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CHAPTER 1

Scrapie, Kuru, Cannibalism, and "Mad Cow" Disease

The story of prions goes back a long way. Scrapie, the disease of sheep, was already known in the eighteenth century by farmers in England and Germany. Sick animals would continuously rub themselves against fence posts, damaging their fleece, hence the name *scrapie*. They also showed other disturbing behaviors and some neurological symptoms such as tremors. Already in those days people knew that it had to be a brain disease. The animals were used mainly to produce wool, a valuable product, and the farmers were losing their income to scrapie. They dealt with their economic difficulties in the time-honored fashion by blaming somebody else for it, preferably someone from the south. In this case they accused Spaniards, from whom they had bought Merino sheep years before. With hindsight, they were probably right. Two centuries later, in the late 1930s, two French veterinarians, Jean Cuillé and Paul-Louis Chelle, published a series of articles that showed convincingly that scrapie was infectious, that it could be transmitted from

sheep to sheep by intraocular injection, and that the incubation period was greater than one year. They also demonstrated that natural transmission occurred between animals housed together.

Sometime before this pioneering work, toward the end of the nineteenth century, Icelandic farmers had noticed that some of their sheep were coming down with a new disease, which they named *rida*.[1] In this case the farmers blamed a single ram that had been imported from Denmark some years before for bringing the disease to their flocks.[2] Needless to say, this origin was never proven. Rida was later shown to be scrapie. Then, in the 1930s, Iceland decided to import sheep from European countries and to cross them with their local animals to increase the quality of the wool and meat. This turned out to be a very unfortunate decision, as over the following years several previously unknown infectious diseases began to cause serious economic problems. One of them was visna, the neurological disease I was working on when Prusiner was looking for a name for the scrapie agent. The imported sheep had been quarantined and examined before they were released to farmers, but, because the diseases had unusually long incubation periods and protracted courses, infected animals went unnoticed. Furthermore, the imported sheep, having been exposed to their pathogens for centuries, were relatively resistant, whereas the Icelandic animals, which had been living in com-

1. *Rida* in Icelandic means "to tremble," "to stagger."

2. P. A. Palsson, "Rida in Iceland and Its Epidemiology," in *Slow Transmissible Diseases of the Nervous System*, edited by S. B. Prusiner and W. J. Hadlow (New York: Academic Press, 1979), 1:357.

plete isolation from the rest of the world, were highly susceptible.[3] These diseases were studied by Bjorn Sigurdsson, a talented veterinarian. He thought that they were all caused by viruses, including rida, and proposed calling them "slow viruses," to reflect the long incubation period and protracted course of the diseases.

Following Sigurdsson's work, slow viruses became an active field of research. When I became a virologist and started to work on visna, it was considered a frontier in virology. Visna was an interesting model for human multiple sclerosis. The virus looked like a tumor virus, but the disease was not a cancer. It became the prototype of a new group of viruses called *lentiviruses* (*lent* is French for "slow"), of which HIV became the most infamous member. But when Sigurdsson coined the name *slow viruses* he certainly did not realize that he would subject some of us to an easy joke that I heard far too many times at the beginning of my career: "There are no slow viruses, only slow virologists!" Colleagues are not always helpful.

Scrapie before Prions

After the pioneering work of Cuillé and Chelle in France, research on scrapie moved mainly to the United Kingdom and the United States. Some remarkable work was done despite enormous technical difficulties. Measuring the amount of the pathogen required using animals. Researchers injected

3. Infections play a major role in the evolution of species. They exert what is called a strong "selection pressure." Resistant individuals tend to reproduce more successfully than susceptible ones, and their presence in a population increases with time.

the animals with increasing dilutions of the sample and counted how many came down with disease and died from it. Several animals had to be injected for each dilution, and the amount of pathogen, its *titer*, was set as the dilution that killed 50 percent of the animals. This meant inoculating large numbers of sheep and waiting a year or more to get the result. Obviously, progress had to be slow. Important results were nevertheless obtained, including on the genetic susceptibility of various breeds of sheep and on the physicochemical properties of the agent. It was discovered that infectivity resisted treatments that inactivated all known parasites, bacteria, and viruses. This was a bewildering and important result.

