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# Prions, BSE and food

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**Abstract**

Biochemical and biophysical properties of prions including possible inactivation methods are reviewed. Possible molecular markers of transmissible spongiform encephalopathy (TSE) and mechanisms behind infectivity and correlation with clinical symptoms are discussed. The risk of Bovine Spongiform Encephalopathy (BSE) for humans i.e. variant Creutzfeldt–Jakob Disease (cCJD) is addressed in detail. The consequences of the emergence of the new cCJD and the lack of information on the infectivity of cCJD at the clinical stage of the disease in relation to the need to reconsider the biological concepts currently used in microbiology.


*Keywords:* Prions; TSE; BSE; Molecular markers; Risk assessment; Variant of Creutzfeldt–Jakob Disease (cCJD)

The transmissible spongiform encephalopathy (TSE) agent (TSE agent), also named prion, is responsible for fatal neurodegenerative diseases in humans and animals. Human transmissible spongiform encephalopathies include the Creutzfeldt–Jakob Disease, fatal familial insomnia, kuru and Gerstmann–Sträussler–Scheinker syndrome. In animals, TSE includes natural scrapie in sheep and goats, bovine spongiform encephalopathy, feline spongiform encephalopathy, transmissible mink encephalopathy, and chronic wasting disease. The analysis of purified infectious fractions removed from infected brains shows that they are mostly composed of one host-encoded protein, the prion protein (PrP), and are free of any detectable specific nucleic acid (Prusiner, 1982). In vivo, the infectivity titer is always associated with a proportional accumulation of PrP in central nervous system (CNS) and, in some species, in lymphoid tissues. Amino acid sequences of PrP obtained from normal and infected brains are identical, and these two proteins differ only by their biochemical and biophysical behavior: in particular, PrP from TSE individuals resists proteinase K digestion. PrP-c is the PrP found in normal individuals; PrP-sc is the proteinase K resistant PrP identifiable in infected individuals. Moreover, PrP-sc accumulation is never associated with an increase of PrP messenger RNAs: this accumulation is post-translational (Bolton and Bendheim, 1988). Lastly, infected organisms accumulate their own PrP-sc and not the PrP-sc used for infection. These data point out that infectivity is strongly associated with de novo PrP-sc synthesis. Today, it is not known if PrP-res (The resistant C-terminal fragment of PrP-sc) accumulation is a neuropathological event related to spongiosis and neuronal death, or if it is the agent of the disease. This may be a new type of disease, induced by a post-translational modified host-encoded protein. Scrapie-associated fibrils (SAF) are

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identifiable in brain homogenates from infected individuals (Merz et al., 1981). They are composed of PrP-res; antibodies raised against PrP recognize SAF, and PrP-res precipitates in rods resembling SAF in particular physicochemical conditions (Prusiner, 1983).

## 1. Biochemical and biophysical properties of prions

### *1.1. Inactivation of prions*

Prions resist almost all the procedures generally used to inactivate conventional viruses. Due to their biophysical properties, prions behave differently depending on the biophysical and biochemical characteristics of their environment. Moreover, the sensitivities to physical and biochemical disinfectants are different from one strain to another.

For instance, the following procedures or compounds do not totally inactivate the scrapie agent: (1) dry temperatures exceeding 160 °C for 24 h (Dickinson and Taylor, 1978) and at 360 °C for 1 h; (2) autoclaving at 121 °C for 1 h reduces infectivity titer by 7.5 logs (Brown et al., 1983); (3) sodium hypochlorite 0.5% during 1 h (4 logs of infectivity reduction) (Brown et al., 1983); (4) hydrogen peroxide 3% for 1 h (0.8 log) (Brown et al., 1983); (5) ethanol treatment (no partial inactivation).

Moreover, radioresistance of TSE agents is very unusual: ultra-violet 37% inactivation dose is 42,000 Jm-2 (Latarjet et al., 1970) and a dose of 25,000 Gray (2.5 Mrad) of X-rays only partially inactivates TSE agents (Latarjet, 1979).

Total decontamination may be obtained (1) by autoclaving at 132 °C for 1.5 h; (2) by treatment with sodium hydroxide (1 M) for 1 h at 20 °C (Ernst and Race, 1993); (3) by treatment with sodium hypochloride (2% chloride) for 1 h at 20 °C. One should note that formaldehyde treatment before autoclaving reduces the efficacy of thermic inactivation (Taylor and McConnell, 1988).

