

医薬品 研究報告 調査報告書

<p>識別番号・報告回数</p>			<p>報告日</p>	<p>第一報入手日 2005. 12. 20</p>	<p>新医薬品等の区分 該当なし</p>	<p>機構処理欄</p>
<p>一般的名称</p>	<p>(製造承認書に記載なし)</p>		<p>研究報告の公表状況</p>	<p>Peretz D, Supattapone S, Giles K, Vergara J, Freyman Y, Lessard P, Safar JG, Glidden DV, McCulloch C, Nguyen HO, Scott M, Dearmond SJ, Prusiner SB. J Virol. 2006 Jan;80(1):322-31.</p>	<p>公表国 米国</p>	
<p>販売名(企業名)</p>	<p>合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)</p>					
<p>研究報告の概要</p>	<p>○酸性ドデシル硫酸ナトリウムによるプリオンの不活化 酢酸中の分岐ポリアミン・ dendリマーにプリオンを曝露するとプロテアーゼ感受性になるという発見 (S. Supattapone, H. Wille, L. Uyechi, J. Safar, P. Tremblay, F. C. Szoka, F. E. Cohen, S. B. Prusiner, and M. R. Scott, J. Virol. 75:3453-3461, 2001)を受けて、弱酸における酸性ドデシル硫酸ナトリウム (SDS) によるプリオンの不活化の検討を行った。ゴールデンハムスターの脳ホモジネート中のSc237プリオンを室温で1% SDS及び0.5% 酢酸に曝露したところ、プリオン力価が約107分の1に減少した。しかし、バイオアッセイに用いたハムスターすべてが最終的にプリオン病を発症した。SDS及び酢酸のさまざまな濃度について実験を行ったところ、曝露期間と温度が相乗的に作用し、これによりハムスターのSc237プリオンとヒトの孤発性クロイツフェルト・ヤコブ病(sCJD)プリオン双方の不活化が起こった。脳ホモジネート中のプリオン及びステンレス鋼線材に付着したプリオンの不活化を、遺伝子組み換えマウスのバイオアッセイで評価した。sCJDプリオンは、不活化に対してSc237より10万倍以上の耐性があり、齧歯類のプリオンで評価されている不活化の手順がヒトのプリオンの不活化に適用できないことが示された。脳ホモジネート中のプリオン力価を有意に減少させた手順には、ステンレス鋼線材に付着したプリオンにはわずかな影響しか与えないものもあった。15分間のオートクレーブと酸性SDSの併用により、ステンレス鋼線材に付着したヒトsCJDプリオンが除去された。この知見は、手術器具、またその他の医療機器、歯科用機器のプリオン不活化に適した非腐食性システムの基礎となる。</p>					<p>使用上の注意記載状況・ その他参考事項等</p>
<p>報告企業の意見</p>			<p>今後の対応</p>			
<p>15分間のオートクレーブと酸性ドデシル硫酸ナトリウムの併用により、ステンレス鋼線材に付着したヒトsCJDプリオンが除去されたとの報告である。</p>			<p>今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。</p>			

Inactivation of Prions by Acidic Sodium Dodecyl Sulfate

David Peretz,^{1,2†‡} Surachai Supattapone,^{1,2†§} Kurt Giles,^{1,2†} Julie Vergara,¹ Yevgeniy Freyman,¹ Pierre Lessard,¹ Jiri G. Safar,^{1,2} David V. Glidden,³ Charles McCulloch,³ Hoang-Oanh B. Nguyen,¹ Michael Scott,^{1,2||} Stephen J. DeArmond,^{1,4} and Stanley B. Prusiner^{1,2,5*}

Institute for Neurodegenerative Diseases¹ and Departments of Neurology,² Epidemiology and Biostatistics,³ Pathology,⁴ and Biochemistry and Biophysics,⁵ University of California San Francisco, San Francisco, California 94143

Received 14 March 2005/Accepted 16 September 2005

Prompted by the discovery that prions become protease-sensitive after exposure to branched polyamine dendrimers in acetic acid (AcOH) (S. Supattapone, H. Wille, L. Uyechi, J. Safar, P. Tremblay, F. C. Szoka, F. E. Cohen, S. B. Prusiner, and M. R. Scott, *J. Virol.* 75:3453–3461, 2001), we investigated the inactivation of prions by sodium dodecyl sulfate (SDS) in weak acid. As judged by sensitivity to proteolytic digestion, the disease-causing prion protein (PrP^{Sc}) was denatured at room temperature by SDS at pH values of ≤ 4.5 or ≥ 10 . Exposure of Sc237 prions in Syrian hamster brain homogenates to 1% SDS and 0.5% AcOH at room temperature resulted in a reduction of prion titer by a factor of ca. 10^7 ; however, all of the bioassay hamsters eventually developed prion disease. When various concentrations of SDS and AcOH were tested, the duration and temperature of exposure acted synergistically to inactivate both hamster Sc237 prions and human sporadic Creutzfeldt-Jakob disease (sCJD) prions. The inactivation of prions in brain homogenates and those bound to stainless steel wires was evaluated by using bioassays in transgenic mice. sCJD prions were more than 100,000 times more resistant to inactivation than Sc237 prions, demonstrating that inactivation procedures validated on rodent prions cannot be extrapolated to inactivation of human prions. Some procedures that significantly reduced prion titers in brain homogenates had a limited effect on prions bound to the surface of stainless steel wires. Using acidic SDS combined with autoclaving for 15 min, human sCJD prions bound to stainless steel wires were eliminated. Our findings form the basis for a noncorrosive system that is suitable for inactivating prions on surgical instruments, as well as on other medical and dental equipment.

Prions are infectious proteins that cause fatal neurodegenerative illnesses, including Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE), and scrapie in sheep (37, 60, 97). In mammals, prions are comprised solely of the disease-causing isoform of the prion protein (PrP), designated PrP^{Sc}. PrP^{Sc} is formed from the cellular precursor PrP^C by a process involving a profound conformational change. While PrP^C is a protein with three α -helices and little β -sheet, PrP^{Sc} is rich in β -sheet structure. It seems likely that the infectious prion monomer consists of a trimer of PrP^{Sc} molecules based on an ionizing radiation target size of 55 kDa and electron crystallography studies (3, 7, 30, 99). Limited proteolysis of PrP^{Sc} results in N-terminal truncation to form PrP 27-30, which retains infectivity and polymerizes into amyloid fibrils (49, 67). Electron crystallography combined with molecular modeling suggests that both PrP^{Sc} and PrP 27-30 contain a β -helix (30, 99). The conformation and extraordinarily small size of the prion are probably responsible for its extreme resistance to inactivation.

Reports in the mid-1960s on the resistance of prions to inactivation by both ionizing and UV radiation served to accentuate the mysterious nature of the infectious agent causing scrapie of sheep (2, 3). Two decades earlier, the resistance of the scrapie agent to inactivation by formalin was recognized when more than 1,500 sheep, immunized against looping-ill virus with a formalin-treated vaccine contaminated by the scrapie agent, developed scrapie several years after vaccination (29). With the transmission of the scrapie agent to mice and later Syrian hamsters (16, 46), studies were undertaken to define conditions for inactivation. The results of numerous studies designed to probe the molecular nature of the scrapie agent and define conditions for inactivation concluded that protein denaturants were effective at reducing infectivity titers but that complete inactivation required extremely harsh conditions, such as 5 h of autoclaving at 134°C or treatment with 2 N NaOH (65, 66). It is important to note that these conditions, on which current guidelines are based, were determined for the rodent strains, before it was known that prion strains may exhibit different stabilities to denaturation by heat, as well as chaotropes (56, 91). Defining conditions for inactivation of prions is an important undertaking in view of the human forms of prion disease that were elucidated by studies demonstrating the experimental transmission of prions from patients who died of kuru or CJD to apes and monkeys (26, 27). Radiation inactivation of CJD prions and studies of human PrP argue that human prions, like those causing scrapie and BSE, are resistant to inactivation (10, 28). More recently, the number of cases of iatrogenic CJD; the transmission of BSE prions from

* Corresponding author. Mailing address: Institute for Neurodegenerative Diseases, 513 Parnassus Ave., HSE-774, San Francisco, CA 94143-0518. Phone: (415) 476-4482. Fax: (415) 476-8386. E-mail: stanley@ind.ucsf.edu.

† D.P., S.S., and K.G. contributed equally to this study.

‡ Present address: Chiron Corp., Emeryville, CA 94608-2916.

§ Present address: Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

|| Present address: Department of Zoology, University College, Belfield, Dublin 4, Ireland.

cattle to humans, causing variant (v) CJD (97, 98); and the probable transmission of vCJD by blood transfusion (45, 55) highlight the pressing need for effective prion decontamination.

In the course of studies on the expression of PrP genes in prion-infected cultured cells, we found that branched polyamine dendrimers rendered PrP^{Sc} susceptible to degradation (83). This enhanced susceptibility to degradation could be mimicked in vitro by incubating prions with polyamine dendrimers at pH ~3.5 (84). Intrigued by the ability of weak acids such as acetic acid (AcOH) in combination with dendrimers to render prions susceptible to proteolytic degradation, we explored prion stability upon exposure to a variety of protein denaturants under weakly acidic conditions. Of all the detergents and chaotropes examined, sodium dodecyl sulfate (SDS) combined with AcOH proved to be the most potent reagent for the inactivation of prions. This finding was unexpected since SDS at neutral pH exhibits only a modest ability to inactivate prions in our experience (63). The experiences of others are noteworthy: 3% SDS at neutral pH has been reported to destroy prion infectivity in brain homogenates when samples were boiled or autoclaved (36, 87, 88). However, prion infectivity in macerated brain samples survived boiling for 15 min in 5% SDS at neutral pH (90). These findings suggest that SDS solutions at neutral pH, even when exposed to high temperatures, cannot be used for the complete inactivation of prion infectivity.

