Wen-Quan Zou
Pierluigi Gambetti *Editors*

Prions and Diseases

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Contents

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Chapter 1
Transmissible Spongiform Encephalopathy: From its Beginnings to Daniel Carlton Gajdusek

Paul Brown

Abstract Scrapie was the original member of what has become a family of both animal and human spongiform encephalopathies. Described clearly in the eighteenth century in both England and Germany as a fatal contagious disease of sheep, it was not experimentally transmitted until 1936, and became the subject of wide-ranging research in a number of laboratories in Great Britain. The human analog was first described in 1920 by the German neurologists Creutzfeldt and Jakob, and experimentally transmitted by Gajdusek in 1968, following a similar success in transmitting another analogous human disease (kuru) 2 years earlier. The evolving story of these and other members of the transmissible spongiform encephalopathy family (including "mad cow" disease) has led through a maze of studies involving many unexpected twists and turns, and eventually culminating in the discovery of a new category of infectious disease caused by the misfolding of a normal host protein (PrP^{TSE}).

Keywords Transmissible spongiform encephalopathy (TSE) • Scrapie • Kuru • Creutzfeldt–Jakob disease (CJD) • Transmissible mink encephalopathy (TME) • Chronic wasting disease (CWD) • TSE history

1.1 In the Beginning, …

…there was scrapie. How far back in time is unknown, but it is thought to have originated somewhere in Europe during the late Middle Ages. Whatever the historic beginnings, we know that by the eighteenth century it was prevalent in both England and Germany and that its introduction into England probably came from the importation

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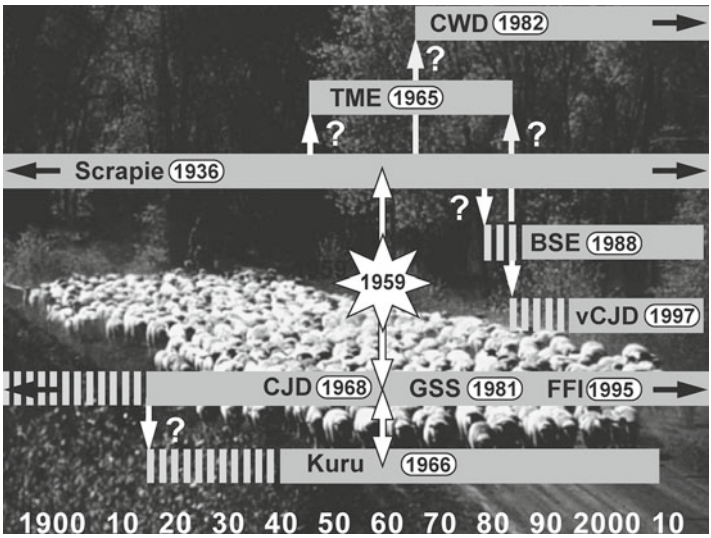

Fig. 1.1 The chronology of TSE. The position and length of the *bars* are keyed to the time line at the *bottom of the figure*. *Striped regions* represent the possible or probable (but unproven) preexistence of the disease. The date of the first reported experimental transmission of each disease is shown within the *bars*. The year 1959 is emphasized to draw attention to its importance as the year in which the kuru–scrapie–CJD connection was made

of Spanish merino sheep that, although highly susceptible to scrapie, had wool of exceptional quality.

At least two centuries elapsed between recognition of the disease and the first attempts to investigate it scientifically. The veterinary literature was limited to its incidence, clinical description, and speculation about its cause until the end of the nineteenth century, when Charles Besnoit and Charles Morel, colleagues in veterinary medicine at Toulouse, France, recognized the regular presence of spongiform change in the spinal cord and adjacent nerves, but considered it to be part of a wider pathology which they thought most likely represented a toxic peripheral neuropathy (Besnoit and Morel 1898). Besnoit also directed a number of transmission experiments in sheep that, unfortunately, were destined to fail because of a surveillance period limited to 9 months (Besnoit 1899), an oversight that a half-century later was also to delay recognition of the transmissibility of the human disease, kuru. Among the younger faculty members at that time was Jean Cuillé, who would later recognize this need for an extended period of postinfection observation, and publish with Paul-Louis Chelle a superb set of experiments between 1936 and 1938 that established beyond any doubt that scrapie was indeed a transmissible disease (Cuillé and Chelle 1936, 1938) (Fig. 1.1).

