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## Comparative analysis of scrapie agent inactivation methods

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### Summary

A scrapie-infected hamster brain homogenate was subjected to several different potential inactivation methods. Methods included autoclaving for various lengths of time, either alone or in combination with different concentrations of sodium hydroxide or LpH, an aqueous acid phenolic derivative (Calgon Vestal Laboratories in St. Louis, MO). Inactivation treatments utilizing either NaOH or LpH alone were also evaluated. It was determined that several of the treatments inactivated all of the detectable infectivity.

Scrapie; LpH; Spongiform encephalopathy; Neuro-degenerative; Kuru; Gerstmann-Straussler-Scheinker disease; Creutzfeldt-Jakob disease

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### Introduction

Spongiform encephalopathies are neuro-degenerative diseases that can be transmitted orally by ingestion of infected tissues or by the inoculation of infected tissue suspensions into susceptible species (Pattison et al., 1972; Hadlow et al., 1984; Gibbs et al., 1980; Gibbs and Gajdusek, 1973). These encephalopathies include scrapie of sheep and goats (Hadlow et al., 1982; Cuille and Chelle, 1936; Chandler, 1961; Sigurdsson, 1954), chronic wasting disease of deer and elk (Williams and Young, 1980), transmissible mink

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encephalopathy (TME) (Kimberlin and Marsh, 1975; Hartsough and Burger, 1965; Marsh and Kimberlin, 1975), bovine spongiform encephalopathy (BSE) (Wells et al., 1987), and kuru (Gajdusek, 1977; Gajdusek and Zigas, 1957; Gajdusek et al., 1966; Gajdusek et al., 1967), Gerstmann-Straussler-Scheinker disease (GSS) (Masters et al., 1981), and Creutzfeldt-Jakob disease (CJD) (Bernoulli et al., 1977; Gibbs et al. 1968; Duffy et al., 1974) of humans. A wide range of other animal species are susceptible to experimental inoculation of the agent(s) which cause these encephalopathies (Gibbs and Gajdusek, 1973; Williams and Young, 1982; Chandler and Fisher, 1963). It is likely, for example, that the current outbreak of bovine spongiform encephalopathy in Great Britain arose from supplemental feed rations rendered from infected sheep tissues which were not adequately treated to inactivate the scrapie agent (Wilesmith et al., 1988). The agent(s) which cause these diseases are resistant to inactivation methods that are effective against most other micro-organisms (Chatigny and Prusiner, 1979; Taylor, 1991). Because of this resistance, investigators and health professionals, have questioned the efficacy of some inactivation techniques (Gajdusek et al., 1977; Chatigny and Prusiner, 1979; Taylor and McBride, 1987; Taylor, 1991).

Many different methods and/or disinfectants have been tested for their ability to inactivate the agent(s) causing spongiform encephalopathies (Kimberlin et al., 1983; Brown et al., 1990; Millson et al., 1976; Brown et al., 1982b; Brown et al., 1986). Methods have included autoclaving at 121°C for 1 h (Kast, 1976; Rohwer, 1984; Brown et al., 1986), ashing (Brown et al., 1990), exposure to 6 M urea (Brown et al., 1986; Hunter et al., 1969), exposure to 1 M NaOH (Brown et al., 1984), exposure to periodate or iodate (Hunter et al., 1969), 1 M KSCN or NaSCN treatment (Prusiner et al., 1981), 0.5% sodium hypochlorite treatment (Brown et al., 1982a; Brown et al., 1986), exposure to 1 M NaOH followed by autoclaving (Taguchi et al., 1991), and many others (Chesebro, 1990; Hunter et al., 1971; Dees et al., 1985; Bellinger-Kawahara et al., 1987; Tateishi et al., 1991; Chatigny and Prusiner, 1979; Pocchiari et al., 1988; Prusiner et al., 1981). Of these treatments only autoclaving at 121°C for 1 h, and 1 M NaOH treatment inactivated more than  $10^6$  ID<sub>50</sub>/1 mg of tissue from scrapie-infected hamster brain homogenates (Chesebro, 1990). A recent study in which NaOH and autoclaving were combined showed complete inactivation of  $10^{5.8}$  ID<sub>50</sub> of mouse adapted CJD agent from a mouse brain homogenate (Taguchi et al., 1991). However, considering that the titer of scrapie agent in hamster brain can be as high as  $10^8$  ID<sub>50</sub>/1 mg of tissue, we sought to identify other inactivation methods that could inactivate this high amount of infectivity.

Inactivation methods included various concentrations of NaOH, and LpH, as well as autoclaving for various periods of time. NaOH and autoclaving were tested because of conflicting conclusions from earlier reports, whereas LpH was tested because of its known disinfectant properties against other infectious agents.