A breakthrough occurred in 1961 when Richard Chandler at the Agricultural Research Council facility at Compton in the United Kingdom succeeded in adapting sheep scrapie to the laboratory mouse. This was a major advance. Infectivity assays still required observing the animals for a year or more, but they were small animals in cages, not sheep in barns. The work with mice took two directions. One was to study the mechanism of the disease, its *pathogenesis*: which organs besides the brain are infected and in what order, which cells carry the infection, and so on. These studies showed, for example, that when animals are inoculated in muscles, the organs of the immune system, in particular the spleen, become infected before the agent reaches the brain. The other direction was the characterization of the scrapie agent. Was it a virus or something else? The remarkable resistance of the agent to chemicals and radiation that inactivated viruses was puzzling. Its resistance to ultraviolet radiation was particularly unsettling. UV radiation interacts with nucleic acids, DNA and RNA. Resistance

implied that nucleic acids were not essential for its infectivity. This was so unorthodox that it was met with enormous skepticism among biologists. Many of them tried to make models with viruses protected from the effect of UV radiation by unknown mechanisms. Even to this day, some—admittedly, a small minority—still cling to the idea that the scrapie agent contains a nucleic acid and that a virus is there that has just not yet been discovered.

Kuru in New Guinea

While the research on scrapie was ongoing in the United Kingdom and the United States, in 1954 Carlton Gajdusek, a pediatrician with a wide range of interests in biology, was spending the year in the laboratory of Sir Macfarlane Burnet at the Walter and Eliza Hall Institute of Medical Research in Australia. During a trip to New Guinea, he met Vincent Zigas, who was a medical doctor from Australia working in the Eastern Highlands of New Guinea. Zigas described to him a strange neurological disease that appeared to be epidemic in some isolated human populations in the Highlands, including a group that called themselves the Fore. The disease, which the Fore called *kuru*, was causing numerous deaths, mainly in women and children.[4]

4. The story of kuru and the work of Carleton Gajdusek in New Guinea are well described in P. P. Liberski, B. Sikorska, and P. Brown's "Kuru: The First Prion Disease" (chapter 12 in *Neurodegenerative Diseases*, edited by Shamin and Ahmad [New York: Springer, 2012]) and Liberski's "Kuru: A Journey Back in Time from Papua New Guinea to the Neanderthal's Extinction" (*Pathogens* 2 [2013]: 472–505). Vincent Zigas gave his own account of the early days of kuru research in *Laughing Death:*

Access to the Eastern Highlands of New Guinea is difficult. The mountains are high and separated by deep valleys with torrential rivers. As a result, the population is very fragmented, with small groups living in isolation. Gajdusek accompanied Zigas on one of his expeditions in the Highlands and became fascinated by the kuru disease and by the Fore people, who at the time were living in complete isolation, subsisting on hunting and gathering and farming root vegetables.[5] With great difficulty the two managed to set up an outpost where they could examine patients and work out the history of the disease, its *epidemiology*. They published their first findings in 1957. Gajdusek and Zigas worked under extreme physical hardship and amid great personal danger. The early studies of kuru would not have been possible without the unusual and extremely strong personality of Carleton Gajdusek. Sir Macfarlane Burnet, in whose laboratory Gajdusek was working at the time, gave this description of him: "I had heard that the only way to handle him was to kick him in the tail, hard. Somebody else told me he was fine but there just wasn't anything human

The Untold Story of Kuru (Clifton, NJ: Humana, 1990). Michael Alpers has written an important and enjoyable paper that gives all sorts of information on the Fore people and other kuru-affected groups, and on the early work done in the 1960s by him, Gajdusek, and others: "The Epidemiology of Kuru: Monitoring the Epidemic from Its Peak to Its End," *Philosophical Transactions of the Royal Society B* 363 (2008):3707–13.

5. Carleton Gajdusek was a prolific writer. No matter the circumstances, he would always find time to keep a daily account of his activities in his journal. A selection of notes taken during the early days of his work on kuru has been published: D. Carleton Gajdusek, *Kuru: Early Letters and Field-Notes from the Collection of D. Carleton Gajdusek*, edited by J. Farquhar and Gajdusek (New York: Raven 1981).

about him. My own summing up was that he had an intelligence quotient up in the 180s and the emotional immaturity of a 15-year-old. He is completely self-centered, thick-skinned, and inconsiderate, but equally won't let danger, physical difficulty or other people's feelings interfere with what he wants to do."[6]

In their outpost, Gajdusek and Zigas managed to perform autopsies and to ship brain samples to neuropathologists at the National Institutes of Health (NIH) in Bethesda and elsewhere. Later, when Gajdusek moved to the NIH, his laboratory worked almost exclusively on kuru. However, for many years he still spent extended periods of time living and trekking in the Highlands of New Guinea.

