Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 2793–2797, April 1993
Medical Sciences

Attempts to restore scrapie prion infectivity after exposure to protein denaturants

(protein renaturation/SDS/PAGE/urea/guanidinium)

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ABSTRACT **A wealth of experimental evidence argues that infectious prions are composed largely, if not entirely, of the scrapie isoform of the prion protein. We attempted to restore scrapie infectivity after exposure to protein denaturants including urea, chaotropic salts, and SDS. None of the procedures restored infectivity. Dialysis to remove slowly chaotropic ions and urea failed to restore scrapie infectivity. Attempts to create monomers of the scrapie isoform of the prion protein under nondenaturing conditions using a wide variety of detergents have been unsuccessful, to date, except for one report claiming that scrapie infectivity could be recovered from 12% polyacrylamide gels after SDS/PAGE [Brown, P., Liberski, P. P., Wolff, A. & Gajdusek, D. C. (1990) *Proc. Natl. Acad. Sci. USA* 87, 7240–7244]. We found that <0.001% of the infectious prion titer could be recovered from the region of a polyacrylamide gel where the denatured proteinase K-resistant core of the scrapie isoform of the prion protein and other 30-kDa proteins migrate. We conclude that under the denaturing conditions used for SDS/PAGE, the scrapie isoform of the prion protein is denatured and little or no renaturation occurs upon injection of fractions eluted from gels into animals for bioassays.**

The discovery of the protease-resistant polypeptide of the scrapie isoform of the prion protein (PrP^{Sc}) designated PrP 27-30 in fractions enriched for scrapie infectivity (1, 2) suggested that characterization of this protein might advance understanding of scrapie and related neurodegenerative diseases. Indeed, a wealth of experimental evidence argues that infectious prion particles are composed largely, if not entirely, of PrP^{Sc} from which PrP 27-30 is derived by limited proteolysis (3). The PrP is encoded by a chromosomal gene and PrP^{Sc} is produced from the cellular isoform of PrP by a post-translational process (4).

Physical studies of PrP^{Sc} have been difficult because the protein has resisted solubilization with detergents under nondenaturing conditions. To circumvent these difficulties, phospholipids alone or in combination with detergents have been used to solubilize PrP^{Sc} under conditions where prion infectivity is either preserved or enhanced (5). Attempts to create monomers of PrP^{Sc} or PrP 27-30 under nondenaturing conditions using a wide variety of detergents have been unsuccessful although there have been occasional reports to the contrary. In one set of studies, it was thought that scrapie infectivity could be electrophoresed through a composite agarose/polyacrylamide gel in the presence of SDS and eluted in fractions where it could be inactivated upon digestion with DNases (6, 7). Neither the recovery of high levels of infectivity from the electrophoretic gels nor the sensitivity to DNase could be confirmed (8). More recently, a study (9) has reported that scrapie infectivity could be recovered in fractions comigrating with PrP^{Sc} in SDS/PAGE with an apparent molecular mass of ≈30 kDa (9).

Because nondenatured PrP^{Sc} has been inseparable from scrapie infectivity, the report of experiments demonstrating recovery of fractions after SDS/PAGE with high prion titers (9) was particularly intriguing. Thus, we attempted to reproduce the conditions of those experiments as closely as possible but repeatedly failed to recover >0.001% of the infectious prions after SDS/PAGE. We also report here on studies designed to renature PrP^{Sc} and restore prion infectivity after denaturation during SDS/PAGE or by exposure to urea and chaotropes such as guanidinium (Gdn), thiocyanate (SCN), and Gdn·HCl. Attempts to renature PrP^{Sc} by dispersion in phospholipids or stepwise removal of the denaturant failed to restore scrapie infectivity or protease resistance.

MATERIALS AND METHODS

All chemicals were of highest grades commercially available. SDS was obtained from Bio-Rad, Gdn salts were from Fluka, urea was from Schwarz/Mann, acrylamide was from Bio-Rad, and phospholipids were from Avanti (Polar Lipids).

A Syrian hamster-adapted scrapie prion inoculum, designated Sc237, that causes disease in ≈70 days in adult Syrian hamsters was used in these studies (10). This inoculum (11) was originally obtained from Richard Marsh (University of Wisconsin, Madison) and is similar to the inoculum labeled 263K (12) that was used in the studies by Brown *et al.* (9).