About the same time that Cuillé and Chelle published their studies, transmissibility was accidentally confirmed when a formalinized louping ill vaccine prepared from sheep CNS tissues was identified as the cause of a mini-epidemic of scrapie in

Scotland (Gordon 1946). Investigation of the outbreak revealed that one batch of vaccine had included material from Cheviot lambs born of ewes that subsequently developed scrapie. These observations laid the groundwork for a flowering of experimental research that was mostly concentrated in Great Britain during the next 30 years, although scrapie was also under study in Iceland, where it had the name "Rida," and in the USA, where it became a growing concern following its diagnosis in Suffolk sheep imported from Great Britain via Canada in 1947.

1.2 Working Out the Biology (in Sheep)

All of the early work on scrapie was conducted in sheep, an extremely inconvenient bioassay animal requiring observation periods of several years in carefully monitored farms, which meant that research remained limited to the very few facilities capable of performing such experiments. Worse still, the unpredictable response of sheep to the same experimental inoculum made it difficult and at times impossible to conduct quantitative titration studies.

Thus, the pioneering work of David R. Wilson at the Moredun Institute in Edinburgh during the 1940s, largely overshadowed by the personalities and careers of the many researchers who followed him, was a remarkable achievement. Conducting experiments almost single-handedly in sheep that had only a 25% transmission rate, he added transmissibility via intradermal and intravenous routes to those reported by Cuillé and Chelle; studied the pathogen's filtration and sedimentation behavior; and discovered its surprising resistance to a variety of chemical and physical treatments, including heat (100 °C for 30 min), exposure to phenol, chloroform, and formaldehyde, and UV irradiation (in retrospect perhaps the most interesting finding). He also documented the survival of infectivity in dried brain tissue after a 2-year storage. A great deal of experimental work published during the next several decades built upon the foundation laid down by Wilson.

The fact that scrapie was of lesser concern to the sheep industry than several other diseases, and was not known (then or now) to be a human pathogen, resulted in little governmental interest in the disease. That indifference changed when, in the early 1950s, North America, Australia, and New Zealand placed embargos on the importation of British sheep in response to the existence of undiagnosed scrapie in their exported sheep. (Never underestimate the power of commercial interests on the funding of scientific research, which recently surfaced again when "mad cow disease" appeared on the scene). Increased funding from the UK expanded the program at Moredun under the continuing direction of Wilson, and later John Stamp, and at Agriculture Research Council (ARC) facility at Compton, England, under the direction of William Gordon.

Gordon conceived and executed a massive study using over 1,000 sheep to investigate the breed susceptibility to scrapie (the "twenty-four breed experiment"), leading to the selection for experimental purposes of two flocks of the Herdwick breed: one highly susceptible and the other relatively resistant. He also put together

a very active group of scientists, including Gordon Hunter, Geoffrey Millson, Richard Kimberlin, Carol Walker, and Iain Pattison, who produced a flood of research papers during the 1960s to the 1980s dealing with genetic susceptibility, pathogenesis, and the nature of the scrapie agent.

Meanwhile, at Moredun, Stamp and Alan Dickinson began a wide-ranging study of scrapie strains in Cheviot sheep, producing, for the first time, sound experimental evidence for the maternal transmission of infection and spread of disease through close contact, and in a remarkable set of classical genetic analyses established that a single gene (*Sip*) with two alleles controlled the incubation period in sheep. Dickinson later became the founding Director of the ARC and MRC Neuropathogenesis Unit, also in Edinburgh, where he was soon joined by Kimberlin, Hugh Fraser, Moira Bruce, and David Taylor (and later by Jim Hope, Nora Hunter, and Jean Manson)—who as a group with wide-ranging expertise in pathogenesis, disinfection, molecular biology, and molecular genetics would advance knowledge in each of these areas in the years that followed.

1.3 The Mouse that Roared

In 1961, at Compton, Richard Chandler succeeded in adapting sheep scrapie to the mouse (Chandler 1961). This accomplishment immediately opened the door to studies that would have been prohibitive if limited to bioassays in sheep, and later made possible all of the genetic engineering that is crucial to so much work being done today. Pattison describes the event with his customary flair (Pattison 1972):

> I still feel the urge to genuflect as I pass the spot at our Institute (Compton) beside the boiler house, where my colleague R.L. Chandler paused 1 day in 1960 to suggest to me that he might inoculate three strains of mice (C57, CBA and Swiss) with brain material from two clinical types of goat scrapie (drowsy and scratching). Chandler had already found that the three strains of mice had different susceptibilities to *M. johnei*. He subsequently injected the two strains of scrapie i/c and he transmitted the drowsy strain in 7 months in the Swiss strain and to the other two strains a few weeks later. These mouse strains of scrapie bred true with an incubation period of 4 months. Thus occurred the greatest single advance in scrapie research since experimental transmission of the disease by Cuillé and Chelle in 1936.

