The hamster as a secondary reservoir host of lymphocytic choriomeningitis virus

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SUMMARY

Exposure of weaned hamsters to an environment contaminated with LCM virus shed by tolerantly infected mice led to short subclinical infections. If infection occurred in early pregnancy, the young appeared normal at birth but their tissues were highly infective. For two to three months their bites and urine were also highly infective. A viraemia did not persist long enough for successive vertical transmissions of the infection to be likely. However, the viruria persisted in most prenatally infected hamsters for at least eight months and under simulated field conditions was a potent virus source for contact infections, leading to further generations of prenatally infected young. In the absence of the natural reservoir host, such long-term carriers could have been a major factor in causing the build-up of infection in colonies of hamsters which, when purchased as household pets, led to a recent spate of human clinical infections in Germany and the U.S.A.

INTRODUCTION

Interest in lymphocytic choriomeningitis (LCM) has been heightened by recent reports of the infection resulting from contact with the Syrian hamster, *Meso-cricetus auratus*, kept as a pet (Ackermann et al. 1972; Center for Disease Control, 1974; Hirsch, Moellering, Pope & Poskanzer, 1974; Biggar, Woodall, Walter & Haughie, 1975; Deibel, Woodall, Decher & Schryver, 1975). Formerly, cases outside the laboratory had usually been attributed to the house mouse, the natural reservoir host of the virus (Ackermann, 1973). Human infections in the United Kingdom have led to the identification of two foci of wild house mouse tolerantly infected with LCM virus (Duncan, Thomas & Tobin, 1951; Smithard & Macrae, 1951; Komrower, Williams & Stoner, 1955).

In West Germany (Ackermann, 1973) and the U.S.A. (Lehmann-Grube, 1971) the foci have been numerous, and it is from these countries that pet hamsters have now been reported as the source of human infections. In Germany the stocks of eleven commercial breeders were monitored and six were found to be infected with the virus (Forster & Wachendorfer, 1973). In the USA the source of about 100 human infections was traced to a single breeder whose stock was destroyed (Center for Disease Control, 1974).

We had earlier studied the routes by which laboratory mouse colonies could become infected by wild mice (Skinner & Knight, 1973, 1974) and we are reporting
here the application of the same procedures to hamsters. The study reveals the ease with which transfer of infection can occur and confirms that hamsters thus infected can provide an unsuspected source of LCM virus especially hazardous in the home, school or pet shop where handling takes place.

MATERIALS AND METHODS

Hamsters

Hamsters were bred by us under barrier conditions in a colony derived from six pregnant hamsters purchased from a British breeder in December 1972. The gestation period of 16 days is assumed. Experimental cages with wire grid floors were used to house 2–4 compatible hamsters up to the age of about 4 months, after which they were caged singly. As a precaution against bites and cross infections, two pairs of long forceps were used for handling infected animals. These were washed after each handling. To induce micturition, hamsters were handled over clean greaseproof paper. Samples of the urine were diluted at once 1/10 in phosphate buffer, pH 7.6. From about 20 weeks of age this technique often failed, and freshly excreted urine was then collected from Petri dishes placed under the experimental cage. Terminal collections were usually direct from the bladder. Carbon dioxide was used for euthanasia and for anaesthesia before experimental infection by a scratch or bite. At the end of an experiment, individual serum samples were prepared from all hamsters and were tested for complement-fixing antibody (CFA) using antigen supplied by the Standards Laboratory, Central Public Health Laboratory, Colindale.

Virus source and infectivity titrations

Freshly killed Pirbright P(PTI) strain mice were the source of infective urine and blood and were used to make experimental bites. These mice are tolerantly infected with LCM virus and have been used before to represent the natural reservoir host (Skinner & Knight, 1973).