Incubation time bioassays and end-point titrations were performed as described using Syrian hamsters (13). Weanling random-bred Syrian hamsters (Lak:LVG) from a closed colony were purchased from Charles River Breeding Laboratories and inoculated intracerebrally with 50 μl. Dilutions of samples were factored into the prion titers reported here except for those samples which did not produce illness in hamsters; in those cases, the titer is designated as <1.0 log ID_{50} unit/ml. This was done because of the difficulties in estimating low titers (13). Differences in prion titers of a factor of <30 were generally not considered significant.

Purified prions and partially purified fractions were prepared from Syrian hamster brains as described (14) using detergent extraction, limited proteolysis, $(NH_4)_2SO_4$ fractionation, and discontinuous-sucrose-gradient centrifugation. Fractions were evaluated for the presence of PrP 27-30 by both silver staining and immunoblot analysis. Total protein in the fractions was determined by a dye binding assay, with bovine serum albumin as standard (Pierce). The sucrose

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Abbreviations: PrP, prion protein; PrP^{Sc}, scrapie isoform of the prion protein; PrP 27-30, proteinase K-resistant core of PrP^{Sc}; Gdn, guanidinium; SCN, thiocyanate.

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gradient fraction was ethanol-precipitated with 10 vol and the proteins were resuspended to 250 μg/ml in sample buffer containing 5% (vol/vol) 2-mercaptoethanol, 2% (wt/vol) SDS, 10% (vol/vol) glycerol, and 0.01% bromophenol blue dye, buffered with 0.5 M Tris·HCl (pH 6.8).

Electrophoresis using a 1.5-mm 12% polyacrylamide minigel in the presence of 0.1% SDS was performed as described by Laemmli (15). Prior to electrophoresis, 0.4-ml samples were boiled for 2 min in a buffer containing 2% SDS. Electrophoresis was performed for 1 h at 150 V, until the dye front was at the bottom of the gel. PrP was localized by either silver staining an adjacent lane or using prestained markers. The molecular mass values for proteins were determined by using prestained Bio-Rad low molecular weight markers. The gel was cut into four sections. Each section was homogenized in 5 ml of phosphate-buffered saline and passed through a 22-gauge needle prior to inoculation.

Phospholipids were dried onto the wall of glass tubes to which prion samples were added (5). Dispersion of prions was accomplished by a bath sonicator (model G112SPT, Lab Supplies, Hicksville, NY). Sonication was performed for 10–20 min until clear.

RESULTS

Syrian hamsters were inoculated intracerebrally with $\approx 10^7$ ID_{50} units of Syrian hamster Sc237 prions. Syrian hamsters exhibiting clinical signs of experimental scrapie were sacrificed ≈70 days after inoculation and their brains were removed. Brains were stored at −70°C prior to purification. Typically, 1000 frozen brains were homogenized in 0.32 M sucrose using a Polytron device (Brinkman). Purification of prion rods composed of PrP 27-30 was accomplished by detergent extractions, limited proteolysis, $(NH_4)_2SO_4$ fractionation, and sedimentation through a discontinuous sucrose gradient (14). Fractions obtained from different steps in the purification scheme were used in these studies.

Chaotrope Inactivation of Prions. The chaotropic salts GdnHCl and GdnSCN were used to inactivate prions in purified fractions in an effort to identify conditions for renaturation of PrP^{Sc} and restoration of scrapie infectivity. The diminution of scrapie prion infectivity upon exposure to 6 M GdnSCN was rapid and complete almost immediately (Table 1). Complete inactivation was achieved with 3 M GdnSCN after a prolonged exposure of 24 h, whereas 4 M GdnSCN sterilized the prions in purified fractions after 1 h of exposure.

To restore protease resistance and regain prion infectivity, fractions were subjected to stepwise dilution of the chaotrope or its removal by dialysis. Preparations were incubated or dialyzed for up to 4 days to investigate the possibility that refolding of denatured PrP^{Sc} is a slow process. No protease-resistant PrP was found under these conditions and no prion infectivity was recovered (Table 2).