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## Materials and Methods

### *Infectivity bioassay*

The amount of infectivity was measured by an infectivity titer bioassay in Syrian hamsters (Race et al., 1988; Reed and Muench, 1938). To determine infectivity titers using the bioassay, treated homogenates were serially diluted using ten fold dilutions, and 50  $\mu$ l was inoculated IC for each dilution into each of six Syrian hamsters to determine the amount of agent removed after treatment. The 10% suspension of the 263 K strain of scrapie-infected hamster brain used in these studies contained  $10^{9.4}$  ID<sub>50</sub>/50  $\mu$ l. However, a 10-fold dilution of the original 10% homogenate, created by treatment with LpH or NaOH, was toxic when inoculated intracerebrally (IC). Therefore, a 100-fold dilution of the original 10% homogenate containing  $10^{7.4}$  ID<sub>50</sub>/50  $\mu$ l was the maximum amount which was bioassayed (Fig. 1). Titers were determined by endpoint titration according to the method of Spearman and Karber (Dougherty, 1964).

### *Autoclaving*

To test the inactivation potential by autoclaving, 2 ml of a 10% scrapie-infected hamster brain homogenate in 0.32 M sucrose was added to each of eight 25-ml glass centrifuge tubes with screw-on caps. The homogenate was adjusted to a final volume of 20 ml using 0.32 M sucrose, and the tubes were vortexed for 30 s. Duplicate pairs of tubes were autoclaved at either 121°C or 132°C for either 1 h or 1.5 h in an AMSCO Eagle series 2031 Gravity Sterilizer that does not initiate sterilization time until preset temperature and pressure have been attained. Volume amounts remained constant after autoclaving. The infectivity bioassay provided the data to determine the titer of agent removed from the original homogenate, as previously indicated.

### *NaOH*

Suspensions of hamster brain were made in each of six 25-ml glass centrifuge tubes with screw-on caps by adding 2 mls of a 10% scrapie infected hamster brain homogenate in 0.32 M sucrose to 18 ml of either 0.1 N or 1.0 N NaOH. These homogenates were vortexed for 30 s at high speed, then left at room temperature for either 0.5 hour, 2 h, or 16 h. At the end of each time period, homogenates were equilibrated to pH 7.0 using glacial acetic acid. The homogenates were vortexed for 1 min and then sonicated 1 min in a cuphorn sonicator (Model W-385; Heat Systems-Ultrasonics Inc.). The titer of agent was determined as described.

*LpH*

LpH is an aqueous acid phenolic disinfectant which contains 12.6% glycolic acid, 3.0% p-tertiary amyphenol, 6.1% o-benzyl-p-chlorophenol, 0.5% #2-phenylphenol, <5% hexylene glycol, and <10% isopropanol by weight. Four suspensions of infected brain were made as described for NaOH inactivation testing with final LpH concentrations of 90, 81, 9, and 0.9% (Fig. 1). These suspensions were vortexed for 30 s at high speed, incubated at 22°C for 30 min, and then serially diluted using 10-fold dilutions. The titer of agent was determined as described.

*LpH or NaOH combined with autoclaving*

We tested several NaOH or LpH concentrations in conjunction with autoclaving. Treatments included; 0.09 N or 0.9 N NaOH for 2 h, or 9% or 0.9% LpH for 30 min, followed by autoclaving for 1 h at 121°C. After the appropriate incubation period at room temperature, the homogenates containing NaOH were neutralized to pH 7.0 using glacial acetic acid. The

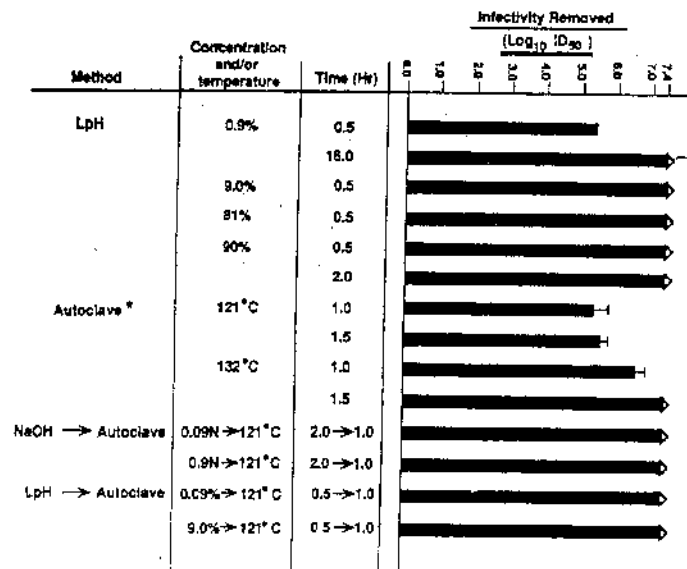


Fig. 1. Infectivity titers in hamster brain homogenates. Bars indicate the amount of infectivity ( $\log_{10}$  ID<sub>50</sub>) removed from a 10% scrapie-infected hamster brain homogenate in 0.32 M sucrose using the inactivation methods listed. The column labeled time represents the incubation period that the inactivation method was tested. Error bars in the autoclave section indicate the standard deviation for multiple experiments. An arrow at the end of a bar indicates that greater than  $10^{7.4}$  ID<sub>50</sub> of infectivity was removed. NaOH → Autoclave and LpH → Autoclave refer to inactivation methods combining either NaOH or LpH followed by autoclaving. Infectivity bioassays were conducted as described. \*These conditions were repeated two times.

titer of agent was determined as described.

