

# Infectivity of Scrapie Prions Bound to a Stainless Steel Surface

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Communicated by C. Weissmann. Accepted February 23, 1999.

## Abstract

**Background:** The transmissible agent of Creutzfeldt-Jakob disease (CJD) is not readily destroyed by conventional sterilization and transmissions by surgical instruments have been reported. Decontamination studies have been carried out thus far on solutions or suspensions of the agent and may not reflect the behavior of surface-bound infectivity.

**Materials and Methods:** As a model for contaminated surgical instruments, thin stainless-steel wire segments were exposed to scrapie agent, washed exhaustively with or without treatment with 10% formaldehyde, and implanted into the brains of indicator mice. Infectivity was estimated from the time elapsing to terminal disease.

**Results:** Stainless steel wire (0.15 × 5 mm) exposed to scrapie-infected mouse brain homogenate and washed extensively with PBS retained the equivalent of about 10<sup>5</sup> LD<sub>50</sub> units per segment. Treatment with 10% formaldehyde for 1 hr reduced this value by only about 30-fold.

**Conclusions:** The model system we have devised confirms the anecdotal reports that steel instruments can retain CJD infectivity even after formaldehyde treatment. It lends itself to a systematic study of the conditions required to effectively inactivate CJD, bovine spongiform encephalopathy, and scrapie agent adsorbed to stainless steel surfaces such as those of surgical instruments.

## Introduction

The agent responsible for transmissible spongiform encephalopathies, the prion, is far more resistant to physical and chemical inactivation than conventional pathogens. These properties are reflected in the difficulties encountered in

sterilizing prion-containing material by conventional procedures, in particular heat sterilization and formaldehyde treatment (1-5). More than 100 cases of proven or suspected iatrogenic transmissions to humans have been catalogued (6).

A particularly well-documented and disturbing report concerns an electrode that had been inserted into the cortex of a patient with unrecognized Creutzfeldt-Jakob disease (CJD) and, following a decontamination procedure involving treatment with benzene, 70% ethanol, and formaldehyde vapor after each use, was employed in succession on two additional patients who subsequently came down with CJD. Following these events, the tip of the electrode was implanted into the brain of a chimpanzee where it again caused lethal spongiform encephalopa-

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thy (7,8). The electrodes in question had a complex structure: a steel shaft of about 6 mm in diameter, with multiple silver contacts separated by rings of insulating plastic, allowing for the existence of crevices into which infectious material might have penetrated and become inaccessible to mechanical cleansing.

Instruments used for tonsillectomy or appendectomy on unrecognized nvCJD sufferers could become contaminated with the agent (9,10), so the question of how surgical instruments could be sterilized effectively without being damaged has assumed increased importance. In view of the report that scrapie-infected tissue dried onto surfaces is more resistant to inactivation than a suspension of the same material (quoted in ref. 4), extrapolation from available data on sterilization of suspensions may be unreliable. It thus became of interest to generate a model system for the sterilization of stainless steel instruments. We here show that mouse-adapted scrapie prions can firmly bind to stainless steel wire and give rise to infection when implanted into the brain of indicator mice, even after treatment with 10% formaldehyde.

## Materials and Methods

### *Scrapie Infection and Diagnosis*

RML is a mouse-adapted scrapie isolate (11) that was passaged in Swiss CD-1 mice (Charles River Laboratories, Wilmington, MA). Inoculum stock was prepared as a 10% (w/v) homogenate of RML scrapie-infected CD-1 mouse brains in 0.32 M sucrose; RML4.1 had a titer of 8.7 log LD<sub>50</sub> units/ml and 20 mg/ml total protein. Inoculated mice were checked for the development of scrapie symptoms every other day and, once they developed disease, every day (12). Apparent prion titers were estimated from incubation times (13) using the parameters derived for *Tga20* indicator mice (14).

### *Scrapie Agent-Exposed Wire*

Stainless steel wire segments (diameter, 0.15 mm; length, 5 mm; stainless steel suture monofilament wire, Art. Nr. 01614037, USP 4/0, B. Braun Melsungen AG, Melsungen, Germany; batch 1/7502) were incubated with 10% homogenate of RML scrapie-infected CD-1 mouse brain in phosphate-buffered saline (PBS) for 16 hr, washed five times for 10 min in 50 ml PBS, all at room temperature. The wire segments were air

dried and stored at room temperature for 1 day, and one segment was inserted into the brain of each of four *Tga20* mice, while in deep anesthesia, by pushing it through a 25-gauge injection needle.