In 1959, Klatzo, Gajdusek, and Zigas published a lengthy description of the brain lesions in kuru.[7] They described widespread loss of neurons and the proliferation of glial cells called *astrocytes*. They compared these lesions with those of other neurological diseases and concluded that the only resemblance was with Creutzfeldt-Jakob disease, a rare human disease of unknown cause. They discarded the possibility of a viral infection because of the absence of infiltration by cells of the immune system, and the negative results of their inoculations to laboratory animals.

6. Quoted in Jay Ingram, *Fatal Flaws: How a Misfolded Protein Baffled Scientists and Changed the Way We Look at the Brain* (New Haven, CT: Yale University Press, 2013), 18.

7. I. Klatzo, D. C. Gajdusek, and V. Zigas, "Pathology of Kuru," *Laboratory Investigation* 8 (1959):799–847.

Kuru and Scrapie

The story of prions took another serendipitous turn a few years later. William Hadlow, a veterinarian from the NIH Rocky Mountain Laboratories, happened to be in England. He was alerted by a colleague to the presence of an exhibit in London about New Guinea, kuru, and Gajdusek's findings. Hadlow was interested in neuropathology and was an expert on scrapie. He went to the exhibit and was struck by the pathology micrographs taken by Gajdusek and his coworkers, which showed microscopic holes inside neurons and in the tissue between them, not mentioned by Klatzo, Gajdusek, and Zigas in their 1959 article. Hadlow thought that they closely resembled those observed in scrapie.

Following his visit to London, Hadlow wrote a short letter to the *Lancet* pointing out the similarities between the lesions of scrapie and those of kuru. He suggested inoculating nonhuman primates to determine if the disease was transmissible, possibly with a long incubation period like that of scrapie in sheep. The letter was a turning point. It prompted Gajdusek and his associate Joe Gibbs to perform a new round of inoculations, including of chimpanzees. As predicted by Hadlow, the chimpanzees came down with a kuru-like disease, but only one to two years after inoculation, depending on the animal. This was soon followed by the transmission of Creutzfeldt-Jakob disease to chimpanzees, again by Gibbs in the Gajdusek's laboratory. For these discoveries Carlton Gajdusek was awarded the Nobel Prize in Medicine in 1976.

The numerous microscopic holes in the brains of sheep with scrapie and humans with kuru, Creutzfeldt-Jakob disease,

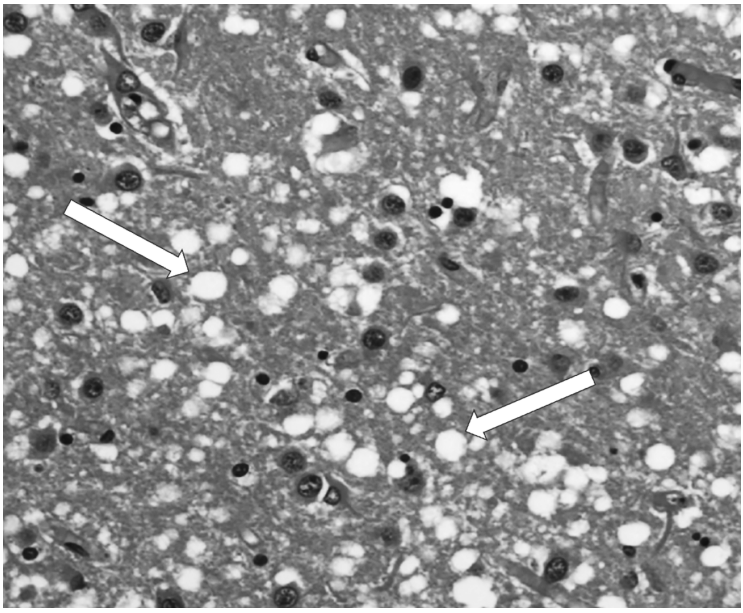

FIGURE 1.1. Section of a brain sample from a patient who died of Creutzfeldt-Jakob disease, as seen under the microscope. The arrows indicate vacuoles. The large number of vacuoles gives the brain the look of a sponge. From *Neuropathology Simplified: A Guide for Clinicians and Neuroscientists* by David A. Hilton and Aditya G. Shivane; © 2015 Springer International Publishing Switzerland

and some other diseases are called *vacuoles* (figure 1.1). These vacuoles, which are mainly located inside neurons, make parts of the brain look like a sponge under the microscope, hence the name *transmissible spongiform encephalopathies* (TSEs) now given to this group of diseases.