This technical advance nearly, but not quite, extinguished all further experimental studies in sheep: the exceptions being studies in which non-rodent species are used to confirm the results in mice, or where there is a need for large amounts of tissues or fluids (blood, for example), or most recently, in studies designed to explore the behavior of bovine spongiform encephalopathy (BSE) infection in sheep. Three of the most important early studies in mice were conducted at the following laboratories:

– At the NIH Rocky Mountain laboratory in Montana, Carl Ecklund and William Hadlow initiated an exhaustive study of the distribution and level of infectivity in a wide variety of tissues and fluids in Chandler's strain of mouse-adapted scrapie, and in mice inoculated with material from naturally and experimentally infected sheep and goats.

– At Compton, Kimberlin and Walker extended these pathogenesis studies to the dynamics of peripheral infection, implicating lymph nodes and spleen along a pathway through visceral sympathetic nerves to the thoracic spinal cord and thence to the brain.
– At the ARC unit in Edinburgh, Dickinson's group applied the same classical genetic approach they had used in sheep, discovering that a similar gene (*Sinc*) controlled the incubation period in mice. They also showed that distinctive patterns of brain lesion distribution were reproducibly associated with different scrapie strains. The conjunction of these two observations led to a method of TSE strain identification that would later serve as the most persuasive evidence for a close strain similarity between BSE and vCJD (Bruce et al. 1997).

1.4 The Nature of the Beast

Amidst all of this work, two crucial questions stood out: what was the relative importance of an infectious versus genetic origin of the naturally occurring disease and, assuming the existence of an infectious agent, what were its biochemical components? The first question was a major topic of discussion at a 1964 meeting convened by the USDA in Washington DC. After listening to 3 days of heated debate, novitiates in the audience were left wondering if all medical meetings were going to be similarly confrontational (they would not be disappointed). Two participants were in almost diametrical opposition: H.B. (James) Parry, an Oxford veterinarian who argued for genetics as the exclusive cause of the naturally occurring disease, and Dickinson, who argued that scrapie was caused by an infectious agent that was influenced by genetic susceptibility. In due course, Dickinson's position would be fully validated. In fact, the *Sip* and *Sinc* genes that Dickinson had identified by classical genetics were none other than the prion-encoding *Prnp* alleles later identified by molecular genetics.

The other question—biochemical characterization of the infectious agent—was (and continues to be) a subject of intense research interest and importance. Although the burden of evidence for different strains of the scrapie agent clearly implied the existence of a nucleic acid genome, there were indications as early as the 1960s that nucleic acid was not only unlikely to be the sole constituent of the scrapie pathogen but, based on radiation resistance data, unlikely even to be present. The first clue came from the early inactivation studies by Wilson, noted above, that included a resistance to standard sterilizing doses of UV radiation. Then came the set of inactivation studies by Hunter, Millson, and Kimberlin that, in conjunction with their demonstration of a firm association of infectivity with cell membranes, led Gibbons and Hunter to propose that the infective entity was a modified glycoprotein subunit of membranes that multiplied by inducing similar chemical or conformation changes in newly "infected" cell membranes (Millson et al. 1976).

The "coup de grace" came from a set of rigorously controlled irradiation studies published by Tikvah Alper and colleagues between 1966 and 1971, in which both

the resistance of scrapie brain extracts to very high doses of ionizing and UV radiation and the UV inactivation profile were inconsistent with any known virus or nucleic acid. One paper in particular began with the following point-blank abstract: "Scrapie is a slowly developing disease of the nervous system. Experiments on the effects of ultra-violet irradiation of suspensions of infected mouse brain extracts confirm that the agent responsible for it does not depend on a nucleic acid for its ability to replicate. No evidence is obtained, however, to indicate whether the agent is associated with a protein" (Alper et al. 1967).

No one doubted the validity of Alper's radiation resistance work, but no one knew how to deal with it—in other words, how to accommodate a clear indication of the absence of nucleic acid in the pathogenic agent, and still satisfy the dogma of nucleic acid-directed replication. Explanations invoking protection or repair of nucleic acid eased acceptance of her data, but her conclusions remained in a kind of limbo for years.

1.5 The Transition from Biology to Molecular Biology

In 1967, the mathematician John Stanley Griffith suggested three ways by which a protein might self-replicate, remarking that "there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down" (Griffith 1967). He presented free energy equations for the polymerization of protein subunits on preexisting dimerized molecules, i.e., a template mechanism, as had been suggested by Gibbons and Hunter. He went on to say that "there is an obvious analogy between the idea presented here and the idea that a gas can only condense on nuclei which are already present: many of the more general schemes could be summed up by saying that the subunits can only polymerize by utilizing condensation nuclei of polymers which are already there." He concluded that scrapie could be "a protein or a set of proteins which the animal is genetically equipped to make, but which it either does not normally make or does not make in that form. It may be passed between animals but be actually a different protein in different species. Finally, in either case there is the possibility of spontaneous appearance of the disease in previously healthy animals."