As described here, acidic SDS was superior to all other protein denaturants examined. The PrP^{Sc} molecule or a higher-order multimer such as a trimer is susceptible to denaturation by acidic SDS. To study the inactivation of prions by acidic SDS, Sc237 and sCJD prions from Syrian hamsters and humans, respectively, were used. Sc237 prions originated in sheep with scrapie and were isolated on passage from rats to Syrian hamsters (46). sCJD prions were from a patient who did not have any PRNP gene mutations and appeared to have developed prion disease spontaneously. Both immunoblotting and bioassays in rodents were used to assess the inactivation of prions in brain homogenates by acidic SDS, as well as those adhering to a steel surface (100). Our studies identified conditions under which it is possible to inactivate all detectable prion infectivity by a combination of acidic SDS and 15 min of autoclaving.

MATERIALS AND METHODS

Inocula. sCJD was confirmed by histopathology, immunohistochemistry, and detection of human PrP^{Sc} by Western blotting. Genomic sequencing of the open reading frame revealed no mutations and methionine homozygosity at position 129. The Sc237 hamster prion strain was a gift from Richard Marsh and was repeatedly passaged in golden Syrian hamsters (LVG:Lak) purchased from Charles River Laboratory (Wilmington, MA).

Preparation of brain homogenates and acidic buffers. Crude brain homogenates (10% [wt/vol]) in calcium- and magnesium-free phosphate-buffered saline (PBS) were prepared by repeated extrusion through syringe needles of successively smaller size, as previously described (78). Nuclei and debris were removed by centrifugation at 1,000 × g for 5 min. Incubations of brain homogenates with various solutions were performed with continuous shaking at 100 cycles/min. Glycine buffer was made as a 1 M stock titrated to pH 3.0, whereas AcOH and peracetic acid were added directly without adjustment. In other experiments, 10% (wt/vol) brain homogenates were prepared in calcium- and magnesium-free PBS with two 3.2-mm stainless steel beads using the Mini-BeadBeater-8 apparatus (BioSpec, Bartlesville, OK) for two cycles of 45 s each and then placed on ice. Homogenates were either precleared at 500 × g for 5 min (see Fig. 2B) or used without centrifugation, and the protocol was performed without shaking (see Fig. 1C and 2A and Table 1 to Table 4). The final pH value for each sample

was measured directly on parallel, uninfected samples with a calibrated pH electrode (Radiometer, Copenhagen, Denmark) during each experiment and is provided in the appropriate figure legends.

PrP^{Sc} detection by immunoblotting. PrP^{Sc} in neutralized samples was measured by limited proteinase K (PK) digestion and immunoblotting as described previously (83). After incubations, an equal volume of 4% Sarkosyl-100 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES; pH 7.5)-200 mM NaCl was added to neutralize each sample. Protease digestion was performed with 20 µg of PK/ml (Invitrogen, Carlsbad, CA) for 1 h at 37°C. Digestions were terminated by the addition of 8 µl of 0.5 M phenylmethylsulfonyl fluoride in absolute ethanol (Roche, Indianapolis, IN). Digested samples were then mixed with equal volumes of 2× SDS sample buffer. All samples were boiled for 5 min prior to electrophoresis. SDS-polyacrylamide gel electrophoresis (PAGE) was performed on 1.5-mm 12% polyacrylamide gels (39). After electrophoresis, Western blotting was performed as previously described (78). Membranes were blocked with 5% nonfat milk protein in PBST (calcium- and magnesium-free PBS plus 0.1% Tween 20) for 1 h at room temperature. Blocked membranes were incubated with 1 µg of recombinant, humanized antibody fragments (Fab) D13 (Fig. 1 and 2) or D18 (Fig. 1B)/ml. After incubation with the primary Fab, membranes were washed 3 × 10 min in PBST, incubated with horseradish peroxidase-labeled, anti-human Fab secondary antibody (ICN) diluted 1:5,000 in PBST for 45 min at room temperature, and washed again four times for 10 min each time in PBST. After enhanced chemiluminescent (ECL) detection (Amersham Bioscience, Piscataway, NJ) for 1 to 5 min, blots were sealed in plastic covers and exposed to ECL Hypermax film (Amersham). Films were processed automatically in a Konica film processor.

Preparation and bioassay of prion-coated stainless steel wires. Four-millimeter segments of 3-0 stainless steel suture wire (Ethicon, Cornelia, GA) were coated with prions and bioassayed by a modification of a procedure described previously (100). Wire segments were incubated with 10% prion-infected brain homogenate in PBS at room temperature for 16 h in a 10-cm petri dish, washed five times for 10 min each time at room temperature with PBS, followed by a 10-min wash with H₂O, and air dried in a ducted class II, type B2 biosafety cabinet (Baker, Sanford, ME) overnight. Coated wires (10 to 15) were incubated with 1 ml of H₂O, SDS, AcOH, or an SDS-AcOH solution at different temperatures for various durations. After incubation, wires were washed briefly in PBS and implanted into the right cerebral hemisphere. Mice were premedicated with buprenorphine hydrochloride (Buprenex; Reckitt Benckiser Healthcare, Berkshire, United Kingdom), anesthetized using isoflurane (AErrane; Baxter Healthcare, Deerfield, IL), and kept immobilized in a stereotaxic apparatus. A 1-cm skin incision was performed under aseptic conditions and a 0.9-mm bore hole was drilled through the skull, ca. 1 mm caudal and 1.2 mm right of the skull reference point bregma. The stainless steel wire was inserted into the brain with forceps. The skin was then closed by using surgical glue (Nexaband; Abbott Laboratories, Abbott Park, IL). The wires remained embedded in the brains of the mice for the duration of the experiment.

Bioassay for prion infectivity in brain homogenates. Brain homogenates were diluted 1:10 into sterile, calcium- and magnesium-free PBS plus 5 mg of bovine serum albumin/ml. Brain homogenates treated with various solutions, at final concentrations of 2.5%, were diluted 1:10 (see Tables 1 and 3) or 1:25 (see Tables 2 and 4). New, sterile, individually packaged needles, syringes, and tubes were used. All work was carried out in laminar flow hoods to avoid cross-contamination.

Brain homogenates containing hamster Sc237 prions were bioassayed in hamsters or transgenic (Tg) mice expressing Syrian hamster (SHa) PrP, designated Tg7 mice. Brain homogenates containing human sCJD prions were bioassayed in mice expressing a chimeric mouse-human PrP transgene designated MHu2M(M165V, E167Q), in which the most rapid incubation times are ca. 120 days (38); for simplicity, these mice are designated Tg22372 mice. For negative controls, brain homogenates of uninoculated *Prnp*^{0/0} mice were used. Hamsters and weanling mice were inoculated intracerebrally with 50 and 30 µl, respectively, of diluted samples. Inoculation was carried out with a 26-gauge, disposable hypodermic needle inserted into the right parietal lobe. After inoculation, mice were examined daily for neurologic dysfunction. Standard diagnostic criteria were used to identify animals affected by prion disease (13, 62). Animals whose deaths were imminent were sacrificed, and their brains were removed for histological and biochemical analysis.

Survival analysis. Prion incubation periods in experimental models have been reported historically as the mean incubation period ± the standard error of the mean. This approach assumes that the data are normally distributed, which is a reasonable approximation for high-titer samples. When prion titers are low, as in prion inactivation studies, the distribution of incubation periods becomes asymmetric, and not all animals succumb to disease. In such cases, the calculation of

a mean underestimates the incubation period because mice that do not become ill are excluded. Applying survival analysis methods overcomes these issues and also incorporates previously censored data on animals that die without showing clinical signs of prion disease (asymptomatic on the last inspection). We calculated median incubation periods and their standard error based on survival curves calculated by the method of Kaplan and Meier (34).

The effects of a treatment protocol on two different prion strains were analyzed by separate Cox models (19) with terms for \log_{10} dilution and inactivation and compared by stratification (33). All calculations were performed with Stata 8 (Stata Corp., College Station, TX).

RESULTS

Prions in SHa brain homogenates were chosen for our initial inactivation studies for three reasons: (i) SHa prions are the most well characterized with respect to physical properties, (ii) the titers of prions in SHa brain are 10- to 100-fold higher than those found in the brains of other species, and (iii) high-titer samples produce disease in ~70 days in hamsters and 45 days in Tg7 mice. The high titers of prions in SHa brain homogenates and short incubation times create a large range over which measurements can be made, and thus, provide the most sensitive system available for evaluating low levels of infectivity.

Acidic SDS denatures PrP^{Sc}. To examine whether solutions of 1 to 4% SDS could denature PrP^{Sc} more effectively at different pH values, we used 50 mM sodium acetate and Tris acetate buffers to maintain the pH between 3.5 and 10.0 for homogenates prepared from the brains of Syrian hamsters infected with Sc237 prions. Our studies demonstrated that aqueous solutions of $\geq 1\%$ SDS could denature PrP^{Sc} completely at pH values of ≤ 4.5 or ≥ 10.0 , as judged by immunoblotting for PrP after the samples were subjected to limited proteolysis (Fig. 1A). We also found that acidic solutions other than AcOH enable SDS to denature PrP^{Sc} in Sc237-infected brain homogenate: PrP^{Sc} was denatured in the presence of 1% SDS plus either 0.5% AcOH (pH 3.6), 50 mM glycine (pH 3.7), or 0.2% peracetic acid (pH 3.4) for 15 min at 37°C (Fig. 1B).