The size of the prions is estimated to be between 15 and 40 nm (Latarjet, 1979; Latarjet et al., 1970), though this size might be compatible with a conventional agent. An important biophysical property of this class of agents is their hydrophobicity (Prusiner, 1983). These agents may easily aggregate.

### *1.2. Biochemical components of the infectious fractions in TSE*

The inactivation processes which have been demonstrated to be efficient against scrapie agents are those denaturing or hydrolysing protein components: treatment with high doses of proteinase K or trypsin (Prusiner, 1983), SDS, diethylpyrocarbonate, guanidium thiocyanate; and urea alters infectivity (Brown et al., 1986; Dickinson et al., 1984; Kimberlin and Walker, 1978; Prusiner et al., 1980, 1981) .Procedures that interact with nucleic acids do not modify infectivity titers: nucleases, UV treatment, $Zn^{2+}$ hydrolysis, and psoralens (McKinley et al., 1983; Prusiner, 1982; Prusiner et al., 1980).

Therefore, one may hypothesize that prions are either constituted of proteins or harbor a small nucleic acid which is protected into a ‘‘shell’’. Today, the ‘‘protein only’’ hypothesis is supported by the majority of experimental data, although none of them is 100% conclusive.

## 2. Molecular markers of TSE

### *2.1. Prion protein is a normal host protein*

The partial sequence of PrP was identified in 1985 (Oesch et al., 1985). There is no specific DNA or RNA detectable in the infectious fractions; DNA and RNA specific for PrP are present in the infected brains; RNA and DNA are present in similar amounts in non-infected and infected brains. These results were crucial in demonstrating that PrP was a normal component of the host, accumulating according to a post-translational mechanism in the brains of infected individuals (Hope and Baybutt, 1991; Prusiner et al., 1987). The amount of PrP is 50 times greater in the brain than in other organs (Hope and Baybutt, 1991; Oesch et al., 1985, 1991; Prusiner et al., 1987). PrP amino acid and gene sequences have been identified: in man, PrP is a 253-amino acid protein. This protein is associated with cell membranes, through a C-terminal hydrophobic GPI anchor. Its half-life is 5 h for the normal PrP, and greater than 25 h for the PrP associated with TSE. The PrP gene is located on chromosome 20 in humans (one copy per genome); it contains two exons, the second one containing the

whole coding sequence. PrP-mRNA is 2.1 kb long, and is detected in almost all the organs including brain, lung, spleen and heart (lowest expression in liver and testicle) (Oesch et al., 1991). In the brain, PrP-mRNA is localized mostly in neurons, and is also detectable in astrocytes.

### 2.2. PrP-res is a pathological molecular isoform

Differences between the PrP isolated from normal individuals (PrP-c) and PrP isolated from infected individuals have been investigated. There are no differences in the sequence in amino acids: the primary structures are identical. On the other hand, the sensitivity to proteolytic enzymes is different, since normal PrP is totally degraded by proteinase K concentrations that do not alter the 27–30-kDa C-terminal fragment (PrP-res) of the pathologic isoform, PrP-sc (Prusiner et al., 1993). The prion hypothesis postulates that differences between PrP-c and PrP-sc are at the level of their tridimensional structures. The presence of the PrP-res is specific for TSE. Its detection is the basis for the molecular diagnosis of this disease. The kinetics of PrP-res accumulation has been studied in a number of experimental animal models. Results have confirmed that it is proportional to the increase in infectivity. In rodent TSE models, the presence of prion is detectable in the spleens of infected animals early after their peripheral inoculation (Kimberlin and Walker, 1979). In the CNS, the infectivity develops almost exponentially until the appearance of clinical signs and death. These facts illustrate two main characteristics of TSE: (1) certain organs of the infected individual, and particularly the brains, are highly infectious long before clinical signs appear; (2) these diseases develop without interruption and without any disappearance of the infectious agent during clinical latency.

The tridimensional structure of the PrP-c has now been determined in mouse and in hamster (Riek et al., 1996, 1997); the PrP molecule is composed of a globular core (composed of three alpha helices and two small anti-parallel β sheets) attached to the cell membrane by a GPI anchor, and a long flexible tail (amino acids 1–121) that can adopt several conformations depending on the physicochemical conditions. Recently, crystals of PrP-c have been obtained and analysed; they suggest that PrP-c could exist under dimeric forms (swapping of the C-terminal helix) (Knaus et al., 2001).

