

## Prions

Prions are proteinaceous infectious particles that lack nucleic acids.<sup>(1)</sup> Prions are composed largely, if not entirely, of an abnormal isoform of a normal cellular protein. In mammals, prions are composed of an abnormal, pathogenic isoform of the prion protein (PrP), designated PrP<sup>Sc</sup>. The "Sc" superscript was initially derived from the term scrapie because scrapie is the prototypic prion disease. Since all of the known prion diseases (Table 6) of mammals involve aberrant metabolism of PrP similar to that observed in scrapie, use of the "Sc" superscript is suggested for all abnormal, pathogenic PrP isoforms.<sup>(2)</sup> In this context, the "Sc" superscript is used to designate the scrapie-like isoform of PrP.

A chromosomal gene encodes PrP and no PrP genes are found in purified preparations of prions. PrP<sup>Sc</sup> is derived from PrP<sup>C</sup> (the cellular isoform of PrP) by a posttranslational process whereby PrP<sup>Sc</sup> acquires a high  $\beta$ -sheet content.<sup>(3)</sup> Neither prion-specific nucleic acids nor virus-like particles have been detected in purified, infectious preparations. In fungi, evidence for three different prions has been accumulated.<sup>(4)</sup> The mammalian prions cause scrapie and other related neurodegenerative diseases of humans and animals (Table 6). The prion diseases are also referred to as the transmissible spongiform encephalopathies (TSEs).<sup>(5)</sup>

**Table 1. The Prion Diseases**

Disease abbreviation)	Natural Host	Prion	Pathogenic PrP Isoform
Scrapie	sheep and goats	scrapie prion	OvPrP <sup>Sc</sup>
Transmissible mink encephalopathy (TME)	mink	TME prion	MkPrP <sup>Sc</sup>
Chronic wasting disease (CWD)	mule deer and ell	CWD prion	MdePrP <sup>Sc</sup>
Bovine spongiform encephalopathy (BSE)	Cattle	BSE prion	BoPrP <sup>Sc</sup>
Feline spongiform encephalopathy (FSE)	Cats	FSE prion	FePrP <sup>Sc</sup>
Exotic ungulate encephalopathy (EUE)	nyala and greater kudu	EUE prion	UngPrP <sup>Sc</sup>
Kuru	humans	kuru prion	HuPrP <sup>Sc</sup>
Creutzfeldt-Jakob disease (CJD)	humans	CJD prion	HuPrP <sup>Sc</sup>
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans	GSS prion	HuPrP <sup>Sc</sup>
Fatal familial insomnia (FFI)	humans	FFI prion	HuPrP <sup>Sc</sup>

*Species-specificity of prions.* Unlike many viruses, the properties of prions change dramatically when they are passaged from one species to another. The results of transgenic (Tg) mouse studies indicate that when human prions are passaged into mice, their potential non-Tg pathogenicity for humans probably declines drastically.<sup>(6)</sup> The prions that are propagated in the non-Tg mice are now mouse prions, not human prions. The mouse prions contain mouse PrP<sup>Sc</sup>, not human PrP<sup>Sc</sup>. This species-specific change in the PrP<sup>Sc</sup> molecule is accompanied by an alteration in the pathogenicity of the prion. In contrast to the human prions, mouse prions are highly pathogenic for mice. Our understanding of these species-specific changes in prion pathogenicity is derived largely from studies of mice expressing a variety of PrP transgenes. Because the PrP<sup>Sc</sup> produced in the mouse is from mouse PrP<sup>C</sup>, it is not possible to determine the origin of the prion initially inoculated into the mouse.<sup>(7)</sup>

It is noteworthy that the susceptibility of a particular species to prions from another species can be profoundly affected by different prion strains.<sup>(8)</sup> The properties manifested by prion strains such as incubation times and neuropathology profiles seem to be enciphered in the conformation of PrP<sup>Sc</sup>. Such considerations of the basic principles of prion biology help to form the basis for the biosafety classification of different prions.