Acidic SDS denatured PrP^{Sc} in Sc237-infected brain homogenate in 30 min at room temperature, as judged by Western blotting (Fig. 1C, paired lanes 1). We next investigated whether dilute acid in the presence of detergents other than SDS could also denature PrP^{Sc}. We incubated Sc237-infected brain homogenates with various detergents, in the presence or absence of AcOH, for 30 min at room temperature. The detergents cholic acid, deoxycholic acid, Triton X-100, NP-40, Tween 20, CTAB (cetyltrimethylammonium bromide), Zwittergent, and CHAPS {3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate} failed to denature PrP^{Sc} at low pH (Fig. 1C, paired lanes 2 to 9, respectively). We next tested the ability of alkyl sulfates and alkyl sulfonates of various alkyl chain lengths to denature PrP^{Sc} in the presence of 0.5% AcOH. Alkyl sulfates with alkyl chains of 9 to 12 carbon atoms and alkyl sulfonates with alkyl chains of 10 to 13 carbon atoms denatured PrP^{Sc} substantially in the presence of dilute acid (Fig. 1D).

Physicochemical properties of PrP^{Sc} treated with acidic SDS. To investigate whether the inactivation of prions by acidic SDS is reversible, we added a neutralization buffer containing the nonionic detergent NP-40 to a sample of Sc237-infected brain homogenate initially exposed to 1% SDS and 0.5% AcOH for 15 min at 37°C. We then dialyzed the neutralized sample containing mixed micelles for 8 h at 4°C to remove the SDS. This

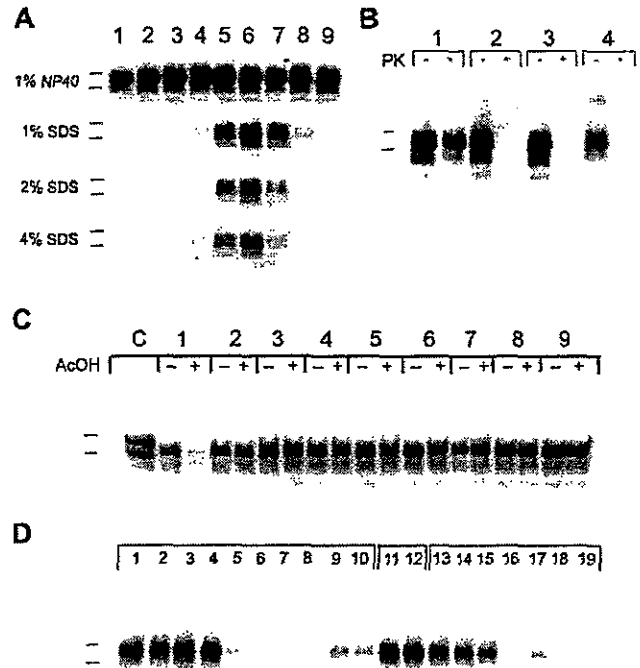


FIG. 1. Western blots of prion-infected brain homogenates treated with different detergents and at different pH values. For all panels, apparent molecular masses based on the migration of protein standards are 30 and 27 kDa (top and bottom markers, respectively). (A) Samples of 1% brain homogenate containing Sc237 prions were incubated for 15 min at 37°C with the indicated detergent at the following pH values: 3.5 (lane 1), 4.0 (lane 2), 4.5 (lane 3), 5.0 (lane 4), 6.0 (lane 5), 7.0 (lane 6), 8.0 (lane 7), 9.0 (lane 8), and 10.0 (lane 9). Sodium acetate buffers (50 mM) were used to maintain pH values 3 to 6 (lanes 1 to 5), and 50 mM Tris acetate buffers were used to maintain pH values 7 to 10 (lanes 6 to 9). After incubation, all samples were neutralized by the addition of an equal volume of 4% Sarkosyl-100 mM HEPES (pH 7.5)-200 mM NaCl and subjected to limited PK digestion (20 μ g/ml for 1 h at 37°C). (B) Samples of 1% brain homogenate containing Sc237 prions were incubated for 15 min at 37°C in 1% SDS plus 50 mM Tris acetate (pH 7.0) (paired lane 1), 0.5% AcOH (pH 3.6) (paired lane 2), 50 mM glycine (pH 3.7) (paired lane 3), or 0.2% peracetic acid (pH 3.4) (paired lane 4). After incubations, samples were neutralized and then left undigested (-) or digested with PK (+) as described in panel A. (C) Samples of 2.5% brain homogenate containing Sc237 prions were incubated with 2% detergent either alone (-) or with (+) 1% AcOH at room temperature for 30 min. Anionic (paired lanes 1 to 3), nonionic (paired lanes 4 to 6), cationic (paired lane 7), and zwitterionic (paired lanes 8 to 9) detergents were used. Lane assignments are as follows: lane 1, SDS; lane 2, cholic acid; lane 3, deoxycholic acid; lane 4, Triton X-100; lane 5, NP-40; lane 6, Tween 20; lane 7, CTAB; lane 8, Zwittergent; lane 9, CHAPS. All paired samples (lanes 1 to 9) were neutralized by the addition of 280 μ l of 2% Sarkosyl-100 mM HEPES (pH 7.5)-100 mM NaCl and subjected to limited PK digestion (4 μ g for 1 h at 37°C). (D) Samples of 1% brain homogenate containing Sc237 prions were incubated with 0.5% AcOH (pH 3.6) and either 1% alkyl sulfates (lanes 1 to 10), NP-40 (lanes 11 and 12), or alkyl sulfonates (lanes 13 to 19) for 2 h at 37°C. Alkyl sulfate detergents with backbones of 5 to 14 carbon atoms (lanes 1 to 10, respectively) were tested. Alkyl sulfonate detergents with backbones of 6 (lane 13), 7 (lane 14), and 9 to 13 carbon atoms (lanes 15 to 19, respectively) were used. All samples were subjected to limited PK digestion.

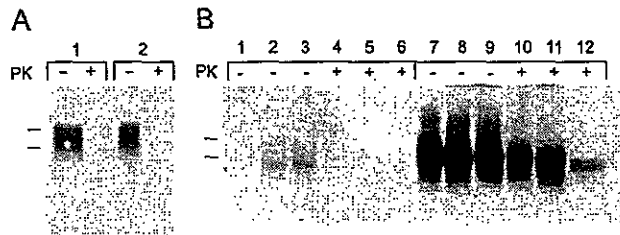


FIG. 2. Physical characterization of Sc237 prions denatured by acidic SDS. (A) A 0.5-ml sample of 1% prion-infected brain homogenate was incubated in 1% SDS-0.5% AcOH (pH 3.6) for 15 min at 37°C and then neutralized by the addition of 0.5 ml of 2% NP-40-100 mM HEPES (pH 7.5)-200 mM NaCl. A 50- μ l aliquot was removed for analysis, and the remainder of the sample was dialyzed two times against 2 liters of 1% NP-40-50 mM HEPES (pH 7.5)-100 mM NaCl for 8 h at 4°C (molecular mass cutoff of 10 kDa). Sample before (paired lane 1) and after (paired lane 2) dialysis. Samples were undigested (-) or subjected (+) to limited PK digestion (20 μ g/ml for 1 h at 37°C). The apparent molecular masses based on the migration of protein standards are 30 and 27 kDa. (B) Samples of 1% prion-infected brain homogenate were incubated at room temperature for 15 min with PBS (lanes 1, 4, 7, and 10), 50 mM Tris (pH 7.0) with 1% SDS (lanes 2, 5, 8, and 11), or 1% SDS-0.5% AcOH (lanes 3, 6, 9, and 12). After incubation, samples were centrifuged at 100,000 \times g for 1 h at 4°C. Supernatant fractions (lanes 1 to 6) were removed and neutralized with an equal volume of 2 \times neutralization buffer (200 mM NaCl, 4% Sarkosyl, 400 mM HEPES [pH 7.5]) and subjected to limited PK digestion (PK-protein [1:50], for 1 h at 37°C) as indicated. Pellet fractions (lanes 7 to 12) were resuspended in 1 \times neutralization buffer and subjected to limited PK digestion as indicated. All samples were mixed with an equal volume of 2 \times SDS sample buffer. The apparent molecular masses based on the migration of protein standards are 36 and 29 kDa.

procedure did not restore the protease-resistance of PrP^{Sc} that had been denatured by acidic SDS (Fig. 2A, paired lanes 2).

To investigate further the biophysical changes in PrP^{Sc} molecules induced by exposure to acidic SDS, we performed ultracentrifugation. We found that PrP^{Sc} molecules become soluble in acidic SDS (Fig. 2B).

Inactivation of Syrian hamster prions by acidic SDS. To determine whether denaturation of PrP^{Sc} caused by acidic SDS correlates with a reduction in prion infectivity, we incubated Sc237-infected brain homogenates for 2 h with different buffers at various temperatures and inoculated the treated samples intracerebrally into Syrian hamsters. Inoculation of a control brain homogenate containing $\sim 10^9$ ID₅₀ units/ml (one ID₅₀ unit is the infectious dose causing illness in 50% of inoculated animals) at neutral pH without detergent caused disease in all animals in 84 ± 0.4 days (Fig. 3). Animals inoculated with samples exposed to either 0.5% AcOH or 1% SDS alone for 2 h at room temperature developed disease in 79 ± 0.9 days and 91 ± 0.4 days, respectively (Fig. 3). In contrast, exposure of the inoculum to a solution of 1% SDS and 0.5% AcOH for 2 h at room temperature prolonged the median incubation time to 200 ± 2.3 days (Fig. 3). Similar results were obtained when samples were incubated at 37°C (data not shown).

