Health Laboratory: mad-cow disease is not caused by a virus, but by something completely different.

The cannibalism connection

Scientists now know that the cow and sheep diseases are similar to some rather rare and unusual human diseases. One of these, kuru, was first described in 1957 by the American scientist Dr Carlton Gajdusek who found that its distribution was limited to the Fore highlanders of Papua New Guinea.

Victims of the condition showed a gradual loss of coordination, and then a sort of premature dementia and an early death. Smart scientific detective work showed that kuru was caused by something that was passed on during ritual cannibalism, where the brains of recently deceased, highly honoured individuals were eaten. Since cannibalism was outlawed kuru has slowly disappeared.

It was clear early on that kuru was indeed infectious: a pathogen (an infectious agent) must have been present in the brain of the dead people, but it had no way of getting from person to person other than through cannibalism. You couldn't 'catch' kuru like a cold.

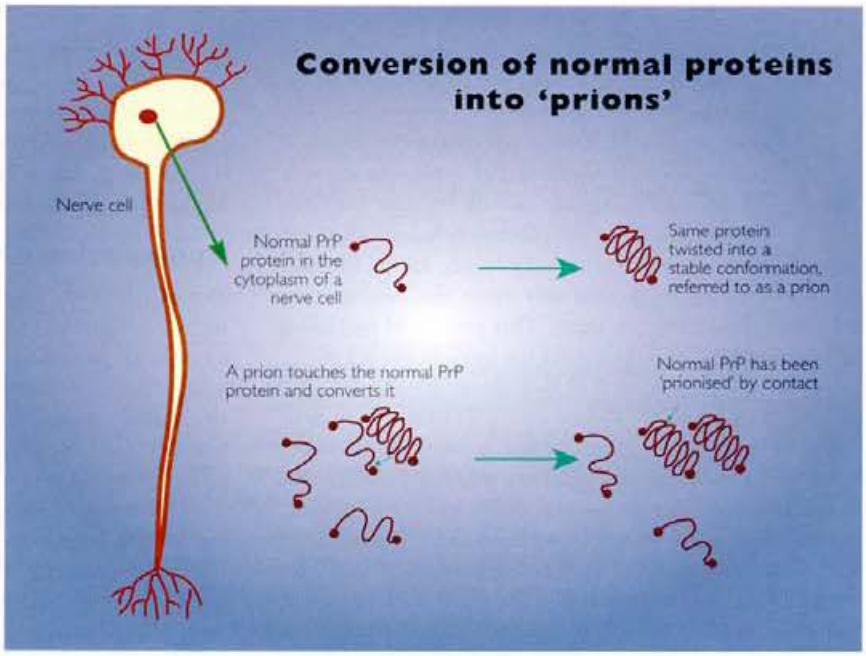
Although human cannibalism no longer takes place, medical procedures sometimes involve something similar: such as giving people human growth hormone extracted from the pituitary gland in the brain of corpses. (Today this hormone is made using genetic engineering techniques.) It so happens that a very rare human condition, called Creutzfeldt-Jakob Disease (CJD), has occurred in a several people who were treated for growth deficiency with these hormone extracts. CJD is also a spongiform encephalopathy with symptoms similar to kuru.

Searching for the mystery bug

For many years no-one knew exactly what kind of microbe caused the spongiform encephalopathies. In the 1970s experiments were starting to show that the infectious agent – whatever it might be – was remarkably

resistant to many forms of sterilisation. Healthy animals could be infected by an extract from diseased animals even after it was zapped with radiation or other treatments that would destroy the genes of any bacterium or virus. It was this ability to survive normal sterilisation that may have enabled the disease to be passed from sheep to cattle during the procedure of manufacturing protein supplement from offal.

By 1982, an American scientist, Stanley Prusiner, had concluded that the 'scrapie-agent', as it was called, had no genes. Clearly, this pathogen was not a virus or any other known organism and was almost certainly not alive in any real sense. Yet it appeared to be have the ability to multiply.



An important clue came with the observation that extracts of dead sheep were no longer infectious if they were treated with substances known to incapacitate proteins. The 'thing' was definitely composed of some sort of protein and Prusiner dubbed it a prion, because it was a 'protein virion'. (A virion is an individual virus particle.)

We now know that the scrapie prion is nothing like a real virion, in which the genes in the nucleic acid specify the structure of the protein surrounding them, which is then made by the cell that the virus infects. Prusiner and his team found that their prion consists of just one protein chain, folded in such a way that it is remarkably strong and resists digestion.

Prusiner faced considerable scepticism about his findings. All known organisms, however small and simple, have both protein and nucleic acid. One cannot exist without the other in a living system. A protein doesn't just appear: something must be making it, and must have access to information for its construction.

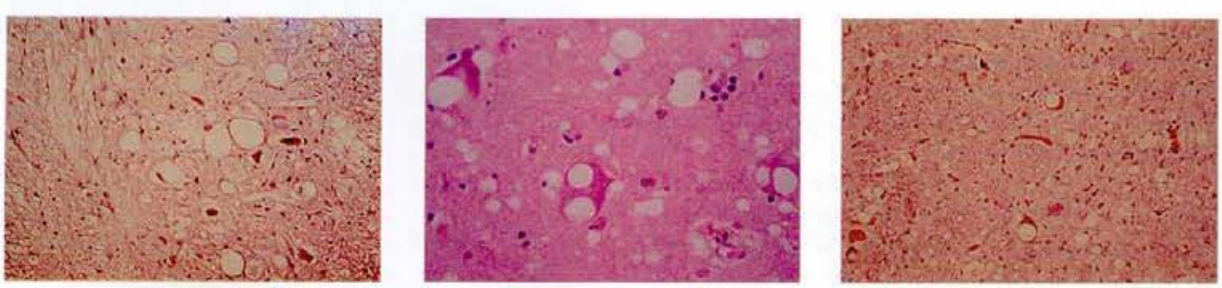
A mucked-up molecule

Latest research shows that the instructions for making prion protein are probably within us all: the gene for it sits in the mammalian chromosome. A prion is merely an unusually folded variant of the normal body protein coded for by this gene. This protein, called PrP or cellular prion protein, occurs mainly in nerve cells.