Table 1. Kinetics of scrapie prion inactivation by GdnSCN

GdnSCN, M	log prion titer (ID_{50} units/ml)		
	0 h	1 h	24 h
0	8.4 ± 0.11	8.6 ± 0.16	8.3 ± 0.10
1	8.3 ± 0.09	8.6 ± 0.17	8.2 ± 0.14
2	8.2 ± 0.07	5.6 ± 0.15	3.7 ± 0.67
3	5.1 ± 0.18	3.0 ± 0.34	<1.0
4	2.1 ± 0.62	<1.0	<1.0
5	<1.0	<1.0	<1.0
6	<1.0	<1.0	<1.0

Fractions enriched for prion rods (0.15 mg of protein per ml) were collected from discontinuous sucrose gradients (2). Samples were diluted ≈1:1000 after the time noted (0 h, 1 h, or 24 h) of GdnSCN exposure and stored at −70°C prior to bioassay. Data are the mean ± SEM.

Urea Inactivation of Prions. Results similar to those obtained with GdnSCN were found when partially purified fractions were exposed to increasing concentrations of urea from 1 to 8 M in the absence or presence of 2% (vol/vol) Triton X-100 for 2 or 24 h (Fig. 1). The urea was removed from an aliquot of each sample by dialysis prior to bioassay, but no increase in scrapie infectivity was found compared to aliquots not dialyzed.

SDS/PAGE of Purified Prions. While the foregoing experiments designed to renature prion infectivity were in progress, the studies of Brown *et al.* (9) were published claiming electrophoresis of infectious prions through SDS/polyacrylamide gels. Puzzled by those results, we purified fractions containing rod-shaped polymers of PrP 27-30 from scrapie-infected hamster brains and subjected them to SDS/PAGE. As shown, the prion rods contain primarily one protein of 27–30 kDa by silver staining that reacted with polyclonal antibody raised against Syrian hamster PrP 27-30 (Fig. 2, lanes 1 and 3). After electrophoresis, the SDS/polyacrylamide gels were cut into four sections and then subjected to either SDS/PAGE again (Fig. 2, lanes 2 and 4) or bioassay of prion titers in Syrian hamsters.

Sucrose gradient fractions contained prions at $>10^8$ ID_{50} units/ml as determined by incubation time bioassay (13). Neither addition of 2% SDS nor boiling appreciably altered the prion titer (Table 3). For this reason, we routinely denature the purified prion rods with 6 M GdnSCN prior to SDS/PAGE, since boiling in SDS alone often reduces the titer by only a factor of 10 and solubilizes 60–90% of the PrP 27-30, as judged by its entry into polyacrylamide gels. We omitted the GdnSCN denaturation to simulate the studies reported by Brown *et al.* (9). The data from three experiments are shown using either the macerated gel or an eluate injected intracerebrally into Syrian hamsters for bioassay. Although $\approx 10^6$ ID_{50} units/ml were recovered from the stacking gel and the top of the separating gel, $<10^3$ ID_{50} units/ml were found in the lower three sections. The third section of the polyacrylamide gel where PrP 27-30 migrates contained prions at $<10^2$ ID_{50} units/ml (Table 3). Thus, the scrapie prion infec-

Table 2. Attempts to restore scrapie prion infectivity after inactivation by GdnSCN

Condition	GdnSCN (6 M)	log prion titer (ID_{50} units/ml) Exp. 1	Exp. 2
Prion rods	−	8.0 ± 0.31	8.4 ± 0.18
Diluted with buffer	+	<1.0	2.9 ± 1.7
Diluted stepwise	+	4.3 ± 0.19	1.6 ± 0.03
Dialyzed against buffer	+		<1.0
Dialyzed stepwise	+		<1.0
Prion DLPCs	−		8.8 ± 0.24
Diluted with buffer	+		2.7 ± 0.05
Diluted stepwise	+		<1.0
Dialyzed against buffer	+		<1.0
Dialyzed stepwise	+		<1.0

Purified prion rods (0.15 mg of protein per ml) were mixed with 6 M GdnSCN. After exposure to GdnSCN for 16 h at room temperature, samples were diluted with buffer containing Tris-buffered saline (TBS), diluted stepwise into TBS resulting in first 3 M GdnSCN, then 1.5 M GdnSCN, 0.38 M GdnSCN, and then 0.09 M GdnSCN. Other samples were dialyzed against buffer containing TBS or stepwise against buffers with 3 M GdnSCN, 1.5 M GdnSCN, 0.38 M GdnSCN, and 0.09 M GdnSCN. Final dilution of all samples containing GdnSCN was ≈1:1000 prior to bioassay. For those samples without GdnSCN, equivalent titers were measured whether or not similar dilutions were made (13). Data are the mean ± SEM. DLPCs, detergent–lipid–protein complexes.