The prolonged incubation times found after acidic SDS treatment of hamster brain homogenates indicate that the prion titers were reduced from $\sim 10^9$ ID₅₀ units/ml to <100 ID₅₀ units/ml. These titers were calculated from standard curves that relate the incubation time to the dose of prions in the inoculum (61, 62). That the prions were not completely

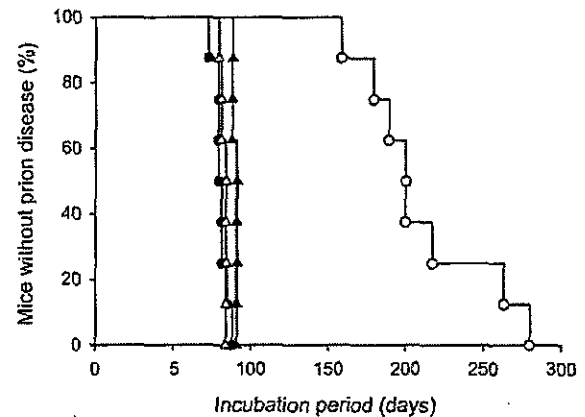


FIG. 3. Effect of acidic SDS and temperature on prion infectivity. Samples of 1% brain homogenate infected with Sc237 prions containing $\sim 10^9$ ID₅₀ units/ml were incubated with continuous shaking for 2 h in the specified solution at room temperature. After incubation, samples were diluted 1:10 into calcium- and magnesium-free PBS plus 5 mg of bovine serum albumin/ml, and 50- μ l aliquots were inoculated into eight Syrian hamsters each, and survival curves were calculated. Symbols: Δ , 50 mM sodium acetate (pH 7.0); \bullet , 0.5% AcOH (pH 3.6); \blacktriangle , 1% SDS and 50 mM sodium acetate (pH 7.0); \circ , 1% SDS and 0.5% AcOH (pH 3.6).

inactivated by the procedure used is clear from the survival curves: all of the hamsters used for bioassay eventually developed disease (Fig. 3). Although the data argue that SDS and dilute acid act synergistically to diminish prion infectivity, complete inactivation necessitated modification of the protocol.

Bioassays in transgenic mice. Although these initial studies proved promising, we were concerned that extensive bioassays in hamsters would be complicated by the relatively short life span of these animals (69); we therefore turned to Tg mice expressing high levels of SHaPrP, designated Tg7 mice (70, 78). Uninoculated Tg7 mice remain healthy for more than 500 days, while those inoculated with $\sim 10^9$ ID₅₀ units/ml of prions develop neurologic dysfunction in approximately 45 days. To make our studies more clinically relevant, we used crude brain homogenates, rather than homogenates precleared by low-speed centrifugation.

A 10% (wt/vol) Sc237-infected brain homogenate was serially diluted, and each dilution was inoculated into Tg7 mice. As the infected brain homogenate is diluted, the percentage of mice succumbing to prion disease decreases (Fig. 4A) and the median incubation period lengthens. These median time points were interpolated with an exponential decay curve to highlight this relationship (Fig. 4A). A similar curve was developed relating the length of the incubation time and the size of the inoculum dose for bioassays of human sCJD prions with Tg22372 mice (Fig. 4B).

Inactivation of Syrian hamster prions by acidic SDS at elevated temperatures. In an attempt to destroy the residual prion infectivity found by initial bioassay (Fig. 3), we increased the concentrations of SDS to 2% and of AcOH to 1% and raised the treatment temperature to 65°C (Table 1). A solution composed of 4% SDS and 2% AcOH was added directly to an equal volume of sample containing a 5% (wt/vol) prion-infected crude brain homogenate, mixed, and incubated without additional agitation.

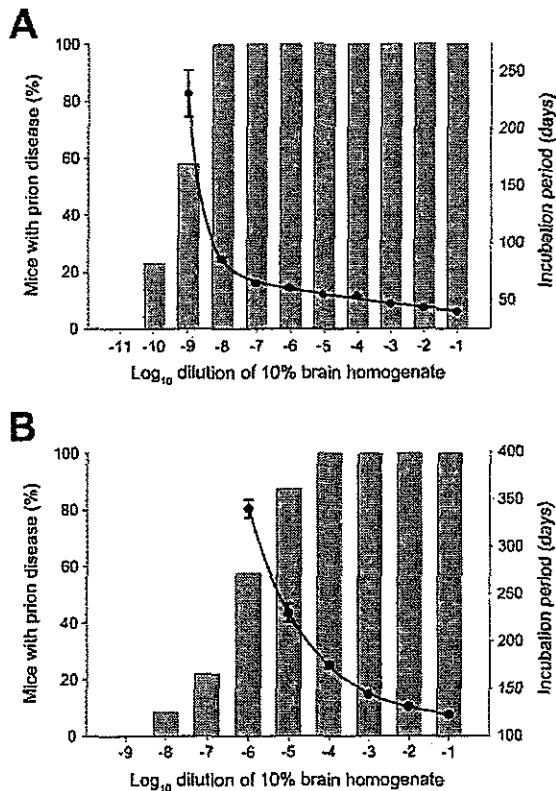


FIG. 4. Proportion and incubation period of transgenic mice succumbing to disease with each dilution of inoculum. Ten percent brain homogenates were serially diluted 10-fold, and each dilution was inoculated into 12 mice. Bars represent the percentage of mice that showed clinical signs of disease when inoculated with the indicated \log_{10} dilution of brain homogenate. The incubation period, the number of days from inoculation to the manifestation of clinical symptoms, was also measured for these mice. When at least half of the animals became ill, the median incubation period was determined by Kaplan-Meier statistics and plotted against \log_{10} dilution (datum point \pm the standard error). Values were interpolated with the best fit to an exponential decay curve. (A) Sc237 prions bioassayed in Tg(SHaPrP)7 mice; (B) sCJD prions bioassayed in Tg(MHu2M, M165V, E167Q)22372 mice.

Inoculation of a control Sc237-infected brain homogenate at neutral pH without detergent produced disease in Tg7 mice with a median incubation time of 46 days (Table 1). Although uninoculated Tg7 mice remained healthy for 500 days, some animals can develop a nontransmissible neuromyopathy after

this time (96). Since mice are inoculated at 8 to 10 weeks of age, bioassays in Tg7 mice were terminated after 400 days to exclude the possibility of illness not caused by prions. After exposure of Sc237-infected brain homogenates to 2% SDS and 1% AcOH at 65°C for 30 min, the inoculum produced prion disease in some of the mice, with significantly prolonged incubation times. Increasing the exposure time of the inoculum to 2 h or 18 h resulted in no animals becoming ill 400 days after inoculation.

Acidic SDS inactivates Syrian hamster prions bound to steel wire. To study the inactivation of prions on surfaces (100), we soaked stainless steel wires in 10% brain homogenates containing Sc237 prions for 16 h at room temperature. The prion-coated wires were implanted into the parietal lobes of Tg7 mice and produced disease in 52 ± 0.3 days. Treatment of the wires at 65°C for 30 min with 2% SDS and 1% AcOH increased the incubation time to 82 ± 0.7 days after implantation into the brains of Tg7 mice; treatment at 65°C for 2 h further lengthened the incubation time, but most of the animals still developed disease in fewer than 400 days. Only treatment at 65°C for 18 h resulted in no animals succumbing to disease before 400 days (Table 1).

Inactivation of human prions by acidic SDS. Because different prion strains exhibit distinct resistances to inactivation by chaotropic salts and heat (56, 57, 81, 89, 91), we investigated the inactivation of sCJD prions by acidic SDS. Brain homogenates were prepared from an sCJD patient homozygous for methionine at residue 129, who carried no mutations in the *PRNP* gene and whose unglycosylated PrP 27-30 migrated at 21 kDa on SDS-PAGE (53). These human prions, designated sCJD(MM1), are the most common strain found in sCJD patients (52, 53). We either inoculated brain homogenate containing sCJD(MM1) prions intracerebrally or implanted wires that were coated with the homogenate into Tg22372 mice (38). It is noteworthy that, like the human PrP^{Sc} inoculum, the PrP transgene in Tg22372 mice encodes methionine at position 129.

Sporadic CJD(MM1) prions in brain homogenates or on steel wires were treated with 2% SDS and 1% AcOH at 65°C (Table 1). For brain homogenates, acidic SDS treatment for 30 min at 65°C prolonged the incubation times from 131 ± 0.7 days to 266 ± 8.5 days. Increasing the time of exposure (2 h or 18 h) to acidic SDS at 65°C lengthened the incubation time (>500 days) and decreased the proportion of Tg mice developing disease (~25%). Studies of sCJD-coated steel wires prolonged incubation times only slightly, and removal of infectivity on these surfaces was less efficient than in brain homogenates.

