Can Scrapie Titres be Calculated Accurately from Incubation Periods?

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SUMMARY

Since endpoint titrations of scrapie material are costly and time-consuming, several workers have estimated titre from the correlation between the incubation period of the disease and the infectivity titre. However, we show here that the relationship between incubation period and titre cannot be assumed to be constant for all scrapie preparations. Our results indicate that sodium deoxycholate treatment of scrapie preparations does not reduce the titre, but can lengthen the incubation period by about 10 days. This is equivalent to a discrepancy of 1 log LD$_{50}$ unit if the estimation of titre was based on the incubation period.

Scrapie research is hampered by the absence of a simple laboratory assay for the infectious agent. The only assay currently available is the calculation of titre by endpoint titration based on development of the disease in susceptible animals. The long incubation period between infection and the subsequent clinical symptoms makes even simple experiments costly and time-consuming. Since the duration of the incubation period decreases with increasing dose of agent (Hunter et al., 1963), several workers have used this relationship to estimate titre in mouse and hamster tissues (Dickinson et al., 1969; Kimberlin & Walker, 1978; Lax et al., 1982a; Prusiner et al., 1981, 1982; Prusiner, 1982).

Nevertheless, it has been noted that various treatments which reduce titre also alter the relationship between titre and incubation period (Dickinson & Fraser, 1969; Kimberlin, 1977; G. D. Hunter, personal communication; A. G. Dickinson, personal communication). Consequently, we have not used this assay to the exclusion of endpoint titrations in our studies on scrapie agent purification. Indeed, it is shown here that sodium deoxycholate treatment, which has been used to obtain scrapie-enriched fractions (Millson & Manning, 1979; Lax et al., 1982a; Prusiner et al., 1980), does alter incubation period without loss of titre.

The 139A strain of scrapie agent and randomly bred, female Compton White mice were used throughout this work. The preparation of membranes from the brains of scrapie-affected mice, and sodium deoxycholate treatment have been described previously (Lax et al., 1982b). Scrapie infectivity was assayed by intracerebral injection of groups of eight young mice with 0·03 ml of 10-fold dilutions prepared in physiological saline. The mice were monitored for clinical signs of scrapie twice weekly, and the time taken for the earliest clinical symptom to appear was recorded for each individual mouse.

Following the dissociation of host cell membrane components by sodium deoxycholate, we found that the incubation period was lengthened on average by 10 days (Table 1). These data were obtained from eight separate experiments which were performed over a number of years. We also calculated for each individual experiment the difference between deoxycholate-treated and control incubation periods for each of the four different doses of scrapie agent inoculated. This varied from -6 to 30 days, and of the 32 paired incubation periods only three were longer for the control sample. A scrapie-enriched fraction can be isolated from sodium deoxycholate-treated preparations upon ultracentrifugation (Millson & Manning, 1979) and full scrapie infectivity can be recovered from this fraction following resuspension in 1% sodium lauroyl lactate.
Table 1. Incubation periods of control and deoxycholate-treated material

<table>
<thead>
<tr>
<th>Infectious units injected*</th>
<th>Control (days)†</th>
<th>Deoxycholate-treated (days)†</th>
<th>Difference (days)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>135 ± 0.97</td>
<td>144 ± 1.5</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>500</td>
<td>149 ± 1.2</td>
<td>156 ± 1.7</td>
<td>7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>50</td>
<td>161 ± 1.7</td>
<td>170 ± 2.2</td>
<td>9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>178 ± 3.2</td>
<td>193 ± 2.7</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Titre (-log LD50/ml)</td>
<td>9.2 ± 0.07</td>
<td>9.3 ± 0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The number of infectious units injected is an approximate value calculated from the titres.
† Results are expressed as mean incubation period (± standard error of the mean). The results were calculated from the individual incubation period of between 46 and 62 mice.

Sarcosinate (Lax et al., 1982a); incubation periods were from 9 to 29 days longer (average 18 days) than those for the corresponding control cell membranes. The estimate of titre by the incubation period/titre relationship of control material would in these cases give values which are falsely low by up to 1 log LD50 unit.

The best-fitting linear equation relating incubation period to titre for the same type of sample has been calculated for hamsters (Prusiner et al., 1980, 1981, 1982; Prusiner, 1982). However, the accuracy with which the titre of a single sample can be calculated from its incubation period is limited by the large biological variation due to unknown factors. For example, the same inocula, injected by different people, can give different incubation periods, but the same eventual titre: in two separate experiments, the incubation periods obtained by one of us (A.J.L.) were on average 8 days longer than those obtained by another (G.C.M.) for the same 10^-3, 10^-4, 10^-5 and 10^-6 dilutions of scrapie membranes. Prusiner et al. (1982), while claiming that this assay is as accurate as endpoint titrations, find that only differences in excess of 2 log LD50 units are significant. In our experience, endpoint titrations reproducibly have an accuracy greater than 0.5 log LD50 units. Our results show that the calculation of the titre of treated samples by the use of the titre/incubation period relationship of control samples (Prusiner et al., 1980; Prusiner, 1982) is even more likely to be subject to error. The use of hamster assays (Prusiner et al., 1980, 1981, 1982; Prusiner, 1982) is likely to produce the same inaccuracies as those we have outlined in the mouse model. Therefore, although the estimate of titre by the incubation period data is a good approximation when all known variables are controlled (Hunter et al., 1963; Dickinson et al., 1969), the assay is not suitable for general use in purification studies where an accurate assessment of infectivity is required.

Since it is believed that scrapie agent infectivity depends on host components as well as scrapie genome information (Hunter, 1972) it is likely that an alteration in host components could alter pathogenesis. Our results are the first demonstration that chemical treatments of scrapie-infected host material can modify the development of the disease without affecting the ultimate infectivity titre.

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REFERENCES


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