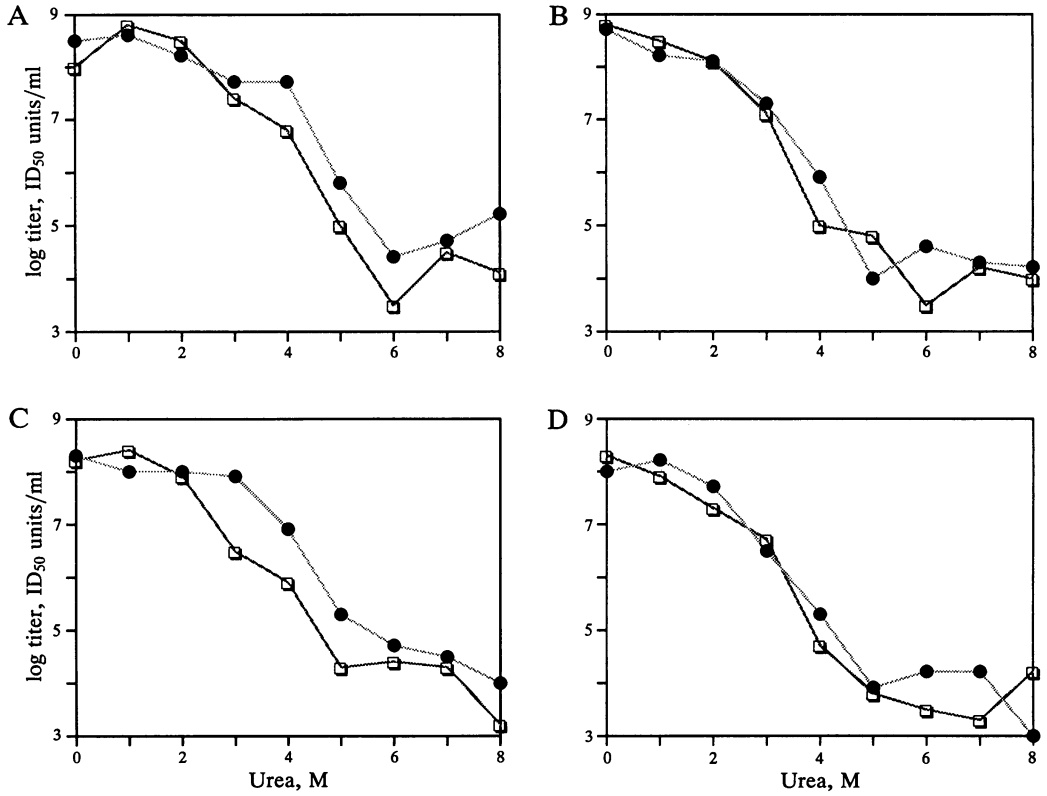

FIG. 1. Urea inactivation of scrapie prion infectivity. Increasing concentrations of urea were added to a partially purified fraction denoted P5 (□), aliquots of which were dialyzed against 100 vol of 20 mM TrisOAc (pH 8.3) containing 1.0 mM EDTA for 1–2 h followed by two additional dialyses under the same conditions (●) after exposure to urea for 2 h (*A*) or 24 h (*B*). The experiment was repeated in the presence of 2% Triton X-100 with exposure to urea for 2 h (*C*) or 24 h (*D*). The data in *C* were previously published in a review article (16), reprinted with permission from ref. 16 (copyright Academic, Orlando, FL).