All proteins are made of a chain of amino acids, and the chain can fold in various ways, which may affect the functioning and stability of the protein. What we call prions are simply molecules of the nerve cell protein PrP folded in such a way that they are very resistant to high temperature, disinfecting agents, and even to protein-digesting enzymes.

A prion can have the same sequence of amino acids as the normal PrP protein of which it is a variant, but something has caused it to fold itself in a different way, giving it a great ability to survive. This 'something' is simply physical contact with another prion. In other words, prions can somehow cause the normal PrP protein molecules to 'flip' into a more stable shape or conformation, which turns them into prions themselves, able to convert other PrP molecules. This gives the appearance of reproduction.

The details of the prion story have recently been confirmed in experiments with genetically engineered mice lacking the gene for normal cellular prion protein. These animals do not have a pool of normal PrP to be



In mammals with spongiform encephalopathies, the brain degenerates. Small holes appear where cells have died, giving brain tissue a spongy appearance under the microscope. These images show the effects of the condition on the brain tissue of sheep (left), cheetah (middle) and deer (right).

'turned' by contact with an acquired prion and so are resistant to prion diseases passed on by extract from infected animals.

Prions appear to be useless proteins, and what, if anything, the normally folded PrP proteins do is a mystery. A cell can't degrade prions because they are resistant to most enzymes, so 'infected' cells tend to shove prions into a sac, which eventually bursts, killing the cell. The released prions presumably enter the surrounding cells and cause havoc in them. This process of spreading cell death probably accounts for the holes in the brain.

Nerve cells outside the brain, lymph tissue, and possibly embryonic tissues like placentas, may also harbour prions, but without such obvious damage. Current knowledge suggests that other tissues are not much affected because normal prion protein is mainly produced in nerve cells. Britain banned the use of brain and certain other offal in human food in 1989. The worry about British beef now is the slight possibility that the small nerves within the muscle tissue would carry prions, and the risk from beef consumption before 1989.

Getting around

Any disease-causing parasite needs to move between its hosts. But a prion is not really a parasite in the strict sense. It's simply something normal gone wrong, so it's difficult for a viable prion to get from one animal to another. A prion is not adapted for this in the way that so many other internal parasites are. Prions can readily pass across from mother to foetus, but the only other way they can reach a new host of the same species is by being eaten, and cannibalism is rare in mammals.

'CJD has been known for decades, and its incidence in the British population is no higher than that in other European countries where there is no mad-cow disease.'

Thus, in the normal course of events, a cow with BSE won't pass it on to the rest of the herd. Although the numbers of BSE cases in Britain increased rapidly – from 17 in 1986 to a peak of about 37 000 in 1992 – this is not a spreading epidemic of a highly contagious disease. The increase merely represents the adoption of the altered treatment regime for offal during its processing into a protein supplement for cows.

But what of carnivores? Surely they would be prion-ridden through eating their prey? The limited evidence suggests not, and the reason seems to be the existence of a species barrier. Theory suggests that prions from one mammal cannot readily infect another species unless the naturally-made, normal prion proteins are quite similar in their amino acid sequence to the infecting prion. If not, they can't be 'turned' by contact with it.

In general, the more closely related species are, the more similar in composition are all their body proteins. How easily cow prions can infect us is still unclear, as our prion protein is known to differ from that of cows in several places, but it's a relief to know that Australia has no scrapie sheep or mad cows!

Human prion diseases

Prusiner believes that all spongiform encephalopathies, including kuru and Creutzfeldt-Jakob Disease (CJD), are caused by prions. The question terrifying Britain is whether some unusual and unexplained cases of CJD are the human manifestation of mad-cow disease, and whether this can be 'caught' by eating digestion-resistant prions in beef.

CJD has been known for decades, and its incidence in the British population is no higher than that in other European countries where there is no mad-cow disease. CJD is usually a disease of older people, unless it is transmitted through medical or surgical procedures, in which case it can occur, it seems, in anyone. What is unusual about the handful of new CJD cases in Britain now is the fact that those affected were relatively young people with no known route of infection other than eating bovine products.

Spontaneous cases of CJD are explained by postulating that the gene for normal prion protein mutates during the course of a lifetime, causing a slight change in the amino acid composition of the protein which will cause it to fold into a prion form. (This mutation is more likely to happen the longer you have lived, hence the age distribution of cases of CJD.) The disease will remain with the affected individual unless the victim is eaten or, possibly, their prion-affected organs are used for transplantation.

There remains the intriguing question of where prions come from in the first place. It's possible that a gene for manufacturing a slightly less stable version of cellular prion protein (which would more easily slip into a prion form) could be inherited. Thus, a predisposition to the spontaneous occurrence of prion diseases could run in families or entire species. If we are careful about avoiding the conditions that allow spread between individuals of the same or different species, prions could be controlled. That's just as well, because there is not likely to be any treatment for prion diseases.

How safe are we in Australia? CJD occurs here at a very low rate, as it does elsewhere. But when it comes to acquiring prion diseases from our food, we are lucky. To help keep us that way, prions and the development of BSE in Britain are being monitored by AAHL. In conjunction with AQIS, AAHL runs scrapie testing on all imported sheep and goats. Provided the prion proteins within your brain stay firmly in their proper shape, so that you do not develop your own spontaneous prion disease, you should be safe living in Australia.