Techniques for testing the infectivity of hamster mouth-swabs and bites, and for collection and titration of tissues, were as used in our mouse studies (Skinner & Knight, 1969, 1973, 1974). Titrations were by the foot-pad method in Pirbright P(SD) SPF mice and infectivity titres are based on individual foot-pad reactions expressed as log_{10} ID50/0.03 g. In Table 2 titres recorded as < 0.5 or < 1.0 indicate that no infectivity was detected at the lowest dilutions tested, namely, 10^{-1} and 10^{-1.5} respectively. When these dilutions only were tested, severe local reactions within 7 days indicated titres > 3.0 and are so recorded in Table 2. If there was no reaction to the original inoculum, and non-reactors were immune to a challenge 3 weeks later, the original material was recorded as having a trace of infectivity (Table 3).
RESULTS

Natural transmission of infection from mice to hamsters

Groups of hamsters were housed in a bin, 76 cm × 60 cm × 53 cm high, over which there rested a mouse rearing cage with a wire grid floor, 84 cm × 28 cm, holding 20 adult P(PTI) mice. Their faeces, urine and food debris dropped on to the bedding of the hamsters below. These were supplied with their own food and water. The mouse cage was removed after 2 weeks and the hamsters were killed 2 weeks later. In three experiments infection rates were: in a group of males 4 months old, 1 out of 7; in a group of females 5 months old, 2 out of 9 (both viraemic when killed); and in a group of females 1 month old, 4 out of 9, 3 of the 4 having a viraemia. Six unexposed controls showed no CFA response.

When groups of 3 and 6 newly weaned hamsters were each housed in a mouse rearing cage with 4 P(PTI) mice for 26 days, and were then isolated from mice for 3 weeks, a total of 4 showed a CFA response. A replica of this experiment, using litter-mates of the hamsters, but SPF mice in place of PTI mice, was run concurrently alongside and none of the nine hamsters showed a CFA response.

Simulation of natural transmission from mice

Earlier studies (Skinner & Knight, 1969, 1973, 1974) showed the infectivity of blood, saliva and urine from P(PTI) mice to be about 10^5 mouse ID50/ml. Fresh serum from these mice was the usual source of virus for experimental infections. Salivary glands were the most infective of many tissues tested, and washings of mouth swabs were consistently infective. Transmission of infection to SPF mice was regularly achieved by biting a lip or tail with the incisors of a freshly killed P(PTI) mouse or by applying fresh PTI mouse urine to scarified epidermis. These results were a guide to the techniques used to simulate natural transmission of infection from mice to weaned hamsters.

The hamsters were aged up to 5 months. Seventeen out of 18 were infected by a bite on the upper lip or a hind limb. Twenty-one out of 25 were infected by a drop of fresh PTI urine given orally after, or before, the inner surface of the lower lip had been scratched. These 38 infections were detected by CFA tests done when the hamsters were killed 2–8 weeks after exposure to the virus. Their blood, spleen, salivary glands (including the sub-mandibular lymph nodes) and saliva were tested for infectivity at the same time. No clinical signs were seen but, when the salivary glands had a high titre, the sub-mandibular lymph nodes were enlarged. Likewise, highly infective spleens were enlarged. Infectivity persisted in the glands after the viraemic phase had ended (Table 1). Infective saliva was detected in only one hamster, which also had a viraemia.

Four controls were given PTI urine orally without a scratch and all tests on them were negative. Seven more litter-mates acting as controls in the same room showed no CFA response.

