The Effect of $\beta$-Propiolactone on the Scrapie Agent

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The transmissible agent of scrapie has not yet been characterized but it is commonly referred to as a virus (Andrewes & Pereira, 1967). However, on the basis of its resistance to acetyleneimine, Stamp et al. (1959) suggested that the agent might not contain nucleoprotein. Pattison (1965), reporting on the effect of formalin, expressed the opinion that the agent might not be a virus and now believes it to be, or to be associated with, a small basic protein (Pattison & Jones, 1967). In a study of the effect of ionizing and ultraviolet irradiation Alper, Haig & Clarke (1966) and Alper et al. (1967) suggested that the agent may lack nucleic acid. On the basis of these results and the inactivation of the scrapie agent with urea and phenol, Gibbons & Hunter (1967) have proposed a membrane hypothesis for its nature. Other workers (e.g. Adams & Caspary, 1967) still believe that the agent is essentially viral in character.

An attempt was made to obtain further information by examining the effect of an alkylating substance on the scrapie agent. $\beta$-Propiolactone was selected because it penetrates tissue well, its action is not inhibited by protein, it has been claimed to inactivate all those viruses against which it has been tested (LoGrippo & Hartman, 1955; LoGrippo, 1959) and is thought to act by combining with free amino groups on both protein and nucleic acid (Newton & Waterson, 1967).

Table 1. The effect of $\beta$-propiolactone on the titre of scrapie mouse brain suspensions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Concentration of $\beta$-propiolactone</th>
<th>$\beta$-propiolactone treated</th>
<th>Untreated control</th>
<th>Average difference from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2%</td>
<td>5.49*</td>
<td>6.11</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>5.44</td>
<td>5.94</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>1.0%</td>
<td>4.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.0%</td>
<td>4.19</td>
<td>5.9</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>4.95</td>
<td>5.39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5%</td>
<td>4.45</td>
<td>5.68</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.23</td>
<td>5.52</td>
<td></td>
</tr>
</tbody>
</table>

* Log. LD50/0.05 ml.

Mice were inoculated with the Chandler (1961) strain of mouse-adapted scrapie. When the mice showed advanced clinical symptoms of disease their brains were homogenized in 10% digest broth in 0.1M-phosphate buffer at pH 8 and coloured with phenol red. Suspensions were centrifuged at 1800 g for 10 min. The supernatant fluids were mixed with an equal volume of buffered broth containing $\beta$-propiolactone (Table 1) and stirred continuously at 37$^\circ$ for 2 hr (LoGrippo & Hartman, 1955). The suspensions were maintained at pH 8 by addition of m-sodium hydroxide during incubation. Each test was carried out in duplicate. After storage overnight at 4$^\circ$ serial tenfold dilutions were made in 0.75% bovine albumin in phosphate buffered saline.
and 0.05 ml. was injected intracerebrally into groups of mice. To control the potency of the β-propiolactone the F1N strain of yellow fever virus was passaged in mice by intracerebral inoculation; brains collected during the terminal stages of the infection were treated and titrated in the same way as those from scrapie-affected mice. All titration end points were determined by the method of Kärber (1931).

Preparations of brains infected with yellow-fever virus were found to have titres of $10^{6.5}$ and $10^{4.1}$, LD$_{50}$/0.05 ml. and were apparently inactivated completely by 0.2% β-propiolactone. In Expts 1 and 2 the titre of scrapie mouse brain preparations was reduced by $10^{6.6}$ LD$_{50}$/0.05 ml. (Table 1) with 0.2% β-propiolactone and although activity was reduced to a greater extent with higher concentrations of β-propiolactone a considerable amount of activity remained. Results were similar in Expt 3 in which a scrapie brain suspension was treated (Brown & Cartwright, 1960) with fluorocarbon (Arcton 113, I.C.I., Ltd) to reduce lipid content before exposure to β-propiolactone.

The resistance of the scrapie agent to β-propiolactone supports our previous conclusion (Alper et al. 1966; Alper et al. 1967) and the view of others (Pattison, 1965; Gibbons & Hunter, 1967) that, according to current virus characterization (Andrewes & Pereira, 1967), the scrapie agent is not a virus.

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REFERENCES

Short communications

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