### Results

Autoclaving at 132°C for 90 min completely eliminated the detectable infectivity, and was at least 100-fold more effective than 121°C for 60 min (Fig. 1). Although earlier reports stated that the scrapie agent contained in hamster brain homogenate can be destroyed by exposure to 132°C for 60 min (Brown et al., 1986), our data show that exposure to 132°C for 60 min did not eliminate all the infectivity from the infected hamster brain homogenate (Fig. 1).

NaOH treatments consistently resulted in large reductions in the scrapie agent titer; however, low amounts of infectivity were detectable after treatment in some instances (data not shown).

LpH concentrations of 90%, 81%, and 9% removed all detectable infectivity from the homogenate after a 30-min incubation period. An overnight incubation with 0.9% LpH solution also removed all of the detectable infectivity. Even a 30-min incubation period with only 0.9% LpH, inactivated  $10^{5.4}$  ID<sub>50</sub>.

All combinations using the low concentrations of LpH or NaOH followed by autoclaving for 1 h at 121°C resulted in complete inactivation of all detectable infectivity (Fig. 1).

### Discussion

There has been considerable concern in recent years regarding inactivation of the agent(s) causing transmissible neuro-degenerative diseases. Although several of the currently published methods of inactivation eliminate large amounts of the agent, most are not completely effective. Common sterilization treatments are not effective at sterilizing the unusually resistant scrapie agent. The difference between our results and the results of others using autoclave methods for scrapie agent inactivation testing may be caused by several factors. The autoclave type, cycle parameters, strain of scrapie, and the strain of hamster were the same. However, there may be differences in the resistance of the agent itself as a result of different passage histories in the separate laboratories. Also, the type of container used to hold the homogenate or buffer in which the homogenates were made might have effected the inactivation potential. Therefore, inactivation potentials by autoclaving may vary in different situations, and can be regarded as a treatment to lower titers of agent, but on the basis of our data, it is not safe to say that autoclaving sterilizes high titers of scrapie agent.

Our analyses showed that 1% LpH solution in contact with the agent for 16 h completely eliminated the detectable infectivity from a 1 in 100 dilution of scrapie-infected hamster brain homogenate containing  $10^{9.4}$  ID<sub>50</sub>/50 μl (Figure

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1). LpH is easy to mix and use, either in a spray bottle for surface decontamination, or in a soaking treatment with contaminated utensils and laboratory glassware immersed in solution. Combination methods utilizing either LpH or NaOH followed by autoclaving are also very effective, but require more time, are tedious, and cannot be performed on the surfaces of stationary objects that may become contaminated with agent.

LpH treatment appears to be very effective at killing the scrapie agent, however, the actual component(s) of LpH that cause agent inactivation is unknown. Previously, phenol extraction has been shown to remove large amounts of scrapie-infectivity (Chatigny and Prusiner, 1979; Marsh et al., 1974; Ward et al., 1974; Chesebro, 1990), but extraction is not considered a decontamination process. Phenolic disinfectants, on the other hand, could be employed to inactivate the scrapie agent, but are not always considered a practical method to use because of toxic properties (Brown et al., 1982a). It is interesting to note that the phenolic derivatives compose <0.1% of a 1% LpH solution. Even at this very low phenol concentration, LpH was very effective at killing the scrapie agent. Thus concerns over the potentially toxic aspects of phenol may not be significant. Although LpH also contains glycolic acid, no information was found pertaining to the inactivation potential of glycolic acid against the scrapie agent. It is known to be very effective at degrading proteins and it has a low pH which may contribute to tissue and agent destruction. Finally, no literature was found citing the use of hexylene glycol or isopropanol, two other components of LpH, as inactivation reagents for spongiform encephalopathy-causing agents. Therefore, their contribution to scrapie agent inactivation as a component of LpH is unknown.

Use of 1% LpH to disinfect surfaces or 1% LpH treatment followed by autoclaving 1.0 h at 121°C on non-stationary objects appear to be effective methods to destroy hamster adapted scrapie agent. Ability to inactivate higher concentrations of infectivity using LpH remains untested. It is likely that LpH treatments could be equally effective against similar titers of infectivity found in tissues containing other agent(s) that cause the previously mentioned spongiform encephalopathies, since the agent(s) have similar properties (Chesebro, 1990). Hence, LpH treatment may eventually provide an effective sterilization procedure for hospital areas and equipment in contact with potentially infectious human neuro-degenerative diseases. However, further tests will have to be performed to confirm the inactivation potential of LpH on other members of this spongiform encephalopathy group.

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