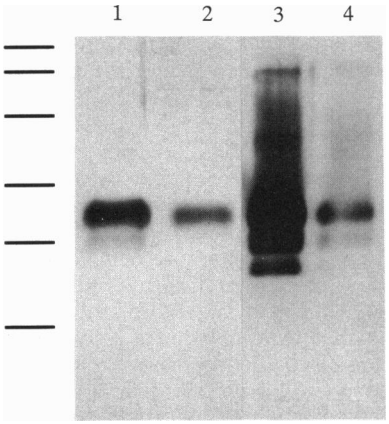

FIG. 2. SDS/PAGE of purified Syrian hamster Sc237 prions. Purified prion rods containing Syrian hamster PrP 27-30 were boiled in 2% SDS according to Laemmli (15). Silver-stained 12% polyacrylamide gel is in lane 1; an adjacent preparative, unstained lane was cut into four sections for bioassays, the results of which are shown in Tables 3 and 4. Immunostaining of SDS/PAGE after transfer to nitrocellulose with Syrian hamster PrP antibodies (lane 3). An aliquot of section 3 with proteins of 30 kDa was subjected to SDS/PAGE for a second time and the 12% gel was stained with silver (lane 2) or PrP antiserum RO73 (lane 4). Gel section 3 was eluted in 5.0 ml of PBS and 0.5 ml was loaded onto lane 2 and 0.1 ml was loaded onto lane 4. Positions of migration of molecular mass protein markers are shown by horizontal lines on the left, from top to bottom: phosphorylase *b*, 106 kDa; bovine serum albumin, 80 kDa; ovalbumin, 49.5 kDa; carbonic anhydrase, 32.5 kDa; soybean trypsin inhibitor, 27.5 kDa; lysozyme, 18.5 kDa.

tivity in the third section of the gel after SDS/PAGE was reduced by a factor of $\approx 10^6$. The results of these studies were highly reproducible as shown by the consistent data for three experiments.

Since the bioassays are based on the length of the incubation period, serial dilutions of the eluted samples from the third section were performed to control for the possibility that a new "strain" or isolate of prions was created during SDS/PAGE with a prolonged incubation time. As shown in Table 4, no infectivity was found in the sample that was

Table 3. Scrapie prion infectivity recovered after SDS/PAGE: Prion infectivity in fractions prepared by SDS/PAGE

Fraction	log prion titer (ID_{50} units/ml)		
	Exp. 1	Exp. 2	Exp. 3
Sucrose gradient	8.2 ± 0.33	8.2 ± 0.23	8.1 ± 0.46
2% SDS	9.1 ± 0.14	8.9 ± 0.16	8.8 ± 0.14
2% SDS at 100°C	8.2 ± 0.36	7.7 ± 0.17	8.4 ± 0.12
SDS/PAGE section			
1	5.9 ± 0.26	7.7 ± 0.28	5.7 ± 0.0
2	2.9 ± 0.09	2.6 ± 0.19	<1.0
3	<1.0	<1.5	<1.0
4	2.8 ± 0.10	2.8 ± 0.41	2.2 ± 1.0

Four SDS/PAGE sections of a 12% polyacrylamide gel correspond to the following molecular mass values: section 1, >116 kDa; section 2, >32 kDa; section 3, >18.5 kDa; section 4, <18.5 kDa. Each preparative, SDS/PAGE lane was loaded with 0.4 ml of prion rods (0.25 mg of protein per ml) that had been boiled for 2 min in 2% SDS and 5% 2-mercaptoethanol. Gel sections were homogenized in 5 ml of PBS and 50-μl aliquots were inoculated for bioassay. When total prion infectivities (titer × volume) were calculated, 13%, 200%, and 5% of the loaded prions were recovered in gel section 1 for experiments 1–3, respectively. Data are the mean ± SEM.

diluted by a factor of $>10^3$ from experiment 3 and only 50% of the animals receiving a sample diluted by a factor of 10^3 in experiment 2 developed illness by 275 days after inoculation. These results demonstrate that a new isolate with different properties was not generated and that the results of the incubation time bioassays are reliable.

Dispersion of SDS/PAGE Fractions in Phospholipids. We attempted to renature fractions eluted from SDS/polyacrylamide gels by dispersing the fractions in a variety of phospholipids in the presence of sodium cholate (Table 5). The phospholipids used included phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine. No increase in scrapie infectivity was found after dispersion of the eluate into liposomes or detergent–lipid–protein complexes by bioassays.

DISCUSSION

A wealth of data argues that prions are composed largely if not entirely of PrP^{Sc} molecules, but it has been difficult to determine the size of the monomeric infectious unit because PrP^{Sc} molecules are so insoluble (17–19). Attempts to find a nondenaturing detergent in which PrP^{Sc} molecules can be dissociated with retention of protease resistance and prion infectivity have been unsuccessful. Functional solubilization has been accomplished only by using large quantities of phospholipids such as phosphatidylcholine to form detergent–lipid–protein complexes (5), but attempts to determine the physical state of PrP^{Sc} molecules within these vesicles have been problematic. Recent studies argue that PrP^{Sc} molecules are not monomeric within these vesicles since measurements by attenuated-total-reflection (ATR)–Fourier-transform-infrared (FTIR) spectroscopy indicate a high degree of β-sheet content, which is largely low-frequency β (20). The presence of low-frequency β indicates intermolecular interactions among PrP^{Sc} molecules. While these ATR–FTIR spectroscopy measurements represent somewhat indirect evidence concerning the multimeric state of PrP^{Sc}, the insolubility of PrP^{Sc} molecules has prevented accurate determination of the number of PrP^{Sc} molecules within each phospholipid vesicle. In addition, such studies are complicated by the fact that there are $\approx 10^5$ PrP^{Sc} molecules per infectious unit (14).