TABLE 1. Inactivation of prions by 2% SDS plus 1% AcOH at 65°C^a

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg22372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)
Negative control	>400	0	>400	0	>500	0	>500	0
Positive control	46 \pm 0.2	100	52 \pm 0.3	100	131 \pm 0.7	100	215 \pm 0.9	100
2% SDS-1% AcOH, 30 min	>400	26	82 \pm 0.7	100	266 \pm 8.5	74	354 \pm 1.6	86
2% SDS-1% AcOH, 2 h	>400	0	269 \pm 3.2	68	>500	26	>500	44
2% SDS-1% AcOH, 18 h	>400	0	>400	0	>500	25	>500	25

^a Median incubation period (IP) \pm the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

TABLE 2. Inactivation of prions by 4% SDS plus 1% AcOH at 65°C^a

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)
Negative control	>400	0	>400	0	>500	0	>500	0
Positive control	55 ± 0.0	100	63 ± 0.1	100	139 ± 0.4	100	181 ± 1.2	100
ddH ₂ O, 18 h	55 ± 0.1	100	67 ± 0.2	94	179 ± 4.5	62	208 ± 3.8	100
1% AcOH, 18 h	60 ± 0.4	100	63 ± 0.1	100	172 ± 0.8	100	196 ± 1.9	100
4% SDS, 18 h	77 ± 0.4	100	106 ± 3.1	62	>500	32	223 ± 1.8	79
4% SDS-1% AcOH, 30 min	>400	0	>400	14	>500	5	278 ± 15	71
4% SDS-1% AcOH, 2 h	>400	0	>400	25	>500	13	259 ± 6.4	64
4% SDS-1% AcOH, 18 h	>400	0	>400	0	>500	0	379 ± 30	60

^a Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

The same treatment protocol (exposure to 2% SDS and 1% AcOH for 30 min at 65°C) resulted in large differences in the level of inactivation for the two prion strains studied (Table 1). Because the titer of Sc237 prions is 10- to 100-fold higher than sCJD prions in brain homogenates (Fig. 4), data were analyzed by a stratified Cox regression (33) in order to quantify differences between hamster and human prions. Taking the ratios of coefficients in the Cox model, it is possible to relate the effect of an inactivation procedure to an approximately equivalent dilution. Applying the stratified Cox regression to the 30-min exposure to acidic SDS, we estimate a 9.0 log₁₀ reduction in infectivity for Sc237 prions and a 3.8 log₁₀ reduction for sCJD prions. The difference in inactivation between the two prion strains is 5.2 log₁₀ (95% confidence intervals, 3.7 to 6.8 log₁₀). This analysis argues that sCJD prions in human brain homogenates are 10⁵-fold more resistant to inactivation than Sc237 prions in SHa brain homogenates.

Two-step inactivation protocol. Concerned that exposure to acid results in the aggregation of proteins and that such aggregates might protect PrP^{Sc} from acidic SDS-mediated denaturation, we developed a two-step protocol: SDS at neutral pH is used to disperse proteins prior to exposure to AcOH. In these studies, exposure to 4% neutral SDS was performed at 65°C for 30 min, followed by acidic SDS (4% SDS and 1% AcOH) at 65°C for 30 min, 2 h, or 18 h (Table 2). All two-step inactivation studies were completed in three independent sets (n = 8) and, since no significant differences were observed, the sets were combined for analysis.

SHa brain homogenates (10% [wt/vol]) containing Sc237 prions were treated by using the two-step acidic SDS protocol. Untreated homogenates produced neurologic dysfunction after 55 days in Tg7 mice (Table 2), whereas the two-step procedure at 65°C for 30 min completely abolished prion infectivity. In experiments with Sc237 prion-coated wires, untreated control wires produced disease in 63 ± 0.1 days after implantation. In contrast to homogenates, exposure of prion-coated wires to the two-step procedure at 65°C for 30 min did not completely inactivate prion infectivity.

Human brain homogenates (10% [wt/vol]) containing sCJD (MM1) prions were treated by using the two-step acidic SDS protocol as described above. Untreated homogenates produced neurologic dysfunction in 139 ± 0.4 days in Tg23372 mice (Table 2). Exposure of these homogenates to the two-step procedure at 65°C for 30 min substantially reduced prion in-

fectivity, while extending the time to 18 h completely abolished infectivity. In three separate experiments with sCJD prion-coated wires, untreated control wires produced disease in 181 ± 1.2 days after implantation. In contrast to homogenates, exposure of prion-coated wires to the two-step procedure at 65°C, even for 18 h, did not inactivate prion infectivity, with the majority of Tg23372 mice still succumbing to disease.

Acidic SDS and autoclaving abolish prion infectivity. Although exposure of Sc237 and sCJD(MM1) prions to 2% SDS and 1% AcOH at 65°C for 2 h was sufficient to destroy more than 99.99% of the infectivity in brain homogenates, complete inactivation was not achieved (Table 1). Also, although the two-step procedure using 4% neutral SDS, followed by a combination of 4% SDS and 1% AcOH, further diminished infectivity levels, residual infectivity could still be measured, particularly on the surfaces of steel wires coated with sCJD(MM1) prions (Table 2).

To determine whether autoclaving for a brief time in the presence of acidic SDS could eliminate prion infectivity, we exposed Sc237 and sCJD(MM1) prions in brain homogenates or on wire surfaces to 121°C in the presence or absence of acidic SDS (Table 3). Neither Sc237 nor sCJD(MM1) prions in brain homogenates or on wire surfaces were detectable by bioassay in Tg mice after exposure to 121°C for 15 min in the presence of 2% SDS and 1% AcOH (Table 3). Similarly, samples first exposed to 4% neutral SDS followed by a combination of 4% SDS and 1% AcOH at 134°C were devoid of prion infectivity (Table 4). The efficacy of acidic SDS-mediated inactivation of prions is strikingly documented by how many prions survived autoclaving at 121°C or 134°C for 15 min, 30 min, or 2 h in the absence of acidic SDS (Tables 3 and 4). The survival of sCJD(MM1) prions bound to steel wires after autoclaving for 2 h at 134°C is especially alarming (Table 4).

To quantify the log₁₀ reduction in prion titer from these procedures, we used a Cox proportional-hazards model based on the serial dilution data (Fig. 4) and derived partial-likelihood ratios based on 95% confidence intervals. Treatment of sCJD prions in brain homogenate for 30 min at 121°C (Table 3) results in a 6.8 log₁₀ reduction (lower 95% confidence interval, 5.3 log₁₀). If no animals succumb to disease, the extent of inactivation cannot be quantified. However, it is still possible to determine a lower 95% confidence interval. For example, treatment of sCJD prions in brain homogenate with the two-step protocol for 15 min at 134°C (Table 4) gives a

TABLE 3. Inactivation of prions by 2% SDS plus 1% AcOH at 121°C^a

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)
Positive control	46 ± 0.2	100	62 ± 0.3	100	146 ± 0.4	100	207 ± 1.1	100
Untreated, 15 min	344 ± 20	73	160 ± 7.3	100	221 ± 1.0	100	>500	22
Untreated, 30 min	>400	12	>400	20	>500	0	>500	0
Untreated, 2 h	>400	12	>400	0	>500	0	414 ± 15	73
2% SDS-1% AcOH, 15 min	>400	0	>400	0	>500	0	>500	0
2% SDS-1% AcOH, 30 min	>400	0	>400	0	>500	12	>500	0
2% SDS-1% AcOH, 2 h	>400	0	>400	0	>500	0	>500	0

^a Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

lower 95% confidence interval of 6.1 log₁₀ reduction in infectivity.

The incubation periods for prion-coated wires cannot be converted into titers since the prions seem to be bound tightly to the surface of the wire (95) and it is not possible to remove them for measurement.

DISCUSSION

The differences in the inactivation profiles of prions and viruses provided the first clues that the scrapie agent was not a slow virus as had been widely thought (6, 23, 41, 48, 58, 59, 80).

Mechanism of acidic SDS inactivation. From the results of the studies described here, exposure to acidic SDS appears to denature PrP^{Sc} (Fig. 1). Although SDS between pH 4.5 and pH 10.0 at room temperature is a poor denaturant, it becomes an excellent denaturant at a pH of ≤4.5 and ≥10 (Fig. 1). Moreover, weak acids are poor denaturants for PrP^{Sc} except in the presence of SDS.

Denaturation of PrP^{Sc} by SDS at pH 4 was dependent on the concentration of SDS, the time of exposure, and the temperature. Equivalent levels of denaturation seemed to be achieved by inversely varying the time and temperature; the time of exposure for complete inactivation of prions was 15 min when a temperature of ≥121°C was used (Tables 3 and 4).

In our initial experiments, denaturation of PrP^{Sc} was measured by the loss of protease-resistance of PrP 27-30 and a decrement in prion infectivity. Immunodetection of PrP 27-30 by using Western blotting has a dynamic range of ~100-fold, whereas bioassays measure prions over an ~10⁹-fold range as described above. In determining the mechanism of acidic SDS-

mediated denaturation of PrP^{Sc}, immunoassays are useful but only prolonged bioassays are adequate to assess whether or not complete inactivation of prion infectivity has been achieved.

Stabilities of different prion strains. The strain phenotype of the prion is enciphered in the conformation of PrP^{Sc} (9, 43, 57, 94), and different strains display distinct conformational stabilities as reflected in their resistance to denaturation by chaotropes and heat (36, 43, 57, 77, 81, 91). Besides the strain of prion, the sequence of PrP, determined by the last host in which the prion was passaged, influences the conformation of PrP^{Sc} and hence its stability (79). The passage of prions through different species may lead to new prion strains with properties different from those of the parental strain (21, 35, 38, 54, 57, 72).