*Biosafety level classification.* Human prions and those propagated in apes and monkeys are manipulated at Biosafety Level 2 or 3, depending on the studies being conducted. BSE prions are likewise manipulated at Biosafety Level 2 or 3, due to the possibility that BSE prions have been transmitted to humans in Great Britain and France.<sup>(9)</sup>

All other animal prions are considered Biosafety Level 2 pathogens. Thus, based on our current understanding of prion biology described above, once human prions are passaged in mice and mouse PrP<sup>Sc</sup> is produced, these prions should be considered Biosafety Level 2 prions, even though the human prions are Biosafety Level 3 under most experimental conditions. An exception to this statement is in the case of mice expressing human or chimeric human/mouse transgenes. These transgenic mice produce human prions when infected with human prions and should be treated as Biosafety Level 2 or 3 in accord with the guidelines described above. The mechanism of prion spread among sheep and goats developing natural scrapie is unknown.<sup>(10)(11)</sup> CWD, TME, BSE, FSE, and EUE are all thought to occur after the consumption of prion-infected foods.<sup>(12)(13)(14)(15)</sup>

*Human prion diseases.* In the care of patients dying of human prion disease, the precautions used for patients with AIDS or hepatitis are certainly adequate. In contrast to these viral illnesses, the human prion diseases are not communicable or contagious.<sup>(16)</sup> There is no evidence of contact or aerosol transmission of prions from one human to another. However, they are infectious under some circumstances, such as ritualistic cannibalism in New Guinea causing kuru, the administration of prion-contaminated growth hormone causing iatrogenic CJD, and the transplantation of prion-contaminated dura mater grafts.<sup>(17)(18)(19)</sup> Familial CJD, GSS, and FFI are all dominantly inherited prion diseases; five different mutations of the PrP gene have been shown to be genetically linked to the development of inherited prion disease. Prions from many cases of inherited prion disease have been transmitted to apes, monkeys, and mice carrying human PrP transgenes.<sup>(20)(21)(22)</sup>

*Surgical procedures.* Surgical procedures on patients diagnosed with prion disease should be minimized. It is thought that CJD has been spread from a CJD patient to two other patients who underwent neurosurgical procedures in the same operating theater shortly thereafter.<sup>(23)</sup> Although there is no documentation of the transmission of prions to humans through droplets of blood or cerebrospinal fluid, or by exposure to intact skin, or gastric and mucous membranes, the risk of such occurrences is a possibility. Sterilization of the instruments and decontamination of the operating room should be performed in accordance with recommendations described below.

Because it is important to establish a 'definitive' diagnosis of a human prion disease and to distinguish between sporadic and familial cases and those acquired by infection as a result of medical procedures or from prion-contaminated food products, unfixed brain tissue should be obtained. For all cases of suspected human prion disease, a minimum of one cubic centimeter of unfixed cerebral cortex should be part of any biopsy. This specimen should be bisected from the cortical surface through to the underlying white matter with one half of the specimen formalin-fixed and the other half frozen.

*Autopsies.* Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions require Biosafety Level 2 precautions.<sup>(24)</sup> At autopsy, the entire brain should be collected and cut into coronal sections about 1.5 inches (~4 cm) thick; small blocks of tissue can easily be removed from each coronal section and placed in fixative for subsequent histopathologic analyses. Each coronal section is immediately heat sealed in a heavy-duty plastic bag. The outside of this bag is assumed to be contaminated with prions and other pathogens. With fresh gloves or with the help of an assistant with uncontaminated gloves, the bag containing the specimen is placed into another plastic bag which does not have a contaminated outer surface. The samples should then be frozen on dry ice or placed directly in a -70C freezer for storage. At the very minimum, a coronal section of cerebral hemisphere containing the thalamus and one of the cerebellar hemisphere and brainstem should be taken and frozen.

The absence of any known effective treatment for prions disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that high concentrations of prions are also found in spleen, thymus, lymph nodes, and lung. The main precaution to be taken when working with prion-infected or contaminated material is to avoid puncture of the skin.<sup>(25)</sup> The prosector should wear cut-resistant gloves if possible. If accidental contamination of skin occurs, the area is swabbed with 1N sodium hydroxide for 5 minutes and then washed with copious amounts of water. Tables 2-5 provide guidelines to reduce the chance of skin punctures, aerosols, and contamination of operating room, morgue surfaces and instruments. Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care at Biosafety Level 3.