The results of studies reported here (Tables 3 and 4) are in accord with investigations reported more than a decade ago (8). In those investigations, we reported (8) that scrapie infectivity was lost after SDS/PAGE. Our results contrasted with those of earlier studies that claimed nearly quantitative recovery of scrapie infectivity after electrophoresis through composite agarose/polyacrylamide gels in the presence of SDS (6). In those studies, it was thought that an essential component of scrapie infectivity was a low molecular weight DNA (7) similar in size to the RNA viroids of plants. Subsequent studies also showed that prion infectivity was resistant to digestion with a variety of nucleases including DNases (21).

Table 4. Scrapie prion infectivity recovered after SDS/PAGE: Serial dilutions of SDS/PAGE section 3

log dilution	Exp. 1		Exp. 2		Exp. 3	
	n/n_0	Days	n/n_0	Days	n/n_0	Days
1	8/8	143 ± 7.8	5/5	219 ± 16.8	8/10	162 ± 13.8
2	4/5	230 ± 42.8			4/4	241 ± 48.0
3	3/3	269 ± 5.0	4/8	275 ± 1.4	0/3	
4					0/3	
5					0/2	
6					0/4	

n, number developing scrapie; n_0, number inoculated with prions; days, time from inoculation to development of clinical signs of scrapie. Data for days are the mean ± SEM.

Table 5. Attempts to restore scrapie infectivity from eluted fractions after SDS/PAGE by dispersion into phospholipids

Prion preparation			log prion titer (ID_{50} units/ml)	
Prion rods	Gel eluate	Phospholipid(s)	Exp. 1	Exp. 2
+		—	8.1 ± 0.23	8.8 ± 0.17
	+	—	3.9 ± 0.31	3.7 ± 0.41
+		Cholate (2%)	8.5 ± 0.19	8.9 ± 0.30
+		PC		8.0 ± 0.35
	+	PC	3.5 ± 0.23	
+		PC + 2% cholate	8.6 ± 0.18	
	+	PC + 2% cholate	1.6 ± 0.64	2.5 ± 0.12
	+	PS		3.9 ± 0.30
+		PS + PC	9.0 ± 0.24	7.2 ± 0.22
	+	PS + PC	3.3 ± 0.17	3.9 ± 0.37
+		PE + oleic acid	6.9 ± 0.32	6.5 ± 0.17
	+	PE + oleic acid	<1.0	2.4 ± 0.13

Purified prion rods in sucrose gradient fractions were precipitated with 4 vol of ethanol at −20°C and collected by centrifugation at 14,000 × *g* for 15 min. The rods were resuspended in 2% SDS sample buffer and boiled 4 min. SDS/PAGE was performed as described by Laemmli (15). The SDS/PAGE eluate equivalent to gel section 3 in Table 3 and the rods were dispersed in phospholipids using a bath sonicator as described (5). PC, phosphatidylcholine; PS, phosphoserine; PE, phosphatidylethanolamine.

In view of the foregoing published data and the results presented here, studies claiming that scrapie infectivity was preserved after electrophoresis through 12% polyacrylamide gels in the presence of SDS (9) remain perplexing. Although we had often obtained results to the contrary (8), we performed the experiments reported here (Tables 3 and 4) to mimic the experimental conditions reported by Brown *et al.* (9) and to determine whether our earlier studies (8, 13) were flawed. We are forced to conclude that our earlier observations are correct. While our investigations were in progress, independent studies undertaken by James Hope (22) also failed to reproduce the recovery of scrapie infectivity after SDS/PAGE.

