Short communication

Exposure to autoclaving or sodium hydroxide extends the dose-response curve of the 263K strain of scrapie agent in hamsters

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An analysis was made of incubation period data from experiments in which samples of brain-tissue infected with the 263K strain of scrapie agent were injected intracerebrally into hamsters following exposure of the tissue to autoclaving or sodium hydroxide. Where there was survival of infectivity, this often produced extended mean incubation periods compared with the maximal incubation periods in controls injected with untreated agent. These results confirmed that, after chemical or physical treatment, infectivity titre should not be calculated by comparing the incubation period from a single dilution-group against a standard dose-response curve for untreated agent.

The sheep disease scrapie is the archetype and model for the transmissible degenerative encephalopathies (TDE) which include bovine spongiform encephalopathy (BSE) and Creutzfeldt–Jakob disease of humans. Among the unusual but well-known characteristics of the TDE is the relative resistance of their causal agents to standard inactivation procedures, and their long incubation periods in both natural and experimental hosts.

The precise relationship between the dose of scrapie agent administered and the incubation period in rodents allows titres of infectivity to be measured from a standard dose-response curve (DRC) for any particular strain of agent (Dickinson et al., 1968; Kimberlin & Walker, 1977; Kimberlin & Walker, 1986). However, the incubation period assay (IPA) cannot be relied upon to measure infectivity which survives chemical or physical treatments because these can result in DRC delays compared with equivalent titres of untreated infectivity, and lead to underestimation of titre (Taylor, 1986, 1993). Although scrapie agent has been exposed to a wide range of chemical and physical procedures, only a small number of experiments have been conducted to investigate their effect on the DRC.

Delayed DRC can be demonstrated in mice by endpoint titration following exposure of the 139A strain of scrapie agent to lithium chloride, sodium dodecyl sulphate (SDS) (Kimberlin, 1977), sodium deoxycholate (Lax et al., 1983) or a combination of octylglucoside and sulphobetaine 3-14 (Somerville & Carp, 1983); a similar phenomenon occurs with the ME7 strain after boiling (Dickinson & Fraser, 1969), and with the hamster-passaged strain Sc237 after heating at 121 °C (Prusiner et al., 1982). The same is described for the 263K strain in hamsters after a combination of Sephacryl chromatography, ultrafiltration and urea treatment, leading to an incubation period delay of 61 days (Pocchiari et al., 1991); the need to maintain hamsters well beyond control endpoints in inactivation experiments generally is recognized (Brown et al., 1986). In an experiment where partially purified Sc237 infectivity is subjected to polyacrylamide gel electrophoresis in the presence of SDS, the infectivity titres of gel fractions are calculated by IPA but endpoint titration data are also presented for one sample (Prusiner et al., 1993). It is difficult to reconcile these authors’ conclusion that their results confirm the reliability of the IPA, because the mean incubation periods for all dilutions of the sample assayed by endpoint titration exceed (by up to 134 days) the maximum mean incubation periods for limiting dilutions of untreated agent (Prusiner et al., 1980); the titres presented, based upon IPA, are up to 4000-fold lower than the values which can be calculated from the endpoint titration (Karber, 1931).

Chemical treatment of the mouse-passaged 139A strain of scrapie agent delays the DRC by around 20 days (Kimberlin, 1977; Lax et al., 1983; Somerville & Carp, 1983) whereas the estimated delay following boiling of the mouse-passaged ME7 strain of scrapie agent is approximately 90 days (Dickinson & Fraser, 1969). The effect with hamster-passaged scrapie agent is generally more profound (Brown et al., 1986; Pocchiari et al., 1991; Prusiner et al., 1993; Taylor, 1993).

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During recent decontamination studies (Taylor et al., 1994) samples of brain tissue infected with the hamster-passaged 263K strain of scrapie agent were exposed to autoclaving or sodium hydroxide, and then injected intracerebrally into hamsters. The incubation period data from these experiments have now been subjected to further analysis which is the basis of this publication.

Fig. 1 shows the DRC for 263K which was either untreated or subjected to porous-load autoclaving at 134 °C for 30 min. The mean incubation periods for the $10^{-1}$ and $10^{-2}$ dilutions of autoclaved agent exceeded that of the maximum mean incubation period for untreated agent. Using Student's t-test, the statistical significance of the incubation period difference between the $10^{-8}$ control group and the $10^{-1}$ autoclaved group can be shown to be $P = < 0.05$ (no statistical analysis was possible with the $10^{-2}$ autoclaved sample because only one animal was positive). Fig. 2 makes a similar comparison for 263K which had been exposed to 1 M-sodium hydroxide for an hour. The mean incubation periods for the $10^{-4}$ and $10^{-3}$ dilutions of the hydroxide-treated samples were significantly greater than for the control $10^{-8}$ dilution ($P = < 0.05$ for both samples). In addition to these titration data, there were occasions where treated materials were injected only at a $10^{-1}$ dilution but could be judged to be at, or close to, their limiting dilutions because only some of the injected hamsters developed scrapie. Table 1 lists such experimental groups, and shows that the extended incubation periods observed were statistically significant when compared with the $10^{-8}$ dilution of the untreated control.

![Graph](image1)

**Fig. 1.** Mean incubation period (±SE) for serial tenfold dilutions of untreated 263K (●) compared with agent autoclaved at 134 °C for 30 min (■).

**Fig. 2.** Mean incubation period (±SE) for serial tenfold dilutions of untreated 263K (●) compared with agent treated with 1 M-NaOH for 60 min (■).

Table 1. Incubation periods for $10^{-1}$ homogenates of 263K-infected brain following exposure to autoclaving or sodium hydroxide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean incubation period (±SE)</th>
<th>Significance of incubation period difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(134 °C/18 min)</td>
<td>298 (82)</td>
<td>$P = &lt; 0.05$</td>
</tr>
<tr>
<td>(134 °C/60 min)</td>
<td>253 (40)</td>
<td>$P = &lt; 0.05$</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 M/30 min)</td>
<td>203 (30)</td>
<td>$P = &lt; 0.05$</td>
</tr>
<tr>
<td>(2 M/30 min)</td>
<td>322 (42)</td>
<td>$P = &lt; 0.005$</td>
</tr>
<tr>
<td>(2 M/60 min)</td>
<td>288 (34)</td>
<td>$P = &lt; 0.005$</td>
</tr>
</tbody>
</table>

* Compared with the mean incubation period of 145 (20) days for untreated agent at its limiting ($10^{-8}$) dilution.

The results reinforce previous doubts about the appropriateness of the IPA to measure infectivity after chemical or physical treatment of scrapie-like agents, and suggest that DRC delay is the rule rather than the exception under such circumstances.

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