*Bovine spongiform encephalopathy.* The risk of infection for humans by BSE prions is unclear. Perhaps the most prudent approach is to study BSE prions in a Biosafety Level 2 or 3 facility depending on the samples to be studied, as noted above for human prions (i.e., brain, spinal cord).

*Experimental rodent prion diseases.* Mice and hamsters are the experimental animals of choice for all studies of prion disease. With the development of transgenic mice that are highly susceptible to human prions, the use of apes and monkeys is rarely needed. The highest titers of prions ( $\sim 10^{9.5}$  ID<sub>50</sub>/g) are found in the brain and spinal cord of laboratory rodents infected with adapted strains of prions;<sup>(26)(27)</sup> lower titers ( $\sim 10^6$  ID<sub>50</sub>/g) are present in the spleen and lymphoreticular system.<sup>(28)(29)</sup>

*Physical properties of prions.* The smallest infectious prion particle is probably a dimer of PrP<sup>Sc</sup>; this estimate is consistent with an ionizing radiation target size of  $55 \pm 9$  kDa.<sup>(30)</sup> Therefore, prions may not be retained by most of the filters that efficiently eliminate bacteria and viruses. Additionally, prions aggregate into particles of non-uniform size and cannot be solubilized by detergents, except under denaturing conditions where infectivity is lost.<sup>(31)(32)</sup> Prions resist inactivation by nucleases,<sup>(33)</sup> UV-irradiation at 254 nm,<sup>(34)(35)</sup> and treatment with psoralens,<sup>(36)</sup> divalent cations, metal ion chelators, acids (between pH 3 and 7), hydroxylamine, formalin, boiling, or proteases.<sup>(37)(38)</sup>

*Inactivation of prions.* Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases,<sup>(39)(40)</sup> results from boiling in sodium dodecyl sulfate and urea are variable. Sterilization of rodent brain extracts with high titers of prions requires autoclaving at 132C for 4.5 hours (h). Denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate or alkali such as NaOH can also be used for sterilization.<sup>(41)(42)(43)(44)(45)</sup> Prions are inactivated by 1N NaOH, 4.0 M guanidinium hydrochloride or isocyanate, sodium hypochlorite (2% free chlorine concentration), and steam autoclaving at 132C for 4.5 h.<sup>(46)(47)(48)(49)</sup> It is recommended that dry waste be autoclaved at 132C for 4.5 h or incinerated. Large volumes of infectious liquid waste containing high titers of prions can be completely sterilized by treatment with 1N NaOH (final concentration) or autoclaving at 132C for 4.5 h. Disposable plasticware, which can be discarded as a dry waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, the biosafety cabinets must be decontaminated with 1N NaOH, followed by 1N HCl, and rinsed with water. HEPA filters should be autoclaved and incinerated.

Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Formaldehyde-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 min in 96% formic acid or phenol before histopathologic processing,<sup>(50)</sup> but such treatment may severely distort the microscopic neuropathology.

*Handling and processing of tissues from patients with suspected prion disease.* The special characteristics of work with prions require particular attention to the facilities, equipment, policies, and procedures involved. The related considerations outlined in the following tables should be incorporated into the laboratory's risk management for this work.

### **Standard precautions\* for autopsies of patients with suspected prion disease**

\*Not to be confused with "Standard Universal Precautions"

1. Attendance should be limited to at least one experienced pathologist and minimal staff. One of the staff avoids direct contact with the deceased but assists with handling of instruments and specimen containers.
2. Standard autopsy attire is mandatory.
  - a. A disposable, waterproof gown is worn in place of a cloth gown.
  - b. Cut-resistant gloves are worn underneath two pairs of surgical gloves or chain mail gloves are worn between two pairs of surgical gloves.
  - c. Aerosols are mainly created during opening of the skull with a Stryker saw. Appropriate respiratory protection should be worn (i.e., PAPR).
3. To reduce contamination of the autopsy suite:
  - a. The autopsy table is covered with an absorbent sheet that has a waterproof backing.
  - b. Contaminated instruments are placed on an absorbent pad.
  - c. The brain is removed while the head is in a plastic bag to reduce aerosolization and splatter.
  - d. The brain can be placed into a container with a plastic bag liner for weighing.
  - e. The brain is placed onto a cutting board and appropriate samples are dissected for snap freezing (see Table 4).
  - f. The brain or organs to be fixed are immediately placed into a container with 10% neutral buffered formalin.
  - g. In most cases of suspected prion disease, the autopsy can be limited to examination of the brain only. In cases requiring a full autopsy, consideration should be given to examining and sampling of thoracic and abdominal organs *in situ*.