To determine the amount of protein bound to a wire segment, ten 5-mm wire segments were treated with brain homogenate, washed and dried as above. They were then incubated in 0.1 ml 2 M NaOH for 1 hr at 20°C and the eluate was diluted with 0.3 ml water. Protein was determined by the Micro BCA Protein assay (Pierce, Rockford, IL), using bovine serum albumin (BSA) dilutions as standards.

## Results

We exposed segments of thin stainless-steel wire to a 10% homogenate of freshly prepared scrapie-infected mouse brain, washed them exhaustively with only PBS or with PBS followed by exposure to 10% formaldehyde for 1 hr, and inserted them into the brains of indicator mice. Aliquots (30  $\mu$ l) of a standard 1% brain homogenate (RML4.1) and of the last PBS wash of the wires were inoculated intracerebrally. The effective infectious dose was estimated by the incubation time method (13) using the parameters determined for *Tga20* indicator mice (14).

As shown in Table 1, prion-exposed wire segments that had been washed exhaustively with PBS caused terminal disease after  $72 \pm 3$  days; this is equivalent to a dose of about  $5.2 \pm 0.4$  log LD<sub>50</sub> units administered as brain homogenate. After formaldehyde treatment, terminal disease occurred after  $87 \pm 9$  days (equivalent to a dose of about  $3.5 \pm 1$  log LD<sub>50</sub> units). Thirty microliters of a 1% brain homogenate (RML4.1) produced disease after  $65 \pm 1$  days (a dose of  $6 \pm 0.1$  log LD<sub>50</sub> units) whereas 30  $\mu$ l of the fifth PBS wash did not cause disease.

Ten segments of 0.5-cm wire segment, exposed to scrapie-infected brain homogenate and exhaustively washed with PBS, were eluted with 0.1 ml 2 M NaOH. No protein was detected in the eluate; the limit of detection was about 50 ng per segment.

## Discussion

Stainless steel wire exposed to scrapie-infected brain homogenate and washed exhaustively with PBS retained a high level of infectivity,

**Table 1. Infectivity of prion-coated wire**

Treatment <sup>a</sup>	Incubation Time to Terminal Disease (no. mice sick/total)	Log LD <sub>50</sub> Units <sup>b</sup>
Wire <sup>c</sup>	72 ± 3 (4/4)	5 ± 0.3
Wire, formaldehyde-treated <sup>d</sup>	87 ± 9 (4/4)	3.5 ± 1
1% scrapie-infected brain homogenate <sup>e</sup>	65 ± 1 (4/4)	6 ± 0.1
PBS wash <sup>f</sup>	>120 (0/4)	<1

<sup>a</sup>Wire segments were inserted into the brain of four *Tga20* mice in deep anesthesia. Liquid samples (30 μl) were injected intracerebrally.

<sup>b</sup>Apparent infectivity titers were estimated from incubation times using the formula  $\log LD_{50} = 13 - 0.11 \times (\text{incubation time in days}) (14)$ .

<sup>c</sup>Stainless steel wire (diameter about 0.15 mm; 5 mm length) exposed to scrapie brain homogenate and washed five times with PBS, as described in Materials and Methods.

<sup>d</sup>Wire exposed to scrapie brain homogenate, washed four times with 50 ml PBS, and incubated in 10% formaldehyde for 1 hr at room temperature.

<sup>e</sup>Thirty microliters of RML4.1 diluted 10-fold in PBS containing 5% BSA.

<sup>f</sup>Thirty microliters of the 5th 50-ml PBS wash of wire segments exposed to scrapie homogenate.

which was diminished about 30-fold but not abolished by formaldehyde treatment.

Our experiments show that the anecdotal clinical findings of Bernoulli et al. (7) could be reproduced under defined laboratory settings, both in regard to the tight binding of prion infectivity to a stainless steel surface and to the considerable resistance of the bound infectivity to formaldehyde treatment. Failure to elute detectable amounts of protein from the exposed wires means that <50 ng per wire segment was bound and/or that the binding is partly or entirely irreversible under our experimental conditions.

The approach we report here enables the performance of sterilization experiments that more closely mimic real-life circumstances than experiments with solutions, suspensions, and homogenates. It is advisable that not only scrapie but also BSE and CJD prions be used in such tests, for which appropriate indicator mice exist (15–17).

Our findings also raise the interesting ques-

tion as to whether the wire-bound prions desorb from the wire when inserted into the brain or whether they initiate infection from the bound state.

## Acknowledgments

We thank Dr. Michael A. Klein for RML4.1 and Mr. Josef Ecsodi for care and surveillance of the mice. This work was supported by the Kanton of Zürich and by grants of the Schweizerischer Nationalfonds and the European Union to C.W.

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