### 2.3. Biological function of the PrP

PrP seems to be a neuronal protein, but at present its precise function remains unknown. Transgenic mice have been grown with the hamster PrP gene: these hamster PrP transgenic mice react as hamsters when infected with hamster (Prusiner, 1993; Prusiner et al., 1990). Weissman et al. reported that transgenic mice that lack a PrP gene may grow normally, without any defect in CNS functions; moreover, these animals are not susceptible to scrapie infection, demonstrating the key role of PrP in the TSE (Büeler et al., 1992, 1993). The fact that PrP is a host-coded protein led certain authors to suggest that PrP is not the infective agent, but that its accumulation was the neuropathological sign of the reaction of the CNS to the infection. Recently, PrP-derived peptides have been shown to exert a toxic function on neurons in culture. These peptides aggregate into fibrils similar to prion rods or SAF. These facts might suggest that PrP accumulation constitutes the starting event of neurodegeneration, and is therefore the critical point of neuronal death (Brandner et al., 1996). The differences between the tridimensional structures of PrP-c and PrP-sc are not known today; recently, it was hypothesized that transconformation occurs through direct interactions between PrP-res and PrP-c and that part of the alpha helices is transformed into beta sheet structures during this conversion process (Cohen et al., 1994). Cellular factors like chaperone molecules can participate in PrP-c transconformation (Telling et al., 1995).

Several authors have reported variations in PrP accumulation location in the brain related to clinical symptoms. This has been shown in both natural and experimental diseases. In children treated with extractive growth hormone who developed iatrogenic Creutzfeldt–Jakob disease (CJD), the main clinical sign is ataxia, dementia being late in the evolution or absent. In these cases, PrP is mainly located in the cerebellum (10 times more than in cerebrum) (J.P. Deslys, personal communication). In sporadic adult CJD, dementia is the main clinical symptom and PrP accumulates preferentially in the cerebrum. There is a correlation between PrP-sc accumulation and clinical symptoms; this has been demonstrated in scrapie-

infected hamsters treated with amphotericine B and derivatives (Adjou et al., 1995, 1996; Demaimay et al., 1994, 1997; Xi et al., 1992). Treated animals had a delayed accumulation of PrP-Sc and a longer incubation time. These facts underlie the close relationships between PrP accumulation and the clinical presentation of the patients.

## 3. Risk of BSE for humans: the variant of Creutzfeldt–Jakob disease

TSE agents are capable of crossing the species barrier. This has been demonstrated in experimental research and is the basis of the rodent models that are currently used in biology. This has also been shown in "natural" situations: transmissible mink encephalopathy (TME) is due to the consumption of infected carcasses of ovine origin by minks and FSE is now recognised as a contamination of cats with the BSE agent. Moreover, as suggested above, BSE agent is easily transmissible by oral route to other species. This raised the hypothesis of a possible infection of humans through the consumption of bovine tissues. This risk has been evoked earlier by the scientific community after the onset of the BSE epidemic.

Human TSEs are rare diseases, CJD being the most common TSE. CJD can be sporadic (90% of the cases), familial (5–10% of the cases) or iatrogenic. Its incidence does not significantly vary among countries where a good epidemiological surveillance is performed (Alperovitch et al., 1994): incidences range between 0.6 and 1.5 per million of inhabitants per year. Several clusters of CJD have been described in the past, especially in Slovakia and in Israel; progress in molecular genetics have demonstrated that these clusters were in fact inherited forms of CJD. It is to be noted that all forms of human TSE are transmissible, including the genetic diseases. The mean age at the onset of the clinical disease is around 65 for the sporadic forms, and close to 50–55 for the familial forms. Then, except for the very rare CJD that has been described under the age of 30 (Brown et al., 1984), the appearance of CJD in young individuals indicates the possibility of iatrogenic contamination (Brown et al., 1992).