Conformational stability profiles, as measured by sensitivity to denaturation by GdnHCl gave half-maximal (Gdn_{1/2}) values of 1.5 and 1.8 M for Sc237 prions (56, 57) compared to a Gdn_{1/2} value of 1.8 M for sCJD(MM1) prions (101). However, the resistances of these strains to denaturation by acidic SDS are significantly different (Table 1 to Table 4), implying that inactivation protocols validated on one strain cannot be extrapolated to another. Exploring the spectrum of strain conformations with respect to inactivation by acidic SDS should be facilitated by the availability of synthetic prions, especially since the MoSP1 strain is the most stable strain studied to date (42, 43).

Limitations on the measurement of prion infectivity. Bioassays for prions are most effective when high-titer samples are being assessed. Under these circumstances, the incubation times are the shortest and the variance among the animals is

TABLE 4. Inactivation of prions by 4% SDS plus 1% AcOH at 134°C^a

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)
Positive control	55 ± 0.0	100	63 ± 0.1	100	139 ± 0.5	100	181 ± 1.2	100
Untreated, 15 min	>400	5	96 ± 0.6	87	>500	0	218 ± 4.1	73
Untreated, 30 min	>400	0	262 ± 10	55	>500	0	242 ± 2.8	63
Untreated, 2 h	>400	0	>400	9	>500	0	>500	46
4% SDS-1% AcOH, 15 min	>400	0	>400	0	>500	0	>500	0
4% SDS-1% AcOH, 30 min	>400	0	>400	4	>500	0	>500	0
4% SDS-1% AcOH, 2 h	>400	0	>400	0	>500	0	>500	0

^a Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

the smallest. As the incubation times lengthen, the variance increases. In studies of prion inactivation, it is possible to measure a reduction in titer over a range of $\sim 10^9$ -fold with Sc237 prions using either Syrian hamsters or Tg(SH_hPrP) mice. Inactivation studies performed with sCJD(MM1) prions using Tg mice permit measurements of prion infectivity over a range of $\sim 10^6$ -fold.

It is clear that the best measurements can be obtained when the incubation times are short, as is the case for high-titer samples. For example, Tg7 mice develop neurologic dysfunction ~ 45 days after inoculation with high-titer samples of Sc237 prions (Fig. 4A).

Prion inactivation with acidic SDS. From the foregoing studies, acidic SDS provides for the first time a means of completely inactivating prions under relatively gentle conditions. The need for noncorrosive inactivation of prions is clearly illustrated by cases of iatrogenic CJD in which prion-contaminated neurosurgical equipment seems to have spread prions from one patient to another (8, 11, 14, 15, 20, 31). This concern has been heightened in Britain, where the finding of high titers of vCJD prions in lymphoid tissues worries authorities that any surgical procedure could result in the spread of prions from one patient to another (18, 32). In Britain, 15 young people, all of whom later developed vCJD, donated blood that was transfused into 48 recipients. Recently, two of these recipients have been identified, in whom it is likely that vCJD prions were transmitted from the blood of the young donors (45, 55).

Conventional hospital disinfectants including ethylene oxide, propiolactone, hydrogen peroxide, iodophors, peracetic acid, chaotropes, and phenolics have little effect on prion infectivity (89), although some modified cleaning reagents have been suggested to have utility in diminishing prion titers (25, 71). In addition, prions are resistant to inactivation by UV irradiation, aldehyde fixation, boiling, standard gravity autoclaving at 121°C, and detergent solubilization (1, 5, 22, 24, 40, 64, 89).

Although prolonged proteolytic digestion diminishes prion titers (17, 47, 68), complete inactivation of prion infectivity is difficult to achieve enzymatically. As with any enzymatic reaction, protease-catalyzed hydrolysis of PrP^{Sc} becomes increasingly less efficient as the concentration of the substrate PrP^{Sc} decreases (44, 50). Moreover, one or more substances in crude suspensions may inhibit an enzyme or mixture of enzymes used to digest PrP^{Sc}.

Currently recommended protocols for prion decontamination include either (i) $>2\%$ available chlorine of sodium hypochlorite for 2 h, (ii) 2 M NaOH for 1 h, or (iii) autoclaving at 134°C for 4.5 h (4, 12, 63, 66, 73, 74, 76, 82, 92, 93). Each of these protocols has important limitations: sodium hypochlorite and NaOH are corrosive at the concentrations required to inactivate prions; NaOH did not inactivate CJD prions completely in some reports (85, 86); and extended autoclaving at high temperature is deleterious to many materials. Currently, some high-risk surgical instruments are soaked in 2 N NaOH for 1 h, rinsed with water, and autoclaved at 134°C for 1 h (75), while many other such instruments are discarded.

In the studies reported here, sCJD(MM1) prions on steel wires were not completely inactivated by autoclaving at either 121 or 134°C in the absence of acidic SDS (Tables 3 and 4); this

contrasts with Sc237 prions that were completely inactivated by autoclaving alone (Tables 3 and 4). It is noteworthy that the prion-coated wires were not subjected to any procedures that might reduce the level of prions, such as washing, shaking, scrubbing, and sonicating. Such cleaning procedures are known to reduce substantially the titers of many different pathogens (74).

The data presented here document the efficacy of acidic SDS combined with autoclaving for complete inactivation of human and hamster prions in brain homogenates and on the surface of steel wires (Tables 3 and 4). Acidic SDS combined with autoclaving should supplant routine autoclaving used to sterilize medical and dental equipment. For equipment such as fiber optic instruments that cannot be autoclaved, submerging such equipment in acidic SDS at 65°C will substantially reduce prion infectivity but not completely eliminate it (Table 2). Acidic SDS at room temperature may also find application in the inactivation of equipment and surfaces. Sc237 prion infectivity was reduced more than 99.99% in studies conducted at room temperature (Fig. 3).

Concluding remarks. The results of inactivation studies described here argue that acidic SDS combined with autoclaving be applied immediately for sterilization of surgical and dental instruments. Because SDS is both a detergent and a protein denaturant, it should prove especially apt for sterilizing instruments with complex shapes, serrations, locks, bores, and crevices. Whether other compounds such as urea that are known to denature PrP^{Sc} (51, 63, 64) will prove useful in combination with a weak acid is unknown.

In addition to inactivating prions on the surfaces of surgical instruments and diagnostic equipment, acidic SDS may prove useful in the sterilization of dental and ophthalmologic instruments, as well as the cleaning of operating theaters and invasive diagnostic suites. Acidic SDS is likely to find use in the cleansing and sterilization of equipment used in the production of biopharmaceuticals. In addition, acidic SDS might be applied in the cleaning of abattoirs, meat-processing plants, butcher shops, kitchens, and wherever mammalian products are prepared for human consumption. Acidic SDS might also be used on equipment used in the rendering of offal. Whether acidic SDS or a biodegradable formulation such as acidic urea can be incorporated into some products derived from rendered offal remains to be determined.

In summary, it is important to recognize that procedures routinely used in medical and dental settings do not inactivate prions. That being the case, it may be prudent to institute acidic-SDS protocols as configured in the studies presented here. Acidic SDS combined with autoclaving completely inactivated prion infectivity, even on steel surfaces. Inactivation of human and hamster prions mediated by acidic SDS occurs rapidly and can be achieved without boiling or autoclaving. Acidic SDS is noncorrosive and offers other practical advantages that make it suitable for widespread use.

ACKNOWLEDGMENTS

This study was supported by grants from the National Institutes of Health (AG02132, AG10770, and AG021601). S.S. was supported by the Burroughs Wellcome Fund Career Development Award and an NIH Clinical Investigator Development Award.

We thank the staff at the Hunters Point animal facility. K.G., J.G.S., and S.B.P. have financial interests in InPro Biotechnology, Inc.