Credit for the discovery of the first disease-specific structure in a transmissible spongiform encephalopthy (TSE) goes to Patricia Merz, working at the Institute for Basic Research in Developmental Disabilities in Staten Island, New York, who in the late 1970s began to study extracts of scrapie-infected mouse brains under the electron microscope. She identified fibrillar structures very similar to the those seen in Alzheimer's disease, which she named "scrapie-associated fibrils" (SAF), and in further studies also found them in the brains of humans and experimental animals infected with CJD (Merz et al. 1981; Merz and Somerville 1983).

What all of these experiments lacked was a molecule that specifically co-purified with infectivity, but this was finally rectified by 1982 in Stanley Prusiner's laboratory,

using the 263 K hamster model of scrapie that had been developed by Kimberlin and Walker in 1977 (Kimberlin and Walker 1977). This model proved to have exceptionally high concentrations of infectivity in the brain (10^{10} LD_{50}/g) after an incubation period of only 2 months, a fortuitous combination that made it possible to undertake the purification of a sufficiently large amount of highly infectious fibrils (renamed "prion rods" by Prusiner) to isolate a peptide subunit that could then be subjected to the tools of modern molecular biology.

The overall contribution of scrapie to the field of TSE was aptly summarized by Pattison in (1972), who concluded his reflections with the statement that "Scrapie is one of four closely similar diseases, the others being kuru, Jakob–Creutzfeldt disease, and transmissible mink encephalopathy. Research on scrapie was responsible for recognition of this group of diseases, to which others may be added in due course, and knowledge of the vagaries of scrapie has been of great value in planning research on them all, for in planning a complicated journey it is reassuring to know that similar ground has already been covered."

1.6 The Discovery of Kuru

In the mid-1950s, a young pediatrician turned research scientist named Carleton Gajdusek was stationed at the Walter Reed Army Medical Center where, in 1954, he was assigned to spend a year in Australia to study the immunology of liver disease in the laboratory of Sir MacFarlane Burnet. Ever the explorer, he traveled widely during his stay, including a trip to Papua New Guinea to satisfy what would become a lifelong interest in primitive cultures, and there met Vincent Zigas, a charming if somewhat eccentric Lithuanian physician who was working as a Medical Officer in the Eastern Highlands. Zigas told him about a strange neurological disease (kuru) that was decimating the Foré-speaking peoples in his area of practice, and invited him to the Highlands to see for himself. He did so and was intrigued by the high incidence, age and sex distribution, and neurological characteristics of the disease (Gajdusek and Zigas 1957). His journals and letters detail the heroic efforts needed to establish a beachhead in Okapa, the administrative center of the Foré region, including a dedicated hospital that for many years operated under the direction of Dr. Michael Alpers, and a native personnel network to identify and transport the continuing stream of new patients to and from Okapa.

He experienced many difficulties with the Australian colonial authorities (Papua New Guinea was then a dependency of Australia), which sometimes resented his dramatic intrusion into their territory. He once remarked that the US government would not be pleased in the converse situation of an Australian research team studying a new disease on an Indian reservation. In fact, one of Gajdusek's most remarkable and generous traits was, with a single exception, his acceptance of people and events that would depress or anger almost anyone else, as part of the "comédie humaine." He was simply incapable of feeling offended or bitter, and never looked back.

He was also an authentic genius, whose interests spanned physics, anthropology, medicine, music, and literature, and his early career was spent in the laboratories of a number of Nobel Laureates. It did not take him long to join their ranks: in 1976 he was awarded a Nobel Prize for his demonstration that kuru, a neurodegenerative disease, had an infectious cause. Kuru had been recognized for decades by the affected population (who considered it to be due to sorcery) and by European locals—everyone from missionaries to bush pilots—who attributed the disease to cannibalism. The difficulty was proving it, as is evident from the innumerable failures to find the cause in toxic, hormonal, nutritional, and infectious causes during the first several years of study.