Veterinarians had shown that in the field scrapie was transmitted from animal to animal by the oral route, through eating

contaminated grass and the placenta of ewes that had just delivered. Kuru could be transmitted in the laboratory to chimpanzees by intracerebral inoculation. But how was it transmitted among the Fore in New Guinea? Because of their extreme isolation, the populations of the Eastern Highlands of New Guinea had attracted the attention of anthropologists, among them Robert Glasse and Shirley Lindenbaum.[8] During their field studies with the Fore tribes, they observed that the geographic spread of kuru coincided with the practice of cannibalism. Deceased Fore were dissected, and their organs, including the brain, were cooked and eaten. This is commonly referred to as *ritual cannibalism* in the scientific literature, with the idea that the practice had something to do with the rebirth of the soul of the deceased in future generations. Shirley Lindenbaum, who studied cannibalism in the Fore group and was the first to suggest that kuru was transmitted by cannibalism, contests the term "ritual" and asserts that cannibalism was adopted to provide women and growing children with protein after the Fore became farmers growing root vegetables. Animal meat, from hunting, was reserved for men.

Even though scrapie was known to be transmitted by the oral route, the transmission of kuru by cannibalism was met at first with skepticism by scientists. However, it has been confirmed by the fact that no case of kuru has been observed in people born after the cessation of cannibalism. It is not possible to precisely determine the origin of the kuru epidemic. One can speculate that it began with a sporadic case of Creutzfeldt-Jakob

8. Shirley Lindenbaum wrote an account of her work with kuru: *Kuru Sorcery: Disease and Danger in the New Guinea Highlands* (London: Routledge, 2016).

disease in the Fore population. The consumption of brain tissue from this initial case and the common practice at the time of cannibalism by women and children may have triggered the epidemic.

Creutzfeldt-Jakob Disease

German neurologists Hans G. Creutzfeldt and Alfons M. Jakob first described the disease that bears their name in the 1920s. The disease is rare; its incidence is one to two cases per million per year. The symptoms consist of dementia with memory loss, hallucinations, and involuntary muscle contractions known as *myoclonus*. It is invariably fatal within a few months to a year, and there is no cure or preventive measure. The pathology of the disease is typical of the other spongiform encephalopathies, including scrapie and kuru. We already mentioned that the disease was transmitted to chimpanzees by intracerebral inoculation in the laboratory of Carleton Gajdusek.

There are rare familial cases of the already-rare Creutzfeldt-Jakob disease. Familial cases of diseases can be a great help to scientists. If geneticists find a mutation that is present in all cases in the family, and if the mutation is transmitted from generation to generation according to Mendel's laws of heredity, one can be almost certain that the product of the mutated gene is causing the disease. Despite its rarity, several families with Creutzfeldt-Jakob disease have been identified across the world. In all of them geneticists have found that the gene responsible for disease was *PRNP*, which is the gene that codes for the PrP protein. We will see later in the book how Stanley Prusiner showed that PrP is the agent of scrapie.

Unfortunately, Creutzfeldt-Jakob disease has occasionally been transmitted accidentally from human to human. The first case was reported in 1974, in a woman who had received a corneal transplant. She developed Creutzfeldt-Jakob disease two years later. It was later determined that the donor of the cornea had died from a neurological disease, which at autopsy turned out to be Creutzfeldt-Jakob disease.

Some years later, two cases were caused by neurosurgery for severe epilepsy. To limit the damage caused by the surgery, surgeons located the area to be removed by recording its abnormal electric activity with electrodes. The electrodes in these cases were sterilized in between patients with alcohol and formaldehyde at concentrations that kill all known bacteria, viruses, and parasites. A patient who had undergone neurosurgery was diagnosed with Creutzfeldt-Jakob disease sometime later. Two other patients for whom the same electrodes were used, after sterilization, also came down with Creutzfeldt-Jakob disease, fifteen and eighteen months respectively after surgery. We now know that the Creutzfeldt-Jakob prion is extremely resistant to sterilizing chemicals, including ethanol and formaldehyde at the concentrations used at the time. Transmission of Creutzfeldt-Jakob disease has also occurred with some batches of dura mater used during neurosurgery. Such accidental transmission is now prevented by measures that take the resistance of the infectious agent into account.