REFERENCES

- Alper, T. 1985. Scrapie agent unlike viruses in size and susceptibility to inactivation by ionizing or ultraviolet radiation. *Nature* 317:750.
- Alper, T., W. A. Cramp, D. A. Haig, and M. C. Clarke. 1967. Does the agent of scrapie replicate without nucleic acid? *Nature* 214:764-766.
- Alper, T., D. A. Haig, and M. C. Clarke. 1966. The exceptionally small size of the scrapie agent. *Biochem. Biophys. Res. Commun.* 22:278-284.
- Baron, H., J. Safar, D. Groth, S. J. DeArmond, and S. B. Prusiner. 1999. Biosafety issues in prion diseases, p. 743-777. In S. B. Prusiner (ed.), *Prion biology and diseases*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Bellinger-Kawahara, C., J. E. Cleaver, T. O. Diener, and S. B. Prusiner. 1987. Purified scrapie prions resist inactivation by UV irradiation. *J. Virol.* 61:159-166.
- Bellinger-Kawahara, C., T. O. Diener, M. P. McKinley, D. F. Groth, D. R. Smith, and S. B. Prusiner. 1987. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. *Virology* 160:271-274.
- Bellinger-Kawahara, C. G., E. Kempner, D. F. Groth, R. Gabizon, and S. B. Prusiner. 1988. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. *Virology* 164:537-541.
- Bernoulli, C., J. Siegfried, G. Baumgartner, F. Regli, T. Rabinowicz, D. C. Gajdusek, and C. J. Gibbs, Jr. 1977. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet* i:478-479.
- Bessen, R. A., and R. F. Marsh. 1994. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J. Virol.* 68:7859-7868.
- Bockman, J. M., D. T. Kingsbury, M. P. McKinley, P. E. Bendheim, and S. B. Prusiner. 1985. Creutzfeldt-Jakob disease prion proteins in human brains. *N. Engl. J. Med.* 312:73-78.
- Brown, P., M. Precece, J. P. Brandel, T. Sato, L. McShane, I. Zerr, A. Fletcher, R. G. Will, M. Pocchiari, N. R. Cashman, J. H. d'Aignaux, L. Cervenakova, J. Fradkin, L. B. Schonberger, and S. J. Collins. 2000. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 55:1075-1083.
- Budka, H., A. Aguzzi, P. Brown, J.-M. Brucher, O. Buglani, J. Collinge, H. Diring, F. Gullotta, M. Haltia, J.-J. Hauw, J. W. Ironside, H. A. Kretzschmar, P. L. Lantos, C. Masullo, M. Pocchiari, W. Schlote, J. Tateishi, and R. G. Will. 1995. Tissue handling in suspected Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol.* 5:319-322.
- Carlson, G. A., D. T. Kingsbury, P. A. Goodman, S. Coleman, S. T. Marshall, S. DeArmond, D. Westaway, and S. B. Prusiner. 1986. Linkage of prion protein and scrapie incubation time genes. *Cell* 46:503-511.
- Centers for Disease Control. 1997. Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts—Japan, January 1979-May 1996. *Morb. Mortal. Wkly. Rep.* 46:1066-1069.
- Centers for Disease Control. 1985. Fatal degenerative neurologic disease in patients who received pituitary derived human growth hormone. *Morb. Mortal. Wkly. Rep.* 34:359-360.
- Chandler, R. L. 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. *Lancet* i:1378-1379.
- Cho, H. J. 1983. Inactivation of the scrapie agent by pronase. *Can. J. Comp. Med.* 47:494-496.
- Collins, S., M. G. Law, A. Fletcher, A. Boyd, J. Kaldor, and C. L. Masters. 1999. Surgical treatment and risk of sporadic Creutzfeldt-Jakob disease: a case-control study. *Lancet* 353:693-697.
- Cox, D. R. 1972. Regression models and life-tables. *J. R. Stat. Soc. Ser. A Method.* 34:187-220.
- de Silva, R., and T. Esmonde. 1994. Iatrogenic transmission of Creutzfeldt-Jakob disease: an update. *CNS Drugs* 2:96-101.
- Dickinson, A. G. 1976. Scrapie in sheep and goats, p. 209-241. In R. H. Kimberlin (ed.), *Slow virus diseases of animals and man*. North-Holland Publishing, Amsterdam, The Netherlands.
- Dickinson, A. G., and D. M. Taylor. 1978. Resistance of scrapie agent to decontamination. *N. Engl. J. Med.* 299:1413-1414.
- Diener, T. O., M. P. McKinley, and S. B. Prusiner. 1982. Viroids and prions. *Proc. Natl. Acad. Sci. USA* 79:5220-5224.
- Ernst, D. R., and R. E. Race. 1993. Comparative analysis of scrapie agent inactivation methods. *J. Virol. Methods* 41:193-202.
- Fichet, G., E. Comoy, C. Duval, K. Antloga, C. Dehen, A. Charbonnier, G. McDonnell, P. Brown, C. I. Lasmezas, and J. P. Deslys. 2004. Novel methods for disinfection of prion-contaminated medical devices. *Lancet* 364:521-526.
- Gajdusek, D. C., C. J. Gibbs, Jr., and M. Alpers. 1966. Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature* 209:794-796.
- Gibbs, C. J., Jr., D. C. Gajdusek, D. M. Asber, M. P. Alpers, E. Beck, P. M. Daniel, and W. B. Matthews. 1968. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. *Science* 161:388-389.
- Gibbs, C. J., Jr., D. C. Gajdusek, and R. Lataret. 1978. Unusual resistance to ionizing radiation of the viruses of kuru, Creutzfeldt-Jakob disease. *Proc. Natl. Acad. Sci. USA* 75:6268-6270.
- Gordon, W. S. 1946. Advances in veterinary research. *Vet. Res.* 58:516-520.
- Govaerts, C., H. Wille, S. B. Prusiner, and F. E. Cohen. 2004. Evidence for assembly of prions with left-handed β -helices into trimers. *Proc. Natl. Acad. Sci. USA* 101:8342-8347.
- Healy, D. L., and J. Evans. 1993. Creutzfeldt-Jakob disease after pituitary gonadotrophins. *Br. J. Med.* 307:517-518.
- Hilton, D. A., A. C. Ghani, L. Conyers, P. Edwards, L. McCardle, D. Ritchie, M. Penney, D. Hegazy, and J. W. Ironside. 2004. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J. Pathol.* 203:733-739.
- Holt, J. D., and R. L. Prentice. 1974. Survival analyses in twin studies and matched pair experiments. *Biometrika* 61:17-30.
- Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53:457-481.
- Kimberlin, R. H., and C. A. Walker. 1979. Pathogenesis of scrapie: agent multiplication in brain at the first and second passage of hamster scrapie in mice. *J. Gen. Virol.* 42:107-117.
- Kimberlin, R. H., C. A. Walker, G. C. Millson, D. M. Taylor, P. A. Robertson, A. H. Tomlinson, and A. G. Dickinson. 1983. Disinfection studies with two strains of mouse-passaged scrapie agent. *J. Neurol. Sci.* 59:355-369.
- Kong, Q., W. K. Surewicz, R. B. Petersen, W. Zou, S. G. Chen, P. Gambetti, P. Parchi, S. Capellari, L. Goldfarb, P. Montagna, E. Lugaresi, P. Piccardo, and B. Ghetti. 2004. Inherited prion diseases, p. 673-775. In S. B. Prusiner (ed.), *Prion biology and diseases*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Korth, C., K. Kaneko, D. Groth, N. Heye, G. Telling, J. Mastrianni, P. Parchi, P. Gambetti, R. Will, J. Ironside, C. Heinrich, P. Tremblay, S. J. DeArmond, and S. B. Prusiner. 2003. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. *Proc. Natl. Acad. Sci. USA* 100:4784-4789.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature* 227:680-685.
- Latarjet, R. 1979. Inactivation of the agents of scrapie, Creutzfeldt-Jakob disease, and kuru by radiations, p. 387-408. In S. B. Prusiner and W. J. Hadlow (ed.), *Slow transmissible diseases of the nervous system*, vol. 2. Academic Press, Inc., New York, N.Y.
- Latarjet, R., B. Muel, D. A. Haig, M. C. Clarke, and T. Alper. 1970. Inactivation of the scrapie agent by near monochromatic ultraviolet light. *Nature* 227:1341-1343.
- Legname, G., I. V. Baskakov, H.-O. B. Nguyen, D. Ritsner, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 2004. Synthetic mammalian prions. *Science* 305:673-676.
- Legname, G., H.-O. B. Nguyen, I. V. Baskakov, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 2005. Strain-specified characteristics of mouse synthetic prions. *Proc. Natl. Acad. Sci. USA* 102:2168-2173.
- Lineweaver, H., and D. Burk. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 56:658-666.
- Llewellyn, C. A., P. E. Hewitt, R. S. Knight, K. Amar, S. Cousens, J. Mackenzie, and R. G. Will. 2004. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 363:417-421.
- Marsh, R. F., and R. H. Kimberlin. 1975. Comparison of scrapie and transmissible mink encephalopathy in hamsters. II. Clinical signs, pathology, and pathogenesis. *J. Infect. Dis.* 131:104-110.
- McKinley, M. P., D. C. Bolton, and S. B. Prusiner. 1983. A protease-resistant protein is a structural component of the scrapie prion. *Cell* 35:57-62.
- McKinley, M. P., F. R. Maslars, and S. B. Prusiner. 1981. Reversible chemical modification of the scrapie agent. *Science* 214:1259-1261.
- McKinley, M. P., R. K. Meyer, L. Kenaga, F. Rahbar, R. Cotter, A. Serban, and S. B. Prusiner. 1991. Scrapie prion rod formation in vitro requires both detergent extraction and limited proteolysis. *J. Virol.* 65:1340-1351.
- Michaels, L., and M. I. Merton. 1913. Die kinetische der invertinwirkung. *Biochem. Z.* 49:333-369.
- Millson, G. C., G. D. Hunter, and R. H. Kimberlin. 1976. The physicochemical nature of the scrapie agent, p. 243-266. In R. H. Kimberlin (ed.), *Slow virus diseases of animals and man*. American Elsevier, New York, N.Y.
- Palmer, M. S., A. J. Dryden, J. T. Hughes, and J. Collinge. 1991. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* 352:340-342.
- Parchi, P., A. Giese, S. Capellari, P. Brown, W. Schulz-Schaeffer, O. Windl, I. Zerr, H. Budka, N. Kopp, P. Piccardo, S. Poser, A. Rojiani, N. Streichenberger, J. Julien, C. Vital, B. Ghetti, P. Gambetti, and H. Kretzschmar. 1999. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann. Neurol.* 46:224-233.
- Pattison, I. H. 1966. The relative susceptibility of sheep, goats and mice to two types of the goat scrapie agent. *Res. Vet. Sci.* 7:207-212.
- Peden, A. H., M. W. Head, D. L. Ritchie, J. E. Bell, and J. W. Ironside. 2004. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 364:527-529.
- Peretz, D., M. Scott, D. Groth, A. Williamson, D. Burton, F. E. Cohen, and S. B. Prusiner. 2001. Strain-specified relative conformational stability of the scrapie prion protein. *Protein Sci.* 10:854-863.