1.7 The Kuru–CJD–Scrapie Triangle

The year 1959 was a banner year for TSE (Fig. 1.1). Since his encounter with kuru, Gajdusek had been spending a good part of each year in the field, establishing a kuru hospital in Okapa, the administrative center of the region, organizing the care of kuru patients, doing autopsies, trying to discover the cause of the disease, and conducting preliminary therapeutic trials based on all the possible causes under study. During this time, he sent brains from a dozen kuru cases to Igor Klatzo, a neuropathologist working at the NIH. In 1959 he published his findings, noting widespread neuronal degeneration (including vacuolation), myelin loss, astroglial and microglial proliferation, scattered perivascular cuffing, and, in half the cases, a predominantly cerebellar location of amyloid plaques. He did not mention spongiform change, and attributed the neuronal vacuolation to postmortem artifact. However, in his discussion comparing kuru to other diseases, he concluded that "Creutzfeldt–Jakob disease appears to be closest in resemblance" (Klatzo et al. 1959).

This astute observation by Klatzo was all the more remarkable because the diagnostic criteria for Creutzfeldt–Jakob disease had been in disarray since its initial description in 1920 and remained so through the late 1960s. Creutzfeldt's original case was described as a "new and unusual type of neurological disease" in a 22-year-old woman with a 1-year illness characterized by tremors, spasticity, pyramidal signs, nystagmus, ataxia, myoclonus, and dementia (Creutzfeldt 1920). Neuropathology showed diffuse neuronal loss and astrogliosis, but vacuolation was neither mentioned nor illustrated. A year later, in 1922, Jakob reported four cases that he thought resembled Creutzfeldt's case (Jakob 1921). A review of the slides from Jakob's cases was undertaken by Colin Masters in 1982 (Creutzfeldt's slides had not survived), who concluded that only one of the cases (a 42-year-old male) satisfied the criteria for what we now call Creutzfeldt–Jakob disease: the histopathology included neuronal loss, astrogliosis and a diffuse spongiform change throughout the cerebrum and cerebellum (Masters and Gajdusek 1982).

Over the next several years, Jakob and his students gradually acquired a fuller appreciation of spongiform encephalopathy as a pathological entity, including the first case of familial CJD, and somewhat later, in the mid-1930s, Gerstmann,

Straüssler, and Scheinker reported the first family with the disease that now carries their names (GSS) (Gerstmann et al. 1936). Nevertheless, the clinical and neuropathological characteristics of CJD remained elusive until the bedrock criterion of transmissibility allowed its clear separation from a host of other neurodegenerative diseases of unknown etiology.

Hadlow's recollection of events that led him to make the kuru–scrapie connection was recounted in a reminiscence published in 2008:

> The unlikely linkage of these two diseases came about fortuitously while I was an employee of the USDA studying the pathology of scrapie at Compton. William Jellison, a friend and colleague from Rocky Mountain Laboratory, Hamilton, Montana, where I had worked before coming to England visited me in Compton and casually mentioned an exhibit he saw the previous day at the Wellcome Medical Museum in London. It had to do with a strange brain disease affecting the primitive people in Papua New Guinea. He thought I might like to see it owing to my interest in neuropathology. Five days later I saw the exhibit in London. Neuronal degeneration and intense astrocytosis likened kuru to scrapie. The likeness was made even more so by the single and multilocular vacuoles in the perikaryon of large neurons. From the start I was drawn to them for they were so much like those in scrapie (Hadlow 2008).

In his letter to Lancet, Hadlow recalled that "scrapie can be induced experimentally in the sheep and in the closely related goat but not in other species so far tested…," and he concluded that "It might be profitable, in view of veterinary experience with scrapie, to examine the possibility of the experimental induction of kuru in a laboratory primate, for one might surmise that the pathogenetic mechanisms involved in scrapie—however unusual they may be—are unlikely to be unique in the province of animal pathology" (Hadlow 1959). He had recognized the twin needs for extended observation periods and the use of a species closely related to humans (Bjorn Sigurdsson, working in Iceland, had in 1954 set out criteria for "slow infections" that included species specificity).

1.8 Experimental Transmission of Kuru

At the NIH, brain tissue had already been inoculated into numerous laboratory rodents, observed for periods of up to several months, with negative results, but now Gajdusek went about organizing a primate colony at the Patuxent Wildlife Center in Laurel, MD, under the able direction of Clarence J. (Joe) Gibbs, Jr., who had served with him at the Walter Reed Army Medical Center. By 1963 all was in readiness, but Gajdusek decided to wait until new autopsy specimens could be obtained under optimal conditions for survival of any infectious agent before initiating a chimpanzee inoculation program. The author well remembers being sent to New Guinea only a few months after joining the laboratory in July 1963 with instructions to get autopsies on any kuru patients who died during his month-long stay. Only one patient died, and in a hut under the flickering light of a hurricane lantern, with the deceased woman's husband hovering nearby, it was necessary to barter for each organ that was taken (coffee, canned goods, flashlights, knives, etc.), and also satisfy his very sharp eye for reassembling the body to its pre-autopsy condition. Gajdusek had set