Our attempts to renature PrP^{Sc} and prion infectivity after denaturation during SDS/PAGE using phospholipids were unsuccessful (Table 5). It is noteworthy that a low molecular mass peak of scrapie infectivity was found by size-exclusion chromatography in the presence of SDS followed by dispersion of each fraction in a mixture of phospholipids isolated from the brains of normal healthy Syrian hamsters (23). Unfortunately, <0.1% of the starting infectivity was recovered in the low molecular mass peak, which necessitates that any conclusions be guarded. Renaturation of prion infectivity after exposure to chaotropic ions or urea was also unsuccessful under a wide variety of conditions. Neither dialysis against decreasing concentrations of chaotropes nor the stepwise dilution of denaturant resulted in the recovery of prion infectivity (Table 2). Whether proteins such as chaperones can assist in refolding denatured PrP^{Sc} and catalyze the restoration of scrapie infectivity remains to be established (24).

While results from numerous experimental studies (3, 19) argue persuasively that PrP^{Sc} is an essential component of the transmissible prion, a few investigators have claimed that PrP^{Sc} can be dissociated from scrapie infectivity. In kinetic studies with Syrian hamsters, prion infectivity was stated to increase more rapidly than PrP^{Sc} levels but infectivity in brain homogenates was compared to PrP^{Sc} in purified fractions (25, 26). A subsequent investigation showed that prion infectivity and PrP^{Sc} levels increased concurrently in Syrian hamsters

inoculated with scrapie prions (27). Repeated injections of amphotericin B into Syrian hamsters were found to delay the onset of disease. After infection with Syrian hamster 263K prions, which are similar to the Syrian hamster Sc237 prions used in the studies reported here, a transient dissociation between the titer of prions and the levels of PrP^{Sc} in brain during the early phase of infection was reported (28). This apparent dissociation was found in amphotericin B-treated Syrian hamsters inoculated with only one isolate, Syrian hamster 263K prions. No dissociation was found in the animals not treated with amphotericin B but inoculated with Syrian hamster 263K prions or in treated or untreated hamsters inoculated with Syrian hamster 139H prions or mice injected with a murine isolate. Whether amphotericin B causes an altered distribution or sequestration of prions early after inoculation with Syrian hamster 263K prions that makes the measurement of PrP^{Sc} problematic remains to be established. Histoblots of PrP^{Sc} distribution in Syrian hamsters after inoculation with Syrian hamster Sc237 prions show that PrP^{Sc} molecules are highly localized and that PrP plaques appear early during the course of infection (29, 30). It will be of interest to learn the results of histoblot studies of Syrian hamsters and transgenic mice expressing the Syrian hamster PrP genes inoculated with Syrian hamster Sc237 prions and treated with amphotericin B.

The reduction of scrapie prion infectivity by a factor of $>10^4$ with urea (Fig. 1) is noteworthy with respect to Creutzfeldt–Jakob disease (CJD) in patients treated with pituitary-derived human growth hormone (hGH) (31, 32). Since 1985, >40 young adults have developed CJD between 3 and 21 years after treatment with hGH. It is thought that preparations of hGH derived from batches of 5000–10,000 human pituitaries are contaminated with human prions since ≈ 1 in 10^4 deaths are due to sporadic CJD. Urea has been used in the purification of hGH from human pituitaries and has been suggested as a reagent for sterilization of hormone preparations (33, 34). Although prion infectivity in partially purified fractions was reduced by 8 M urea (Fig. 1), sterilization was not achieved.

In the studies described here and in earlier investigations (21, 35), denaturation of PrP^{Sc} has been consistently accompanied by a reduction in scrapie infectivity. To restore the protease resistance of PrP^{Sc} and scrapie prion infectivity, we used procedures that have been employed to renature many enzymes. Neither PrP^{Sc} nor prion infectivity could be renatured under a variety of conditions. Although "irreversible" denaturation might be a consequence of separating PrP^{Sc} from an essential second component such as a polynucleotide or complex lipid during SDS/PAGE, this argument seems to be a less likely explanation for the results obtained with Gdn salts and urea. Whereas a few investigators have chosen to interpret a wealth of molecular genetic studies in terms of the cellular isoform of PrP being a receptor for the ubiquitous scrapie "virus" and PrP^{Sc} as a pathologic product of the "viral" infection (36, 37), the studies presented here and many other lines of evidence contend that this is not the case. Indeed, considerable evidence argues persuasively that PrP^{Sc} is an essential and possibly the only component of the transmissible prion particle (3).

We thank Drs. T. O. Diener and Charles Weissmann for helpful discussions during this work and Michael Baldwin for his review of the manuscript. This work was supported by research grants from the National Institutes of Health (AG02132, NS14069, AG08967, and NS22786) and by gifts from Sherman Fairchild Foundation and National Medical Enterprises.

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