A Plasma Lactic Dehydrogenase-Elevating Virus Associated with Scrapie-Infected Mice

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SUMMARY

Mice in our scrapie-infected colony were found to have high levels of plasma lactic dehydrogenase activity. The cause of this appears to be contamination of mouse-passaged scrapie material with a plasma lactic dehydrogenase-elevating virus, similar to that normally found as a contaminant of transplantable mouse neoplasia. Mice inoculated with the Compton strain of scrapie virus or with sheep scrapie virus had normal plasma lactic dehydrogenase activities.

INTRODUCTION

Recent evidence has suggested that the scrapie agent, although strongly attached to particulate matter in brain, is itself of very small size (e.g. Mould, Smith & Dawson, 1965) and the hypothesis has been advanced that it is too small to be a replicating agent of the normal nucleic acid containing type (Alper, Haig & Clarke, 1966; Pattison, 1967). However, there appears to be no general agreement between different investigators concerning the size and nature of the scrapie agent, and precise studies are made difficult by the duration of the only available assay method. However, similar controversies have arisen concerning the size of other replicating agents. For example, a virus which elevates plasma lactate dehydrogenase (PLDH) activity in infected mice was described by Riley et al. (1960), who found the agent in inocula prepared from transplantable mouse tumours. Many subsequent studies have shown it to be a contaminant, and not a tumour-inducing virus. Typically, transmission of the agent by the inoculation of normal mice with small quantities of plasma from an infected (e.g. tumour-bearing) host was followed by a five- to tenfold rise in PLDH activity occurring 2 to 3 days after infection and lasting indefinitely.

According to Riley (1963) the agent was extremely small (10 to 30 Å), while results published simultaneously by Notkins (1963) suggested that it was of conventional size (about 600 Å). From centrifugation and chromatographic studies, Adams & Bowman (1964) suggested that the virus might exist in two forms, one large and one small. Although most investigators now seem to accept that the virus is a 400 to 600 Å particle, this controversy over size and, in particular, the possibility that the virus may be capable of existing as a very small particle, has never been satisfactorily resolved.

Because of the relative simplicity of the assay, and the similarity in the controversy over the size of the scrapie agent and of PLDH-elevating virus, it was felt reasonable to make more studies on PLDH-elevating virus as a model system.
In preliminary experiments, however, mice in our colony which had been inoculated with mouse-passaged scrapie brain preparations were found to have high PLDH levels. Experiments were then done to see whether the scrapie agent itself had PLDH-elevating activity, or whether, as in the case of transplanted mouse tumours, our scrapie preparations were contaminated with a PLDH-elevating virus.

METHODS

Animals. Male and female mice of a Swiss albino strain, bred in this Unit but derived in the first instance from the Moredun Institute, Edinburgh, were used. Most of the investigations were done on animals previously inoculated with preparations of scrapie virus for other purposes.

Plasma for injection. Blood was obtained from the severed brachial artery, under ether anaesthesia. It was heparinized, centrifuged and the plasma injected intraperitoneally.

Blood samples for biochemical investigation. Blood (0.025 to 0.05 ml.) was taken from the cut end of the tail into a heparinized micropipette, and diluted with isotonic saline containing 10 units/ml. of heparin. Dilutions varied from 1/10 to 1/20. The suspension was centrifuged (2000 g for 5 min.) and the supernatant fluid carefully removed with a Pasteur pipette. Samples showing more than the slightest trace of haemolysis were discarded, but this occurred rarely. For calculations of plasma dilution the haematocrit was assumed to be 40%.

Estimation of PLDH activity. PLDH was estimated on groups of 5 to 8 mice by measuring the disappearance of pyruvate during 30 min. at 37° as described in the Sigma Chemical Company Technical Bulletin No. 500 (Berger & Broida, 1960) with the slight modification described by Adams, Rowson & Salaman (1961). After colour development the optical density of the solution was read at 460 mμ in a Unicam SP. 500 spectrophotometer. A minimum interval of 5 days was allowed between inoculation and estimation of PLDH activity.

Source of PLDH-elevating virus. Blood was removed as described above from mice bearing a transplantable Ehrlich ascites tumour (kindly provided by the Chester Beatty Research Institute) diluted 1/10 in heparinized saline, centrifuged, and 0.1 ml. of plasma injected intraperitoneally into recipient mice.