In March 1996, British epidemiologists and neurologists reported the appearance of 10 cases of a new form of CJD, the variant of Creutzfeldt–Jakob disease (vCJD) (Will et al., 1996). A further case was soon described in France (Chazot et al., 1996). All these patients were under the age of 40, 10 of them being under 30. The clinical presentation of these patients was different from classical CJD. Ataxia and psychiatric symptoms were detected early in the course of the disease, and EEG often did not show the typical CJD abnormalities, e.g. pseudoperiodic abnormalities that are seen in 60% of the sporadic CJD. Nevertheless, dementia was the main component of the clinical presentation at the state phase of the disease. The medical history of these 11 patients has been investigated: none of them had known medical and/or surgical risk factors for CJD. Open reading frame of the *PRNP* gene has been sequenced; the sequence was 100% normal, and, in particular, none of the mutations associated with familial TSE could be detected. The codon 129 of the *PRNP* gene has been determined for nine patients: all were homozygous at codon 129 (methionine/methionine). One of the most intriguing features was the neuropathology of these patients. They all exhibited amyloid plaques that were surrounded by vacuoles, the florid plaques; the centre of these plaques was dense and eosinophilic, and the periphery was pale, as described sometimes in kuru patients. Florid plaques have never been observed in humans, neither in TSE nor in young patients who died from undiagnosed dementia. The distribution of the lesions was similar for all patients; the cerebrum and cerebellum were the most common locations, although the lesions were also present in basal ganglia, thalamus and hypothalamus. Spongiosis, astrogliosis and neuronal loss were detectable mainly in basal ganglia and thalamus, and at a lower frequency in the cerebrum and cerebellum. Immunocytochemistry for PrP-res revealed a strong labelling of the florid plaques, a PrP deposition with pericellular distribution in the molecular layer of the cerebellum and in the cortex, and shows the presence of many other smaller plaques distributed in the same areas. It is important to note that PrP deposition was seen in the absence of confluent spongiform changes in the surrounding neuropils (Chazot et al., 1996; Will et al., 1996). In May 2002, a total of 122 cases (114 in UK, 1 in USA, 1 in the Republic of Ireland, 1 in Italy and 6 in France) have been diagnosed; all are homozygous Met/Met at codon 129 of the PrP gene.

Altogether, these clinical and pathological observations indicate (1) the emergence of a new form of CJD in UK and France, (2) and the possibility of a common origin of the contamination by a unique strain of agent, as suggested by the common neuropathological picture (Bruce et al., 1991). The possible link with BSE can be suggested as the major hypothesis because: (1) BSE is endemic in the UK, the country which has 122 cases of the 131 vCJD, and (2) BSE is caused by a single-strain TSE agent, as demonstrated by the strain-typing results (Bruce et al., 1993, 1994).

Several experimental data support the possible link between BSE and vCJD.

(1) The inoculation of macaques with the BSE agent results in a disease that has common characteristics with vCJD (Lasmézas et al., 1996). Intracerebral inoculation of cynomolgus monkeys either at the adult stage or perinatally induces a disease that is characterised, after 3 years of incubation, by a severe cerebellar ataxia, behaviour changes, and wasting syndrome. EEG did not exhibit any pseudoperiodic abnormality that is characteristic of sporadic CJD; it is interesting to note that only 40% of the vCJD exhibited typical EEG alterations. Neuropathology was strictly identical to that of vCJD patients: florid plaques were seen in the brain, mainly in the cerebellum. Immunohistochemistry revealed the same pattern as that observed in vCJD: plaques were labelled with anti-PrP antibodies, and deposition of PrP was seen in the basal ganglia. These patterns were identical in all the animals, the severity of the lesions being more important in the perinatally infected animal. Experimental infection of the same species with several scrapie strains has been made in the past in our lab, and the incubation times obtained were over 4 years (L. Court, personal communication): in TSEs, incubation time results from the combination of infectivity dose in the inoculum and species barrier strength between donor and recipient. One should also note that opposite results have been obtained after the inoculation of marmoset with BSE or scrapie agents (Baker et al., 1993).

(2) In the current state of the knowledge, a unique strain of TSE agent has been identified in British affected-cattle. Several authors have reported in the past that PrP-res electrophoretic pattern can be associated with strain characteristics (Mertz et al., 1989). Recently, Collinge et al. (1996) have shown that sporadic and iatrogenic PrP-res patterns in humans differ from that of vCJD: unglycosalyted PrP-res has a lower apparent molecular weight in vCJD and the quantification of the glycoforms of PrP-res shows a majority of highly glycosylated protein in vCJD that is different from the low glycosylation observed in sporadic and iatrogenic CJD. This PrP profile is referred to as type 4. This type 4 PrP has also been identified in the French patient with vCJD (Deslys et al., 1997). Moreover, type 4 PrP pattern is also seen in cats infected with feline spongiform encephalopathy agent, and in macaques infected with the BSE agent. Although these results have yet to be confirmed by others, and their relevance as a general mode of strain-typing the natural diseases in sheep and humans still to be assessed, these data are a strong argument for the infection of the vCJD patients by the BSE agent.