Table 1.1 Animal species used in TSE experiments

Primates	
Apes	**Chimpanzee**, Gibbon
Prosimians	Bushbaby, Lemur, Slow Loris
Old World monkeys	**African green**, Baboon, Bonnet, **Cynomolgus**, Langur, Mangabey, Patas, **Rhesus**, Pig-tailed, Stump-tailed, Talapoin, Vervet
New World monkeys	**Capuchin**, Marmoset, Owl, **Spider**, **Squirrel**, Wooly
Non-primates	
Rodents	**Guinea pig**, **Hamster**, **Mouse**
Carnivores	Mink, Ferret
Ungulates	Horse
Felines	**Domesticated cat**
Avians	Chicken, Duck, Turkey
Suidae	Domesticated pig
Caprinae	Sheep, goat

The most frequently used species are shown in bold type

up an elaborate logistical system to preserve the viability of any infectious agent that might be present, including canisters of liquid nitrogen at the autopsy site, Land Rovers and Piper Cubs on call, and way-station reservoirs of additional liquid nitrogen at each airport between the middle of New Guinea and Washington DC. As it turned out, the brain from this case was among the first three to transmit kuru to chimpanzees (the two others having been collected by Gajdusek himself). Little did we then know that the transmissible agent could have withstood boiling, standard sterilizing chemicals, and burial in the ground for 3 years and still have remained infectious!

The publication in 1966 (Gajdusek et al. 1966) of the first experimental transmission of kuru from three of seven patients, whose brain tissue homogenates had been inoculated intracerebrally into chimpanzees 18–21 months earlier, was followed by an explosive decade of activity in Gajdusek's NIH laboratory, and as Pattison had said, the earlier studies of scrapie provided a valuable road map for this new exploration of kuru. The first order of business was to validate transmissibility of the disease and, if successful, begin to characterize the properties of what appeared to be a "slow" or "unconventional" virus. Chimpanzee to chimpanzee passage of kuru was accomplished in 1967 (Gajdusek et al. 1967), and a large series of experiments in a variety of primate species was carried out to determine the physical/chemical resistance, filtration size, host range, and pathogenesis of this new "virus" (Table 1.1).

1.9 The Expanding Horizon of Transmissible Spongiform Encephalopathy

The other pressing need, in view of Klatzo's observation of the neuropathological similarities between kuru and CJD, was to find a case of CJD to inoculate, which was not an easy task considering the rarity of the disease and its confusion with

other dementia syndromes. However, a fully typical neuropathologically verified case was soon provided by Peter Daniel and Elizabeth Beck at the Maudsley Hospital in London, England, which transmitted disease to a chimpanzee 13 months after intracerebral inoculation, in 1968 (Gibbs et al. 1968). Ironically, that same year Kirschbaum published a comprehensive review of all known cases of CJD, favoring an etiology of vascular origin (Kirschbaum 1968).

Although interest shifted dramatically from scrapie to CJD in the years following its experimental transmission, two animal diseases, transmissible mink encephalopathy (TME) and chronic wasting disease (CWD) of deer and elk, were recognized as belonging to the TSE family by Dieter Burger and Hartsough (1965) and by Elizabeth Williams and Stuart Young (1980), respectively (Burger and Hartsough 1965; Williams and Young 1980; Williams et al. 1982). Both diseases may have originated from exposure to scrapie-infected sheep that had been present in the USA since the late 1940s, but that epidemiologically plausible hypothesis will never be proven. In fact, one of the more interesting features of TME is its association with the consumption of cattle rather than sheep carcasses on two US mink ranches in 1963 and 1985, leading to speculation about an early undetected occurrence of BSE in the USA (Marsh et al. 1991). No further incidents have occurred in the USA since the second outbreak (TME has also been diagnosed in Canada, Finland, and Russia as late as 1986). In contrast, CWD has assumed more and more importance as it spreads from its origin in Colorado mule deer to other species of deer in regions of the USA that now include the Midwest and both US coastlines. It poses an obvious risk to the comparatively small number of humans who hunt and/or consume venison and other vital organs, and a potentially greater future threat via cross-contamination of wild predators (the cat family is highly susceptible), and eventually to captive animals and livestock. The unique attribute of CWD that makes it important is its presence in free-ranging animals that cannot be subjected to the kinds of preventive or destructive measures applied to animals in captivity.

