THE EFFECT OF TREPONEMA PALLIDUM ON MOUSE SURVIVAL

D. J. M. WRIGHT* AND F. P. WHARTON†
* Department of Microbiology, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF, and † Division of Cytopathology, National Institute for Medical Research, Mill Hill, London NW7 1AA

Experimental infection of mice with Treponema pallidum results in no clinical disease or histological damage (Vaisman, 1936). Nevertheless, Rosahn (1952) suggested that mice infected with T. pallidum died significantly earlier than uninoculated mice. In view of the analogy drawn between the asymptomatic infection in mice and untreated latent syphilis in man (Rosahn, 1952), the survival of laboratory mice infected with T. pallidum was re-examined.

MATERIALS AND METHODS

Mice. The animals were inbred CBA mice fed on an antibiotic-free diet and water ad libitum. Mice aged 6 weeks were infected intraperitoneally with 0.5 ml of a suspension of live T. pallidum (Nichols strain). Control mice were inoculated with either a formalin-killed suspension or suspending medium alone.

Histological examination. Mouse liver, lungs, heart, spleen, kidney and inguinal but not abdominal lymph nodes were examined histologically. The tissues were fixed in neutral buffered formalin and stained by Young's (1969) modification of the silver impregnation technique of Warthin and Starry.

Treponemal suspensions. The treponemal suspensions were prepared from experimentally-infected rabbit testes (Wright, Gaugas and Rees, 1974) and the suspensions for inoculation were adjusted to contain 10⁷ spirochaetes per ml. The suspending medium was the thioglycollate medium described by Wilkinson (1972) but without streptomycin and penicillinase. The formalin-killed suspension was prepared according to the method of Marshak and Rothman (1951) except that the formalin was removed by dialysis against saline.

Examination for treponemal antibodies. The mice were bled from the retro-orbital veins with heparinised 50-μl capillary tubes (Hawksley Ltd). The tubes were sealed with plasticine ("Cristaseal", Hawksley Ltd) and the plasma was separated on a haematocrit centrifuge and stored at −20°C until used. If the animal was to be killed, exsanguination by cardiac puncture was performed. The fluorescent treponemal antibody test (Wright et al., 1974) was performed with saline as a diluent. A titre of 125 was taken as evidence of multiplication of spirochaetes in the mouse (Wright et al., 1974).

Experimental design. Two hundred and twenty mice were infected with T. pallidum. Of 90 control mice, 60 were inoculated with the killed suspension and 10 with suspending medium alone, and twenty uninoculated mice were retained for the duration of the experiment. Ten mice from the infected group were killed at intervals of 6 months, and 10 mice inoculated with dead spirochaetes were killed 30, 36 and 42 months after the beginning of the experiment. Organs of all these mice were examined histologically. After inoculation with living or killed spirochaetes, mice were bled monthly for 3 months and every 3 months thereafter. Blood samples were examined by the fluorescent treponemal antibody test. The mice allowed to die naturally thus comprised 150 infected animals and 60 uninfected animals. The

Received 6 May 1976; revised version accepted 13 Sept. 1976.
TABLE

Failure of T. pallidum infection to influence the life span of CBA mice

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Number of survivors from group of 150 infected mice</th>
<th>Percentage of infected mice surviving</th>
<th>Number of survivors from group of 60 uninfected mice</th>
<th>Percentage of uninfected mice surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>145</td>
<td>96.7</td>
<td>57</td>
<td>95.0</td>
</tr>
<tr>
<td>33</td>
<td>142</td>
<td>94.7</td>
<td>52</td>
<td>86.7</td>
</tr>
<tr>
<td>36</td>
<td>125</td>
<td>83.3</td>
<td>50</td>
<td>83.3</td>
</tr>
<tr>
<td>39</td>
<td>111</td>
<td>74.0</td>
<td>42</td>
<td>70.0</td>
</tr>
<tr>
<td>42</td>
<td>78</td>
<td>52.0</td>
<td>33</td>
<td>55.0</td>
</tr>
<tr>
<td>45</td>
<td>27</td>
<td>18.0</td>
<td>20</td>
<td>33.0</td>
</tr>
<tr>
<td>48</td>
<td>1</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>51</td>
<td>0</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Mean survival times (months): infected mice, 41·24; uninfected mice, 41·38. Log-rank test (Peto and Peto, 1972): \( \chi^2 = 0.81 \); d.f. = 1; \( P = 0·3 \) to 0·5.

uninfected group of mice contained animals inoculated with killed spirochaetes or medium alone, as well as uninoculated mice.

RESULTS

There was no difference between the mean age at which death occurred in the infected and control mice (table). The 150 T. pallidum-infected mice died at a mean age of 41·24 months and the 60 uninfected control mice at 41·38 months (log-rank \( \chi^2 = 0.81 \)). There was no appreciable difference between uninfected mice that received killed T. pallidum, medium alone, or no treatment.

All the mice inoculated with T. pallidum developed a significant fluorescent treponemal antibody titre within 3 months, unlike the mice inoculated with formalin-killed spirochaetes. No significant antibody could be detected 15 months after inoculation.

T. pallidum was not found in any material examined histologically except for one spirochaete seen in a single section of liver 33 months after infection.

DISCUSSION

The failure of infection with T. pallidum to affect the life span of the mice suggested a lack of pathogenicity for these animals. This suggestion was supported by the short duration of significant antibody levels and the finding of only one treponeme by histological examination. It is difficult to account for the contrasting results of Rosahn (1952), who suggested that T. pallidum shortens the life of mice. However, Rosahn's normal mice were shorter-lived than ours, perhaps due to separate caging, and all his controls were inoculated with saline instead of with killed T. pallidum. Possibly mouse-strain differences are crucial (Jahnel, 1937) and the black C57H mice used by Rosahn may have been more susceptible to T. pallidum than our inbred CBA mice.

SUMMARY

Intraperitoneal infection with Treponema pallidum did not shorten the lives of inbred CBA mice. One hundred and fifty infected mice survived to a mean age of 41·24 months and 60 uninfected mice to a mean age of 41·38 months.

We wish to thank Dr K. D. MacRae for statistical advice. D. J. M. W. is in receipt of a grant from the Medical Research Council.
REFERENCES