(3) Comparative strain-typing studies of the BSE agent and vCJD have been performed in the UK (Bruce et al., 1997). Several syngenic mice have been inoculated with sporadic CJD, CJD that was diagnosed in farmers who had BSE-affected cattle in their herds, BSE and vCJD. Incubation time, infection efficiency and neuropathological data indicate that BSE agent and vCJD agent share the same biological properties that are significantly different from those of sporadic CJD and CJD diagnosed in farmers. These data are the most accurate data that can be obtained today to support the transmission of BSE agent to humans.

There is a very strong probability that infection of humans with the BSE agent occurred, but the link between BSE and vCJD is not yet 100% proven on a "basic science" point of view; in fact, only the precise identification of the nature of the TSE agent will permit to compare BSE prion and vCJD prion. Many years will be needed to demonstrate the identity of BSE agent and vCJD agent(s), but, in terms of public health, one must consider that BSE is transmissible to humans.

The consequences of the occurrence of BSE (Wells et al., 1987) in cattle and its transmissibility to humans are very important, mainly due to the fact that there is no therapy applicable to TSEs in humans. Therefore, one should try to minimise the exposure of the human population to the BSE agent through food practices and the usage of biological products (drugs and

medical devices) of bovine origin. This could be achieved by (1) preventing the dissemination of BSE agent in the cattle population, (2) detecting and preventing any propagation of the BSE agent in other species entering the food chain, and (3) identifying infected cattle prior to their entry in the human food chain. This requires (1) a very efficient BSE surveillance in cattle, (2) a very precise knowledge of BSE pathogenesis in order to trace the infectivity in all the different phases of the disease, and (3) the development of biological tools that could help in the diagnosis of TSE in cattle, humans and in species that may have been exposed to BSE agents (sheep and goats, for example). Prevention of the dissemination of the BSE agent in the cattle population requires a strict and controlled ban of any ruminant product of cattle feed; this has been achieved by the successive feed ban and meat-and-bone meal ban that have been put in place in the European Union and in other countries. Prevention of human exposure requires the detection of infected animals at slaughterhouses and precautionary measures such as specified risk material (SRM) ban.

- In the current stage of the knowledge, there is no biological test that permits BSE diagnosis in live animals, though incubation has a mean duration of 5 years. This is due to the lack of possible detection of the specific marker of TSEs (PrP-res) in blood or urine of infected cattle (see below), and to the lack of any surrogate marker of BSE in this species. The only way to detect BSE is, apart from clinical examination of affected animals, to evidence the neuropathological characteristics of BSE (spongiosis, neuronal death, gliosis) or to detect abnormal PrP in the CNS using either immunohistochemistry or Western blot or ELISA. In cattle brain, as in all TSEs, there is a heterogeneity in the PrP-sc accumulation inside the CNS: PrP-res is mainly detectable in the obex (Wells et al., 1994); then, any attempt to detect abnormal PrP in the cattle CNS should be performed on this particular part of the CNS. Today, rapid tests (rapid Western blot, ELISA) can be used in slaughterhouses as they have been validated through a European Union-led study (Moynagh and Schimmel, 1999). Although the specificity of these three tests is 100%, their sensitivity varies, at least in laboratory conditions; the best of these tests can detect a PrP-res amount that corresponds to 1000 cattle infectious units (Deslys et al., 2001a,b; Moynagh and Schimmel, 1999). Other tests are under investigation at the EU level, and better sensitivity is expected in the future. Nevertheless, one should note that a given test could be positive only if (1) the amount of PrP-res in the CNS is above the detection threshold, and (2) the agent has reached the CNS (see below).
- Because of this lack of efficient test to detect infected live animals, or to detect infected animals in which neuroivasion did not occur, one needs to know where the infectious agent is at each time point of the infection. This requires experimental infections and reinoculation of all biological fluids and organs of many animals sacrificed at different times after their oral exposure to the BSE agent. British scientists have performed these experiments and they have shown that infectivity could be evidenced in distal ileum as soon as 6 months post oral exposure (Wells et al., 1996, 1998); infectivity remains detectable at low levels until the appearance of clinical symptoms. Two to 8 months before the full-blown BSE, infectivity can be evidenced in the dorsal root ganglia, and then in the spinal cord and thereafter in the brain. These data support the classification of CNS and distal ileum as SRM and their exclusion from any food chain. In this experimental model, infectivity has been reported in a small number of mice inoculated with bone marrow of infected cattle (Wells et al., 1999): this is now considered as an artefact (G. Wells, personal communication), and bone marrow has never been found infectious in naturally affected cattle. Same experiments have been performed at the clinical stage of the disease for naturally affected cattle: in these cases, infectivity is only found in CNS and the retina (Bradley and Wilesmith, 1993). In particular, no infectivity has been evidenced in lymphoid tissues, in blood and in milk, although some of these experiments have been conducted using a mouse bioassay, which permits to detect only 1000 infectious units per gram due to the mouse-to-cattle species barrier. Recently, infectivity was found in skeletal muscles of mice experimentally infected with two strains of scrapie (Bosque et al., 2002): in this experiment, infectivity is restricted to the hindlimbs of some infected animals, but the infectivity levels reported are unusually high (1/700 of brain-associated infectious load). Attempts to confirm these results in other mouse scrapie models, in BSE-infected mice and in a limited number of clinically affected cattle have been