The most recent addition to the TSE family—BSE—appeared on the scene in 1986 in the UK as a new disease of cattle, and spread through most European and a few non-European countries within the next few years. Strictly speaking, it qualifies for discussion in this historical account, but as its occurrence extends well beyond the era when Gajdusek was actively engaged in the field, and it is sufficiently important to deserve a detailed discussion in a chapter of its own, we will instead return to the human diseases with which Gajdusek was most involved.

As news of the transmissibility of CJD spread through the neurological community, the NIH laboratory became a global clearinghouse of case referrals including hundreds of cases of possible or suspected CJD, all of which were inoculated into primates. The early use of chimpanzees rapidly gave way to a variety of monkeys (Table 1.1), and as features of the disease came to be defined in each species, the squirrel monkey became the preferred assay animal because of a susceptibility greater than 90% (nearly equal to the chimpanzee) combined with a comparatively short mean incubation period of 24 months (Table 1.2; Fig. 1.2). However, the observation that the same inoculum could sometimes produce disease after widely spaced incubation periods in replicate monkeys signaled caution in accepting incubation period length as a measure of infective dose in

Table 1.2 Characteristics of CJD transmissions in the most frequently used primate species

		New World monkeys			Old World monkeys	
	Chimpanzee	Squirrel	Spider	Capuchin	Rhesus	Cynomolgus
No. animals inoculated	29	211	31	45	28	23
Transmission rate (%)	97	93	97	80	68	22
Mean incubation period (months)	17	25	32	40	64	61
Mean duration of illness (months)	1.7	1.3	1.6	2.4	3.2	2.1

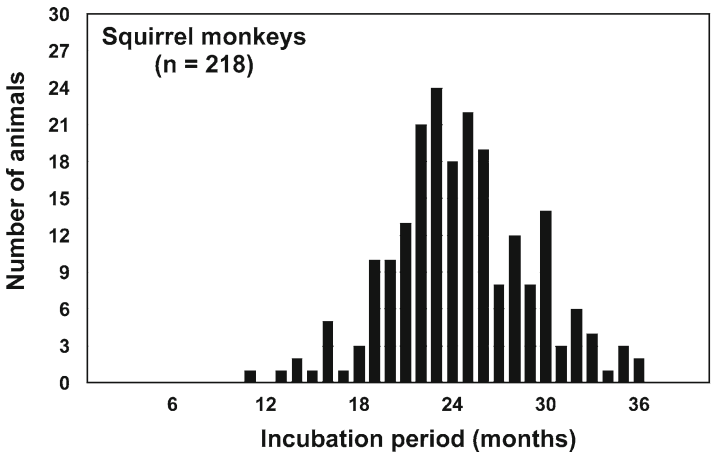

Fig. 1.2 Incubation periods in 218 squirrel monkeys inoculated intracerebrally with human CJD brain homogenates

any experiment using only two or three animals, a point that is sometimes forgotten in current research studies (Fig. 1.3).

The search for additional cases suspected of having CJD or diagnoses of other neurodegenerative diseases, and the laborious task of characterizing the transmissible agent, including its host range and pathogenesis, consumed a much larger number of animals and a much longer period of time, lasting well into the 1980s. Consider the simple matter of estimating the mean lethal dose (LD_{50}) of infectivity in a given tissue. Working with mice or other rodents, the usual technique would be to inoculate groups of 5–6 animals with a spread of dilutions large enough to bracket an unknown end point, typically totaling 40–50 animals, which would be unthinkable when using primates. Even a "stripped down" titration using pairs of animals at successive 100-fold dilutions would require at least eight animals. Add to this the need for observation periods of at least 5 years, and the difficulty of obtaining even the most basic information becomes formidable.

Over the years, the NIH laboratory bought, bred, and housed thousands of monkeys and hundreds of apes used in primary isolation and passage attempts, species

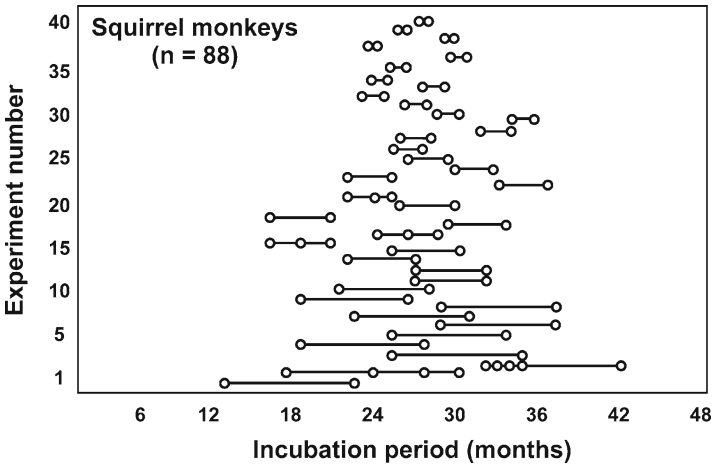

Fig. 1.3 Incubation periods in 40 experiments in which replicate (or in a few cases, more than two) squirrel monkeys were inoculated intracerebrally with the same human CJD brain homogenate