The most dramatic contamination happened with the use of human growth hormone. Growth hormone is a protein made by the pituitary gland, a small gland at the base of the brain. A deficit in growth hormone causes dwarfism, short stature, which can be prevented by treating children with the hormone. For

many years, human growth hormone was extracted from pituitary glands obtained at autopsy. Then, in 1985 the Department of Health and Human Services in the United States was alerted to three cases of Creutzfeldt-Jakob disease in young men treated with human growth hormone for dwarfism. This prompted an investigation, also undertaken in several other countries, into the incidence of Creutzfeldt-Jakob disease among growth-hormone-treated patients. The conclusions were dire. In the United States, 35 cases were found, 80 in the United Kingdom, and 123 in France. The obvious conclusion was that some of the autopsies to obtain pituitary glands had been performed on people with undiagnosed Creutzfeldt-Jakob disease; individuals who died of other causes but had early lesions of Creutzfeldt-Jakob disease in their brain. The amount of growth hormone in the pituitary of elderly individuals is very small. Therefore, the hormone was purified from batches of many pituitaries. A single case of Creutzfeldt-Jakob disease among the donors was sufficient to contaminate the whole batch of hormone, and therefore several patients. Fortunately, this tragedy was followed by the development of synthetic human growth hormone made by genetic engineering. No case of Creutzfeldt-Jakob disease has been reported since the use of synthetic hormone.

The "Mad Cow" Epidemic: Variant Creutzfeldt-Jakob Disease

In the late 1980s, a new disease of cattle appeared in Britain, nicknamed "mad cow" disease by the press. Veterinarians determined that the cows were dying from a neurological disease and that the lesions in the brain were like those of scrapie in

sheep. They named the disease *bovine spongiform encephalopathy* (BSE). In the years following, the disease appeared in several other countries around the world. The UK government knew that scrapie was transmitted by sheep eating contaminated food. They traced the appearance of BSE to a change in the methods of preparation of cattle food supplements from recycled livestock carcasses, a cost-cutting measure. They destroyed the stocks of these food supplements and began the systematic culling of all animals in herds with one or more cases of BSE, with burning of carcasses to destroy the infective agent. The cost to the economy was enormous. Over four million cattle were destroyed to eliminate the disease from the United Kingdom.

For me, "mad cow" disease was the occasion of a humiliating episode. I was not working on spongiform encephalopathies, but I had a keen interest in the field, and my laboratory at the Pasteur Institute in Paris was called the Slow Virus Unit. We were a nice group of scientists, including many students, and we did not mind being called "slow virologists" by our colleagues. I received several calls from journalists at the beginning of the BSE epidemic, many of them asking about the risk of contracting Creutzfeldt-Jakob disease through eating beef. People were worried.

I told all of them that there was absolutely no reason to stop eating beef. Scrapie had been present in Britain and the rest of Europe for a long time. It had a long incubation period. There was no way to screen for asymptomatic animals before they were sent to the slaughterhouse. Therefore, scrapie must have entered the human food chain long ago, and there was

no evidence of a link between eating lamb and Creutzfeldt-Jakob disease. Scientists had done a great deal of epidemiological work, including surveying Libyan shepherds who eat sheep's eyes as a delicacy. No evidence was found of transmission to humans. Period. Besides, when I was in school, we had sheep's brains for lunch quite regularly, and I was fine. So that was that.

Unfortunately, around 1996–97, ten years after the peak of BSE, UK neurologists noticed cases of Creutzfeldt-Jakob disease with unusual brain lesions. They called these cases *variant Creutzfeldt-Jakob*, or vCJD. The number of cases of vCJD peaked around the year 2000 and then diminished. Over the same time period, the incidence of classical Creutzfeldt-Jakob disease remained the same as ever, between one and two new cases per million individuals every year. Variant Creutzfeldt-Jakob was also observed in other countries, but the majority of cases, more than two hundred, were in the United Kingdom, where "mad cow" disease had been much more prevalent than in the rest of the world.