### **Autopsy suite decontamination procedures**

1. Instruments (open box locks and jaws) and saw blades are placed into a large stainless steel dish, soaked for 1 h in 2N sodium hydroxide or 2 h in 1N sodium hydroxide, and then rinsed well in water before autoclaving at 134C (gravity displacement steam autoclaving for 1 h; porous load steam autoclaving for one 18-minute cycle at 30 lbs psi or six 3-minute cycles at 30 lbs psi).
2. The Stryker saw is cleaned by repeated wetting with 2N sodium hydroxide solution over a 1 h period. Appropriate washing to remove residual NaOH is required.
3. The absorbent table cover and instrument pads, disposable clothing, etc. are double bagged in appropriate infectious waste bags for incineration.
4. Any suspected areas of contamination of the autopsy table or room are decontaminated by repeated wetting over 1 h with 2N sodium hydroxide

### **Table 4. Brain cutting procedures**

1. After adequate formaldehyde fixation (at least 10-14 days), the brain is examined and cut on a table covered with an absorbent pad with an impermeable backing.
2. Samples for histology are placed in cassettes labeled with "CJD precautions." For laboratories that do not have embedding and staining equipment or microtome dedicated to infectious diseases including CJD, blocks of formalin-fixed tissue can be placed in 96% absolute formic acid for 30 minutes, followed by fresh 10% neutral buffered formalin solution for at least 48 h.<sup>(51)</sup> The tissue block is then embedded in paraffin as usual. Standard neurohistological or immunohistochemical techniques are not obviously affected by formic acid treatment; however, in our experience, tissue sections are brittle and crack during sectioning.
3. All instruments and surfaces coming in contact with the tissue are decontaminated as described in Table 3.
4. Tissue remnants, cutting debris, and contaminated formaldehyde solution should be discarded within a plastic container as infectious hospital waste for eventual incineration.

### **Table 5. Tissue preparation**

1. Histology technicians wear gloves, apron, laboratory coat, and face protection.

2. Adequate fixation of small tissue samples (e.g. biopsies) from a patient with suspected prion disease is followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 48 hours in fresh 10% formalin.
3. Liquid waste is collected in a 4L waste bottle containing 600 ml 6N sodium hydroxide.
4. Gloves, embedding molds, and all handling materials are disposed of as biohazardous waste.
5. Tissue cassettes are processed manually to prevent contamination of tissue processors.
6. Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated.
7. In preparing sections, gloves are worn, section waste is collected and disposed of in a biohazard waste receptacle. The knife stage is wiped with 1-2N NaOH, and the knife used is discarded immediately in a "biohazard sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned bloc is sealed with paraffin.
8. Routine staining:
  - a. Slides are processed by hand.
  - b. Reagents are prepared in 100 ml disposable specimen cups.
  - c. After placing the coverslip on, slides are decontaminated by soaking them for 1 h in 2N NaOH.
  - d. Slides are labeled as "Infectious-CJD."
9. Other suggestions:
  - a. Disposable specimen cups or slide mailers may be used for reagents.
  - b. Slides for immunocytochemistry may be processed in disposable petri dishes.
  - c. Equipment is decontaminated as described above.