57. Peretz, D., R. A. Williamson, G. Legname, Y. Matsunaga, J. Vergara, D. Burton, S. J. DeArmond, S. B. Prusiner, and M. R. Scott. 2002. A change in the conformation of prions accompanies the emergence of a new prion strain. *Neuron* 34:921-932.
58. Prusiner, S. B. 2004. Development of the prion concept, p. 89-141. *In* S. B. Prusiner (ed.), *Prion biology and diseases*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
59. Prusiner, S. B. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* 216:136-144.
60. Prusiner, S. B. (ed.). 2004. *Prion biology and diseases*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
61. Prusiner, S. B., S. P. Cochran, D. E. Downey, and D. F. Groth. 1981. Determination of scrapie agent titer from incubation period measurements in hamsters, p. 385-399. *In* J. W. Streilein, D. A. Hart, J. Stein-Streilein, W. R. Duncan, and R. E. Billingham (ed.), *Hamster immune responses in infectious and oncologic diseases*. Plenum Press, Inc., New York, N.Y.
62. Prusiner, S. B., S. P. Cochran, D. F. Groth, D. E. Downey, K. A. Bowman, and H. M. Martinez. 1982. Measurement of the scrapie agent using an incubation time interval assay. *Ann. Neurol.* 11:353-358.
63. Prusiner, S. B., D. Groth, A. Serban, N. Stahl, and R. Gabizon. 1993. Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc. Natl. Acad. Sci. USA* 90:2793-2797.
64. Prusiner, S. B., D. F. Groth, S. P. Cochran, F. R. Maslarsz, M. P. McKinley, and H. M. Martinez. 1980. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry* 21:4833-4891.
65. Prusiner, S. B., D. F. Groth, M. P. McKinley, S. P. Cochran, K. A. Bowman, and K. C. Kasper. 1981. Thiocyanate and hydroxyl ions inactivate the scrapie agent. *Proc. Natl. Acad. Sci. USA* 78:4606-4610.
66. Prusiner, S. B., M. P. McKinley, D. C. Bolton, K. A. Bowman, D. F. Groth, S. P. Cochran, E. M. Hennessey, M. B. Braunfeld, J. R. Baringer, and M. A. Chaitigny. 1984. Prions: methods for assay, purification and characterization, p. 293-345. *In* K. Maramorosch and H. Koprowski (ed.), *Methods in virology*, vol. 8. Academic Press, Inc., New York, N.Y.
67. Prusiner, S. B., M. P. McKinley, K. A. Bowman, D. C. Bolton, P. E. Bendheim, D. F. Groth, and G. G. Glenner. 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* 35:349-358.
68. Prusiner, S. B., M. P. McKinley, D. F. Groth, K. A. Bowman, N. I. Mock, S. P. Cochran, and F. R. Maslarsz. 1981. Scrapie agent contains a hydrophobic protein. *Proc. Natl. Acad. Sci. USA* 78:6675-6679.
69. Prusiner, S. B., J. Safar, and S. J. DeArmond. 2004. Bioassays of prions, p. 143-186. *In* S. B. Prusiner (ed.), *Prion biology and diseases*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
70. Prusiner, S. B., M. Scott, D. Foster, K.-M. Pan, D. Groth, C. Miranda, M. Torchia, S.-L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway, and S. J. DeArmond. 1990. Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* 63:673-686.
71. Race, R. E., and G. J. Raymond. 2004. Inactivation of transmissible spongiform encephalopathy (prion) agents by environ L-pH. *J. Virol.* 78:2164-2165.
72. Ridley, R. M., and H. F. Baker. 1996. To what extent is strain variation evidence for an independent genome in the agent of the transmissible spongiform encephalopathies? *Neurodegeneration* 5:219-231.
73. Rosenberg, R. N., L. L. White, III, P. Brown, D. C. Gajdusek, J. J. Volpe, J. Posner, and P. J. Dyck. 1986. Precautions in handling tissues, fluids, and other contaminated materials from patients with documented or suspected Creutzfeldt-Jakob disease. *Ann. Neurol.* 19:75-77.
74. Rutala, W. A. 1996. APIC guideline for selection and use of disinfectants. *Am. J. Infect. Control.* 24:313-342.
75. Rutala, W. A., and D. J. Weber. 2001. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clin. Infect. Dis.* 32:1348-1356.
76. Safar, J., D. Groth, S. J. DeArmond, and S. B. Prusiner. 1999. Prions, p. 134-147. *In* J. Richmond and R. W. McKinney (ed.), *Biosafety in microbiological and biomedical laboratories*, 4th ed. U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, Washington, D.C.
77. Safar, J., H. Wille, V. Itri, D. Groth, H. Serban, M. Torchia, F. E. Cohen, and S. B. Prusiner. 1998. Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat. Med.* 4:1157-1165.
78. Scott, M., D. Foster, C. Miranda, D. Serban, F. Coufal, M. Wälchli, M. Torchia, D. Groth, G. Carlson, S. J. DeArmond, D. Westaway, and S. B. Prusiner. 1989. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* 59:847-857.
79. Scott, M. R., D. Peretz, H.-O. B. Nguyen, S. J. DeArmond, and S. B. Prusiner. 2005. Transmission barriers for bovine, ovine, and human prions in transgenic mice. *J. Virol.* 79:5259-5271.
80. Setlow, R. B. 2002. Shedding light on proteins, nucleic acids, cells, humans, and fish. *Mutat. Res.* 511:1-14.
81. Somerville, R. A., R. C. Oberthür, U. Havekost, F. MacDonald, D. M. Taylor, and A. G. Dickinson. 2002. Characterization of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications. *J. Biol. Chem.* 277:11084-11089.
82. Steelman, V. M. 1999. Prion diseases—an evidence-based protocol for infection control. *AORN J.* 69:946-954; 956-967.
83. Supattapone, S., H.-O. B. Nguyen, F. E. Cohen, S. B. Prusiner, and M. R. Scott. 1999. Elimination of prions by branched polyamines and implications for therapeutics. *Proc. Natl. Acad. Sci. USA* 96:14529-14534.
84. Supattapone, S., H. Wille, L. Ueychi, J. Safar, P. Tremblay, F. C. Szoka, F. E. Cohen, S. B. Prusiner, and M. R. Scott. 2001. Branched polyamines cure prion-infected neuroblastoma cells. *J. Virol.* 75:3453-3461.
85. Tamai, Y., F. Taguchi, and S. Miura. 1988. Inactivation of the Creutzfeldt-Jakob disease agent. *Ann. Neurol.* 24:466-467.
86. Tateishi, J., T. Tashima, and T. Kitamoto. 1988. Inactivation of the Creutzfeldt-Jakob disease agent. *Ann. Neurol.* 24:466.
87. Tateishi, J., T. Tashima, and T. Kitamoto. 1991. Practical methods for chemical inactivation of Creutzfeldt-Jakob disease pathogen. *Microbiol. Immunol.* 35:163-166.
88. Taylor, D. M. 1999. Inactivation of prions by physical and chemical means. *J. Hosp. Infect.* 43:569-576.
89. Taylor, D. M. 2000. Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet. J.* 159:10-17.
90. Taylor, D. M., K. Fernie, I. McConnell, and P. J. Steele. 1999. Survival of scrapie agent after exposure to sodium dodecyl sulphate and heat. *Vet. Microbiol.* 67:13-16.
91. Taylor, D. M., K. Fernie, P. J. Steele, I. McConnell, and R. A. Somerville. 2002. Thermostability of mouse-passaged BSE and scrapie is independent of host PrP genotype: implications for the nature of the causal agents. *J. Gen. Virol.* 83:3199-3204.
92. Taylor, D. M., S. L. Woodgate, and M. J. Atkinson. 1995. Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *Vet. Rec.* 137:605-610.
93. Taylor, D. M., S. L. Woodgate, A. J. Fleetwood, and R. J. G. Cawthorne. 1997. Effect of rendering procedures on the scrapie agent. *Vet. Rec.* 141:643-649.
94. Telling, G. C., P. Parchi, S. J. DeArmond, P. Cortelli, P. Montagna, R. Gabizon, J. Mastrianni, E. Lugaresi, P. Gambetti, and S. B. Prusiner. 1996. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 274:2079-2082.
95. Weissmann, C., M. Enari, P. C. Klohn, D. Rossi, and E. Flechsig. 2002. Transmission of prions. *Proc. Natl. Acad. Sci. USA* 99:16378-16383.
96. Westaway, D., S. J. DeArmond, J. Cayetano-Cañas, D. Groth, D. Foster, S.-L. Yang, M. Torchia, G. A. Carlson, and S. B. Prusiner. 1994. Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. *Cell* 76:117-129.
97. Will, R. G., M. P. Alpers, D. Dormont, and L. B. Schonberger. 2004. Infectious and sporadic prion diseases, p. 629-671. *In* S. B. Prusiner (ed.), *Prion biology and diseases*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
98. Will, R. G., J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiarri, A. Holtman, and P. G. Smith. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 347:921-925.
99. Wille, H., M. D. Michelitsch, V. Guénebaud, S. Supattapone, A. Serban, F. E. Cohen, D. A. Agard, and S. B. Prusiner. 2002. Structural studies of the scrapie prion protein by electron crystallography. *Proc. Natl. Acad. Sci. USA* 99:3563-3568.
100. Zobeley, E., E. Flechsig, A. Cozzio, M. Enari, and C. Weissmann. 1999. Infectivity of scrapie prions bound to a stainless steel surface. *Mol. Med.* 5:240-243.
101. Zou, W. Q., J. Zheng, D. M. Gray, P. Gambetti, and S. G. Chen. 2004. Antibody to DNA detects scrapie but not normal prion protein. *Proc. Natl. Acad. Sci. USA* 101:1380-1385.