Filtration. Brain taken from scrapie-infected mice was homogenized in 10 ml. of sterile 0.9% (w/v) NaCl in an all-glass homogenizer. The homogenate was centrifuged at 10,000 g for 8 min. at 5° in a MSE High Speed 18 refrigerated centrifuge, the upper 4/5 of the supernatant removed, and centrifuged again in the same way. The upper 4/5 of the final supernatant was filtered sequentially through Millipore filters of decreasing average pore diameter (APD) under sterile conditions.

Scrapie-inoculated mice. Except where otherwise stated, the mice used in these studies were inoculated intracerebrally with 0.05 ml. of a 1 in 10 (w/v) homogenate taken from mice already inoculated with a mouse-adapted strain of sheep virus originally derived from sheep and serially passaged several times in mice.
RESULTS

One day after inoculation of mice with scrapie brain material their levels of PLDH activity were normal but from the third day onwards there was up to a fivefold elevation (Fig. 1). The last two groups of mice, i.e. after 6 and 7 months, showed clinical signs of scrapie, the 7-month-old group, in fact, being in the terminal stage. So far as PLDH response is concerned the results were closely similar to those previously reported for mice injected with the contaminant PLDH-elevating virus derived from tumour tissue. Tissues taken from scrapie-infected mice were inoculated intracerebrally, and plasma from the same animals inoculated intraperitoneally into normal mice. Five days after inoculation all the recipient mice had elevated PLDH levels (Table 1). Some experiments were also done on the transmission properties of the PLDH-elevating agent associated with our scrapie-affected mice. PLDH activities were measured in litters born to scrapie-affected parents and of interchanged litters born to scrapie-affected and normal parents. In these experiments the scrapie parents were inoculated 2 to 3 months before mating (Table 2).

Further groups of mice inoculated with brain taken from scrapie-infected rats (themselves inoculated directly from sheep), with normal and scrapie-infected sheep brain, and with multiple-sclerotic human brain, were found subsequently to have normal PLDH levels (Table 3). Scrapie-infected mice kindly provided by the A.R.C. Research Institute, Compton, were also found to have normal PLDH levels (Table 3).

In experiments designed to assess the size of the infective agent, centrifuged scrapie
mouse-brain homogenates were passed through Millipore filters of decreasing size. The agent passed through the 1000 Ǻ filter but not through the 500 Ǻ (Table 4).

So far, infection of mice with PLDH-elevating virus has produced little evidence of pathological changes, although it seems generally accepted that the raised PLDH level

Table 1. Plasma lactic dehydrogenase (PLDH) levels in normal mice, and in mice 5 days after inoculation with tissue from scrapie-infected mice

<table>
<thead>
<tr>
<th>Tissue inoculated</th>
<th>PLDH Level</th>
<th>No of mice in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>2250 ± 175</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>2220 ± 220</td>
<td>5</td>
</tr>
<tr>
<td>Kidney</td>
<td>2550 ± 180</td>
<td>5</td>
</tr>
<tr>
<td>Muscle</td>
<td>2400 ± 170</td>
<td>5</td>
</tr>
<tr>
<td>Plasma</td>
<td>2360 ± 130</td>
<td>6</td>
</tr>
<tr>
<td>Normal mice</td>
<td>585 ± 28</td>
<td>14</td>
</tr>
</tbody>
</table>

Plasma (0.05 ml.) was inoculated intraperitoneally. All tissues (0.05 ml. of 1/10 suspension) were inoculated intracerebrally. PLDH values are given in BB units as averages ± S.E.M.

Table 2. PLDH levels in litters of mice raised as indicated

<table>
<thead>
<tr>
<th>Litter of indication</th>
<th>PLDH activity</th>
<th>No in litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>of scrapie parents—raised with parents</td>
<td>2500 ± 145</td>
<td>7</td>
</tr>
<tr>
<td>of normal parents—transferred to scrapie foster mother at birth</td>
<td>559 ± 72</td>
<td>7</td>
</tr>
<tr>
<td>of scrapie parents—transferred to normal foster mother at birth</td>
<td>2300 ± 126</td>
<td>6</td>
</tr>
<tr>
<td>of scrapie male and normal female—male removed before birth of litter</td>
<td>540 ± 60</td>
<td>9</td>
</tr>
<tr>
<td>of normal male and scrapie female</td>
<td>880, 2300, 2700, 2950, 2450</td>
<td>5</td>
</tr>
</tbody>
</table>

PLDH levels were determined at six weeks of age. PLDH values are given in BB units as averages ± S.E.M.