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## References

1. Prusiner S.B. 1997. Prion diseases and the BSE crisis. *Science* 278:245-251.
2. Prusiner S.B., Baron H., Carlson G., Cohen F.E., DeArmond S.J., Gabizon R., Gambetti P., Hope J., Kitamoto T., Kretzschmar H.A., Laplanche J.-L., Tateishi J., Telling G., Weissmann C., Will R., In press. "Prions." In: *Virus Taxonomy. 7th Report of the International Committee on Taxonomy of Viruses*. Academic Press.
3. Pan K.-M., Baldwin M., Nguyen J., Gasset M., Serban A., Groth D., Mehlhorn I., Huang Z., Fletterick R.J., Cohen F.E., Prusiner S.B. 1993. Conversion of  $\alpha$ -helices into  $\beta$ -sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci USA* 90:10962-10966.
4. Wickner R.B. 1997. A new prion controls fungal cell fusion incompatibility [Commentary]. *Proc Natl Acad Sci USA* 94:10012-10014.
5. Gajdusek, D.C. 1977. Unconventional viruses and the origin and disappearance of kuru. *Science* 197:943-960.
6. Telling G.C., Scott M., Mastrianni J., Gabizon R., Torchia M., Cohen F.E., DeArmond S.J., Prusiner S.B. 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 83:79-90.
7. Prusiner S.B. 1997 (1)
8. Prusiner S.B. 1997 (1)
9. Will R.G., Ironside J.W., Zeidler M., Cousens S.N., Estibeiro K., Alperovitch A., Poser S., Pocchiari M., Hofman A., Smith P.G. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 347:921-925.
10. Foster J.D., McKelvey W.A.C., Mylne M.J.A., Williams A., Hunter N., Hope J., Fraser H. 1992. Studies on maternal transmission of scrapie in sheep by embryo transfer. *Vet Rec* 130:341-343.
11. Dickinson A.G., Stamp J.T., Renwick C.C. 1974. Maternal and lateral transmission of scrapie in sheep. *J Comp Pathol* 84:19-25.
12. Prusiner S.B. 1997 (1)
13. Gajdusek D.C. 1991. The transmissible amyloidoses: genetical control of spontaneous generation of infectious amyloid proteins by nucleation of configurational change in host precursors: kuru-CJD-GSS-scrapie-BSE. *Eur J Epidemiol* 7:567-577.

14. Marsh R.F., 1992. "Transmissible mink encephalopathy." In: *Prion Diseases of Humans and Animals*. Prusiner S.B., Collinge J., Powell J., Anderton B., Eds. Ellis Horwood, London, pp. 300-307.
15. Collinge J., Palmer M.S., 1997. "Human prion diseases." In: *Prion Diseases*. Collinge J., Palmer M.S., Eds. Oxford University Press, Oxford, U.K., pp. 18-56.
16. Ridley R.M., Baker H.F. 1993. Occupational risk of Creutzfeldt-Jakob disease. *Lancet* 341:641-642.
17. Gajdusek D.C. 1977 (5)
18. Public Health Service Interagency Coordinating Committee. 1997. Report on Human Growth Hormone and Creutzfeldt-Jakob Disease. 14:1-11.
19. CDC. 1997. Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts - Japan, January 1979-May 1996. *MMWR* 46:1066-1069.
20. Telling G.C., et al. 1995. (6)
21. Brown P., Gibbs C.J., Jr., Rodgers-Johnson P., Asher D.M., Sulima M.P., Bacote A., Goldfarb L.G., Gajdusek D.C. 1994. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol* 35:513-529.
22. Telling G.C., Parchi P., DeArmond S.J., Cortelli P., Montagna P., Gabizon R., Mastrianni J., Lugaresi E., Gambetti P., Prusiner S.B. 1996. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 274:2079-2082.
23. Brown P., Preece M.A., Will R.G. 1992. "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* 340:24-27.
24. Ironside J.W., Bell J.E. 1996. The 'high-risk' neuropathological autopsy in AIDS and Creutzfeldt-Jakob disease: principles and practice. *Neuropathol Appl Neurobiol* 22:388-393.
25. Ridley R.M., Baker H.F. 1993. (16)
26. Eklund C.M., Kennedy R.C., Hadlow W.J. 1967. Pathogenesis of scrapie virus infection in the mouse. *J Infect Dis* 117:15-22.
27. Prusiner S.B., Groth D.F., Cochran S.P., Masiarz F.R., McKinley M.P., Martinez H.M. 1980. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry* 19:4883-4891.