unsuccessful (T. Baron, J.P. Deslys, 2002, personal communication).

Although the total ban on animal proteins in the ruminant feeding practices in the European Union and the systematic PrP-res testing in slaughterhouses for animals over 24 or 30 months gives support to an efficient control of BSE in the cattle population, several questions remain unsolved: (1) Has BSE spread in the sheep and goat population? Today, there is no BSE diagnosed in sheep although one should keep in mind that BSE diagnosis in sheep will be extremely difficult. If this would occur, it will create an important problem due to the broad distribution of infectivity that occurs in this species when animals are infected with TSE agents (Andreoletti et al., 2000). (2) Is infectivity restricted to distal ileum and CNS in infected cattle? Although there are strong experimental data that support this restricted infectivity distribution, there are still experiments on progress whose results will be critical for public health (cattle-to-cattle inoculation of skeletal muscles, for example). (3) Is a diagnostic test for BSE in live animals possible?

## 4. Conclusion

There are many consequences of the emergence of the new variant of Creutzfeldt–Jakob disease. First, if the link with BSE is proven, it will show that certain strains of animal prions are capable of contaminating humans and inducing clinical spongiform encephalopathies. This will require the maintenance of the specified bovine offal (SBO) ban from human consumption and a careful surveillance of BSE agent prevalent all over the world. Second, whatever the origin of the vCJD is, its emergence raises the question of a specific risk associated with tissue grafting, organ transplantation and blood transfusion. The distribution of infectivity in the organism of infected individual is then of crucial importance; because no test is available for TSE diagnosis, and because tissue distribution of infectivity depends upon the strain and the host, it may be impossible to assess the risk during the asymptomatic phase for a long time. Little is known about the infectivity of vCJD at the clinical stage of the disease; it has been recently reported that PrP-res and infectivity could be identifiable in tonsil (Bruce et al., 2001; Hill et al., 1997; Wadsworth et al., 2001); this differs from all cases of sporadic and familial CJD that were explored, in which infectivity is restricted to CNS. If this is confirmed, the presence of vCJD agent in the lymphoreticular tissues will raise the question of the risk associated with blood transfusion and tissue/organ transplantation. This will require special and careful investigations. Third, the precise determination of the inactivation spectrum of the vCJD agent will be required in order to prevent its dissemination through the daily medical and/or surgical practice. From a biological point of view, prions constitute a very exciting enigma; the only specific event known about transmissible subacute spongiform encephalopathies is the accumulation, proportional to the infectivity titre, of a protein (PrP) of the host in a pathological isoform, the PrP-res. These uncertainties account for the numerous hypotheses put forth with respect to the nature of causal agents. If the ‘‘protein only’’ hypothesis turns out to be true, all the biological concepts that are currently developed in microbiology will have to be reconsidered.

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