Variant Creutzfeldt-Jakob disease illustrates the role of genetic susceptibility in prion diseases. The prion protein responsible for spongiform encephalopathies, PrP, is 230 amino acids long (we will discuss in the next chapter how proteins are made up of small molecules called amino acids). Amino acid 129 is either a methionine or a valine, depending on the genetic background of the individual. We all have two copies of each of our genes, one copy on each chromosome of a pair of chromosomes. For some people, both copies of the gene that codes for the PrP protein have a methionine at position 129 (40% of

Caucasians),[9] for others both have a valine (10% of Caucasians), and for still others one copy of the gene has methionine and the other has valine (50% of Caucasians). It turned out that virtually all the patients with vCJD had methionine on both chromosomes. This is a typical example of genetic susceptibility to a transmissible disease. The mechanism is not understood in the case of vCJD, but a hypothesis will be discussed in chapter 2.

What did I overlook when I was asked by journalists about the risk of BSE infecting humans? Pathogens have what is called a *host range*. For some, it is wide—they infect many different species. Others have a narrow host range. For example, measles virus infects only humans—as far as we know, of course. The PrP prion as known at the time had a narrow host range. Scrapie prion infects only sheep and goats. Careful epidemiological studies have not found evidence of transmission of scrapie to humans through eating lamb, nor even sheep's brains. The kuru and Creutzfeldt-Jakob prions are restricted to humans and some nonhuman primates, including chimpanzees. However, what I overlooked was that goat and sheep scrapie had been "adapted" to mice and hamsters on a few occasions in the 1960s and 1970s, often after several blind passages from mouse to mouse or hamster to hamster. Therefore, the possibility of a change in host range exists, even with the PrP prion.

We do not know by what mechanism a prion can be "adapted" to a new host. But we can build hypotheses based on what we learned about the mechanism of prion multiplication. This also will be discussed in chapter 2.

9. Virtually all the vCJD patients were in Britain and France and were Caucasian.

An Infectious Agent Made of Just One Protein

There are different personal styles among scientists. Some may trust their intuition and proceed to test a bold hypothesis unsupported by preliminary evidence. Others prefer to take a Cartesian approach, starting with what is already known for sure and going one step at a time. Of course, in most cases, progress is made through a mixture of both attitudes, and serendipity can play a big part in getting to the result. When Stanley Prusiner decided to identify the agent of scrapie, he opted for a step-by-step, rational approach. He knew, from the work of others, where to look for it—in the brain—and that it most likely contained proteins but possibly no DNA or RNA. That was not a lot of information.

To purify a component from animal tissue, scientists perform what they call *fractionations*. They start with a complex mixture, such as a piece of brain that has been homogenized in a blender, and try to separate it into its component parts, or *fractions*, keeping track of where the product they want to purify ends up in the process. They may place their mixture in a tube and spin it in a centrifuge, which can spin the tube at various precise speeds. At the right speed, one hopes to separate the product that one wants to purify between either the pellet at the bottom of the tube or the *supernatant*, the liquid at the top. For each speed tested, one needs to find out where the product is, in the pellet or the supernatant, and how much of it is in both. There is nothing more depressing than finding that there is just as much in both. This means that the purification step has achieved nothing. And, in fact, it was often the case for people trying to purify scrapie. Remember that the assay

to detect the agent and quantify it took a year or more, because the only way to do it was to inoculate animals with various dilutions of the material and wait until they got sick and died. No wonder results were coming slowly.

Faced with these difficulties, Stanley Prusiner realized that he needed a starting material that was as rich in the agent as possible, and an assay that was as rapid as possible. After a series of attempts, which are described in detail in his autobiography *Madness and Memory*,[10] he settled on using hamsters instead of mice. The amount of scrapie agent in hamster brain was especially high, and, crucially, with hamsters he could speed up the assay because their disease was more rapid than that in laboratory mice. Furthermore, he devised an assay that did not require waiting until all infected animals had died. In preliminary experiments he determined that measuring the length of time between inoculation and the appearance of the first signs of disease gave an accurate titer of the agent. Using hamsters and a relatively fast assay paved the way to success. The only drawback, but a serious one, was that buying, housing, and observing daily a large number of hamsters was a lot more expensive than buying and housing mice.