28. Prusiner S.B., Hadlow W.J., Eklund C.M., Race R.E., Cochran S.P. 1978. Sedimentation characteristics of the scrapie agent from murine spleen and brain. *Biochemistry* 17:4987-4992.
29. Kimberlin R.H. 1976. *Scrapie in the Mouse*. Meadowfield Press, Durham, England.
30. Bellinger-Kawahara C.G., Kempner E., Groth D.F., Gabizon R., Prusiner S.B. 1988. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. *Virology* 164:537-541.
31. Gabizon R., Prusiner S.B. 1990. Prion liposomes. *Biochem J* 266:1-14.
32. Safar J., Ceroni M., Piccardo P., Liberski P.P., Miyazaki M., Gajdusek D.C., Gibbs C.J., Jr. 1990. Subcellular distribution and physicochemical properties of scrapie associated precursor protein and relationship with scrapie agent. *Neurology* 40:503-508.
33. Bellinger-Kawahara C., Diener T.O., McKinley M.P., Groth D.F., Smith D.R., Prusiner S.B. 1987. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. *Virology* 160:271-274.
34. Alper T., Cramp W.A., Haig D.A., Clarke M.C. 1967. Does the agent of scrapie replicate without nucleic acid? *Nature* 214:764-766.
35. Bellinger-Kawahara C., Cleaver J.E., Diener T.O., Prusiner S.B. 1987. Purified scrapie prions resist inactivation by UV irradiation. *J Virol* 61:159-166.
36. McKinley M.P., Masiarz F.R., Isaacs S.T., Hearst J.E., Prusiner S.B. 1983. Resistance of the scrapie agent to inactivation by psoralens. *Photochem Photobiol* 37:539-545.
37. Prusiner S.B. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* 216:136-144.
38. Brown P., Wolff A., Gajdusek D.C. 1990. A simple and effective method for inactivating virus infectivity in formalin-fixed samples from patients with Creutzfeldt-Jakob disease. *Neurology* 40:887-890.
39. Prusiner S.B., McKinley M.P., Groth D.F., Bowman K.A., Mock N.I., Cochran S.P., Masiarz F.R. 1981. Scrapie agent contains a hydrophobic protein. *Proc Natl Acad Sci USA* 78:6675-6679.
40. McKinley M.P., Bolton D.C., Prusiner S.B. 1983. A protease-resistant protein is a structural component of the scrapie prion. *Cell* 35:57-62.

41. Prusiner S.B., Groth D.F., McKinley M.P., Cochran S.P., Bowman K.A., Kasper K.C. 1981. Thiocyanate and hydroxyl ions inactivate the scrapie agent. *Proc Natl Acad Sci USA* 78:4606-4610.
42. Prusiner S.B., McKinley M.P., Bolton D.C., Bowman K.A., Groth D.F., Cochran S.P., Hennessey E.M., Braunfeld M.B., Baringer J.R., Chatigny M.A., 1984. "Prions: methods for assay, purification and characterization." In: *Methods in Virology*. Maramorosch K., Koprowski H., Eds. Academic Press, New York, pp. 293-345.
43. Prusiner S.B., Groth D., Serban A., Stahl N., Gabizon R. 1993. Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc Natl Acad Sci USA* 90:2793-2797.
44. Taylor D.M., Woodgate S.L., Atkinson M.J. 1995. Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *Vet Rec* 137:605-610.
45. Taylor D.M., Woodgate S.L., Fleetwood A.J., Cawthorne R.J.G. 1997. Effect of rendering procedures on the scrapie agent. *Vet Rec* 141:643-649.
46. Prusiner, S.B., et al. 1984. (42)
47. Prusiner, S.B. et al. 1993. (43)
48. Taylor D.M., Woodgate S.L., Atkinson M.J. 1995. (44)
49. Taylor, D.M. et al. 1997. (45)
50. Brown P., Wolff A., Gajdusek D.C. 1990. (38)
51. Brown P., Wolff A., Gajdusek D.C. 1990. (38)