susceptibility experiments, and pathogenesis bioassays, located at various sites in California, Hawaii, Louisiana, New Mexico, New York, Texas, and Virginia, as well as overseas in Paris and Marseille. Eventually, all primate research was consolidated to Gulf South in the middle of Louisiana Cajun country, and Fort Detrick, about 30 miles north of the NIH in Frederick, MD. Transmission experiments on non-primate species were mostly conducted at a spacious farm-like facility in Otisville in southern New York State. It is to the everlasting credit of Dr. Joseph Smadel, NIH Associate Director who had earlier been Gajdusek's chief at the Walter Reed Army Institute of Research, and Dr. Richard Masland, Director of the NIH Institute of Neurological Diseases and Blindness, to have at its inception approved and assisted in this gigantic undertaking.

1.10 Clinical and Epidemiological Precisions

During the 1970s, the unassailable criterion of transmissibility led to an appreciation of the range of clinical syndromes associated with CJD, and made it possible, finally, to define the essential features with a precision that had hitherto been impossible. This evolving understanding was recorded in several papers based on larger and larger numbers of cases culminating in a synthesis based on 300 transmitted cases of transmissible spongiform encephalopathy published in 1994 (Brown et al. 1994a). During this period, the two remaining members of the quartet of human spongiform encephalopathies were also found to be transmissible: GSS in 1981 (Masters et al. 1981) and fatal familial insomnia (FFI) in 1995 (Tateishi et al. 1995). However, the need for diagnostic verification of cases by transmission studies was, in most

Table 1.3 Disease categories of referrals to the NIH laboratory for transmission studies

Disease category	Number of cases	Number of animals	Observation period (years)	Number of transmissions
TSE	440	1,914	1–21	291
Alzheimer's disease	105	240	1–24	0
Other neurodegenerations	115	224	1–30	0
Other neurological diseases	453	1040	1–26	0
Non-neurological Diseases	53	76	1–30	0
Total	1,113	3,418	–	–

instances, abolished by the twin discoveries of a high level of protein kinase inhibitor (14–3–3) in the spinal fluid with a diagnostic specificity >90%, and of a specific pathognomonic amyloid protein (PrP^{TSE}) in brain tissue that could be detected by ELISA or Western blot.

In stark contrast to the multiple transmissions of each of the spongiform encephalopathies, not a single transmission followed similar inoculations of any non-spongiform neurological disease (including Alzheimer disease, Pick's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis) or a wide variety of non-neurological diseases of unknown etiology like sarcoidosis, lupus erythematosus, Crohn's disease, and rheumatoid arthritis (Table 1.3). It is sometimes forgotten in the present-day impulse to demonstrate transmissibility of Alzheimer's disease using various "seeding" techniques and genetically altered susceptible mice that over 100 cases of neuropathologically verified Alzheimer's disease have been inoculated into primates with uniformly negative results (Brown et al. 1994a). Thus, whatever the similarities between the two diseases (and there are many), inoculation of host species closely related to humans under conditions typically used to demonstrate infectivity simply does not transmit disease, and any claim that Alzheimer's disease is infectious must contend with these consistently negative results. Stated another way, facilitating or accelerating disease in animal models of Alzheimer's disease should not be confused with causing disease in humans.

Given the experimental transmissibility of sporadic CJD, and the increasing repertory of cases referred to the NIH, it was not long before the question of human contagion arose, which led to a burgeoning series of epidemiological studies beginning in 1971 with Giovanni Alemà's search for cases of CJD in Italy (Alemà 1971). This was really only a "sketch" that served to inaugurate the much larger canvases to come, but Alemà deserves credit for first recognizing the need to look at epidemiology, a fact that is almost never cited. Brian Matthews and Robert Will substantially extended the epidemiological exploration of CJD in a systematic 5-year retrospective study in England and Wales (Will and Matthews 1986), and Françoise Cathala and the author followed with an even more intensive 10-year investigation of CJD in France (Brown et al. 1987). With the appearance of variant CJD (vCJD) in 1996, the entire European community, together with individual countries elsewhere in the world (e.g., Argentina, Australia, Canada, and Japan), established a coordinated