At the end of a series of purification experiments, Prusiner concluded that the purest specimen he could obtain contained only protein, with one prominent one. Agents that damage proteins diminished or eliminated the infectivity. Agents acting on DNA and RNA had no effect on infectivity. It looked as though the scrapie agent was made of protein, possibly

10. Stanley B. Prusiner, *Madness and Memory* (New Haven, CT: Yale University Press, 2014).

only one type, and that nucleic acids were not required for infectivity. This is the point where he started looking for a name for the agent and came up with *prion*—a portmanteau from protein and infection.

The identification of the prion protein, abbreviated to PrP, took more time. Prusiner needed the help of molecular biologists. They purified the PrP protein further, were able to determine the sequence of its amino acids, and from there, with the help of more molecular biologists, they determined that the protein was encoded by one of the animal's genes. It was not a foreign protein, not a protein brought in by a microbe. They sequenced the gene coding for the protein and finally were able to obtain mice whose PrP gene had been eliminated by genetic engineering. Remarkably, these mice were totally resistant to inoculation with mouse scrapie. This was of course an important result, showing that the mouse PrP protein was required for the infection. However, it did not prove that the PrP protein by itself caused the disease; it only showed that the gene coding for the protein was needed. This could have been the case if, for example, the PrP protein had been the receptor for a scrapie virus. If there is no receptor for them to bind to, viruses cannot infect cells and cannot cause disease. Eventually, and more recently, Jiyan Ma at East China Normal University in Shanghai and Witold Surewicz from Case Western University in Cleveland, Ohio, showed that PrP prions obtained by genetic engineering could cause scrapie in mice and hamsters. The heretical protein-only hypothesis has been vindicated.[11]

11. This is only a summary of much research that led to the prion concept. Besides those mentioned, other researchers made essential contributions to the

But not for everybody. There are still a few biologists, including Laura Manuelidis at Yale University, who claim that spongiform encephalopathies are caused by a virus that has not yet been discovered, and that the PrP prion protein is a factor in the disease, or could be a consequence of the infection but not its cause. Skepticism is always welcome in science. Dogmas are dangerous, and one should also be wary of fashion. However, at present the overwhelming evidence is in favor of the protein-only original hypothesis.

But you may wonder, since PrP is a protein present in everybody's brain, why do only a few individuals come down with a dreadful spongiform encephalopathy? How can a normal protein in the brain suddenly, without any mutation, turn into a deadly pathogen? This is indeed a very good question. The next chapter will explain this. It requires first giving some background information on proteins and how they fold to acquire a three-dimensional shape.

To Recap

Scrapie is a disease of sheep known of since the eighteenth century. It can be transmitted to healthy animals by inoculation with brain extracts from sick ones. Studying scrapie was difficult because the incubation period can be more than a year, and because sheep are not laboratory animals. Scrapie

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discovery of prions. Adriano Aguzzi, a prion expert, mentions several of them in his article "Prion Science and Its Unsung Heroes" (*Science* 383 [2024], https://doi.org/10.1126/science.adn94).

was transmitted to laboratory mice in the 1960s, making experimental work easier.

The scrapie agent is remarkably resistant to the chemical and physical agents that inactivate all known microbes. Resistance to UV radiation implies that the agent does not contain DNA or RNA.

Carleton Gajdusek and Vincent Zigas studied kuru in the Highlands of New Guinea. William Hadlow pointed out that the brain lesions of kuru and scrapie looked alike. Kuru was transmitted to chimpanzees by intracerebral inoculation. The incubation period was longer than one year. Kuru was transmitted in New Guinea by the practice of cannibalism.

The brains of scrapie-infected sheep and kuru patients both showed numerous microscopic holes, called *vacuoles*, which give the tissue the appearance of a sponge, hence the name *transmissible spongiform encephalopathies* (TSEs) given to this group of diseases.

Creutzfeldt-Jakob disease is a rare human TSE, which was accidentally transmitted to recipients during neurosurgery and through the administration of growth hormone extracted from human cadavers. The "mad cow" epidemic was a variant of scrapie that spread among cattle fed recycled livestock carcasses.

Stanley Prusiner purified the scrapie agent and showed that it was made of a single protein called PrP. PrP is a host protein; it does not come from a microbe. PrP became the prototype prion protein. It causes all spongiform encephalopathies: scrapie, "mad cow" disease, and several rare human diseases including kuru and Creutzfeldt-Jacob disease.

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