Table 3. PLDH levels in mice inoculated with brain taken from various sources, and in Compton normal and scrapie-infected mice

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>PLDH</th>
<th>No of mice in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie rat brain</td>
<td>550 ± 32</td>
<td>6</td>
</tr>
<tr>
<td>Normal sheep brain (Moredun Institute)</td>
<td>570 ± 81</td>
<td>6</td>
</tr>
<tr>
<td>Scrapie sheep brain (Moredun Institute)</td>
<td>430 ± 72</td>
<td>6</td>
</tr>
<tr>
<td>Multiple-sclerotic human brain</td>
<td>510 ± 67</td>
<td>6</td>
</tr>
<tr>
<td>Normal Compton mice</td>
<td>630 ± 22</td>
<td>6</td>
</tr>
<tr>
<td>Compton scrapie-infected mice</td>
<td>470 ± 19</td>
<td>6</td>
</tr>
</tbody>
</table>

Brains (0.05 ml. of 1/10 suspension) were inoculated intracerebrally. PLDH values are given in BB units as averages ± S.E.M.

and plasma infectivity persist for prolonged periods. A mouse inoculated 6 months previously with PLDH-elevating virus derived from a transplanted tumour-bearing mouse was kindly given to us by Dr K. E. K. Rowson. Histological examination of the
Plasma enzyme elevating virus in scrapie mice

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brain and CNS showed none of the changes usually associated with scrapie virus infection. However, it is hoped to study animals infected for longer periods when they become available.

Table 4. Size of PLDH-elevating agent from scrapie brain, determined by serial filtration through graded Millipore filters

<table>
<thead>
<tr>
<th>Average pore diameter (m/µ)</th>
<th>PLDH</th>
<th>No of mice in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 100</td>
<td>All High</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2750±125</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>320±61</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>319±75</td>
<td>5</td>
</tr>
</tbody>
</table>

0.05 ml. of each filtrate was injected intracerebrally. PLDH values are given in BB units as averages ± S.E.M.

DISCUSSION

The results have indicated that all mice in this unit inoculated with our own mouse-passaged scrapie material were also infected by a PLDH-elevating agent. This could occur in three ways. First, the scrapie agent itself may have had PLDH-elevating activity. This seems conclusively disproved by the normal LDH levels in mice injected with sheep- or rat-passaged scrapie, and in mice inoculated with Compton scrapie virus. Secondly, our mouse colony may be infected with the Bartonella-type organism Eperythrozoon coccoides which results in PLDH elevation in infected mice. Thirdly, our preparations may have been contaminated by the PLDH-elevating virus originally described by Riley et al. (1960). The pattern of PLDH elevation resulting from infection by Eperythrozoon or by virus is however markedly different (Riley, Loveless & Fitzmaurice, 1964). In particular, Eperythrozoon produces only a transient elevation of PLDH activity, the level returning to normal within about 14 days, whereas our results have shown a persistent elevation of PLDH activity for 6 months or more. Further, the passage of the PLDH-elevating agent by a membrane of 1000 Å APD but not of 500 Å is inconsistent with its being Eperythrozoon. It appears therefore that much of the scrapie material used in this Unit is contaminated with a PLDH-elevating virus. The absence of raised PLDH levels in our normal mice suggests that the contamination was brought into the Unit associated with mouse scrapie material and was not due to contamination of our normal mouse colony. The transmission pattern of the agent from parents to litters has shown that mice born of infected mothers are usually themselves infected, but that the agent does not pass, or does not infect by passing, in the milk. This resembles the transmission pattern of the contaminant PLDH-elevating virus associated with mouse tumours.

Whilst no pathological significance can be accorded the PLDH-elevating virus at this stage it remains a possibility that it might play some part in the production of the differing pathological pictures with different strains of scrapie virus reported by Zlotnik (1965).

The history of studies on the scrapie agent has been typified by false trails and lack of agreement between different investigators. It is not at present clear whether the injection of PLDH-elevating virus along with the scrapie agent has any influence on the development of the disease, or the replication of the scrapie agent, but experiments are
in progress on this point. In principle, however, it would seem desirable that work on
the scrapie agent should not be complicated by co-infection with another virus and it is
suggested that occasional checks of PLDH activity should be made in scrapie-infected
colonies.

We wish to thank Miss Greta Joyce for histological preparations.

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