Urine from the hamsters in Table 1 was not tested, but a similar group of 22 hamsters, infected by intramuscular inoculation and tested at the same time intervals after infection, gave almost identical test results, and urine tests were
Table 1. Persistence of infectivity in weaned hamsters infected through a bite or scratch

<table>
<thead>
<tr>
<th>Interval after exposure (weeks)</th>
<th>Number of hamsters with infective tissue/total number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>2</td>
<td>6/9</td>
</tr>
<tr>
<td>3</td>
<td>0/2</td>
</tr>
<tr>
<td>4</td>
<td>2/10</td>
</tr>
<tr>
<td>5</td>
<td>0/8</td>
</tr>
<tr>
<td>6</td>
<td>0/6</td>
</tr>
<tr>
<td>8</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Table 2. Infectivity of the young of hamsters infected during pregnancy

<table>
<thead>
<tr>
<th>Day on which the dam was infected before (−) or after (+) conception</th>
<th>−3 to −1</th>
<th>+3 to +5</th>
<th>+6</th>
<th>+8 to +14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of litters reared and studied</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of young studied</td>
<td>41</td>
<td>29</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Infectivity titres of blood (B) or of pooled thorax, heart and lungs (T) from individuals killed 0–26 days old, and the number [ ] showing this titre (log₁₀ ID₅₀/0.03 ml.)</td>
<td>B &gt; 3.0 [11] B 5.0 [1] T &lt; 1.0 [1] B &lt; 0.5 [2]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B 5.0–5.5 [5] T &gt; 5.5 [5]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of young aged 0–26 days with infective tissues</td>
<td>21/21</td>
<td>14/14</td>
<td>1/2</td>
<td>0/9</td>
</tr>
<tr>
<td>Incidence of weaned young aged 4 to 6 weeks with infective saliva or urine</td>
<td>20/20</td>
<td>15/15</td>
<td>5/6</td>
<td>0/31</td>
</tr>
</tbody>
</table>

included. A viruria was present during the viraemic phase but did not persist thereafter except at a low titre in one hamster out of 11 killed 3–8 weeks after infection. This is in marked contrast to the evidence to be presented of persistence of a viruria for several months in hamsters infected prenatally. The persistence of infective saliva was also shorter. In addition to the data in Table 1, the saliva of 3 hamsters infected by a bite when weaned at 23 days of age was monitored by testing mouth swabs every 4–6 days and infectivity persisted in individuals until 15, 25 and 30 days after infection only.

Prenatal infections

Twelve of the hamsters infected by a bite or scratch (Table 1) were pregnant. All developed a CFA response. Infective young were present in all the litters of the 5 dams infected in the first week of gestation but in none of those infected in the second week (Table 2). These litters were reared without significant differences in weight gain or health between those infective and not infective. All the young were
Table 3. Infectivity of urine and kidneys of prenatally infected hamsters

<table>
<thead>
<tr>
<th>Age in weeks of hamsters when tested</th>
<th>4-7</th>
<th>8-11</th>
<th>13-18</th>
<th>20-25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of hamsters with infective urine</td>
<td>32/33*</td>
<td>29/30*</td>
<td>15/16*</td>
<td>26/26</td>
<td>14/16</td>
<td>8/11</td>
<td>5/5</td>
</tr>
<tr>
<td>Incidence of hamsters with infective kidneys</td>
<td>---</td>
<td>4/4</td>
<td>5/6*</td>
<td>8/8</td>
<td>7/8</td>
<td>8/8</td>
<td>4/5</td>
</tr>
<tr>
<td>Number of titrated urine and kidney ( ) samples within each infectivity titre range: log_{10} ID50/0-03 ml. or g.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-0-5-4</td>
<td>11</td>
<td>6</td>
<td>5 (1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-5-3-9</td>
<td>1</td>
<td>7</td>
<td>2 (1)</td>
<td>4 (4)</td>
<td>4 (2)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>1-0-2-4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>11 (3)</td>
<td>6 (3)</td>
<td>4 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Trace to 0-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (1)</td>
<td>4 (2)</td>
<td>3 (4)</td>
<td>4 (2)</td>
</tr>
</tbody>
</table>

* In these groups it was the same hamster, killed 13 weeks old, giving the negative results for infectivity.

monitored individually for infectivity. The 31 young weaned from the non-infective litters were killed when 6 weeks old and no viraemia or CFA response was detected. Simultaneous tests detected high infectivity titres in the blood and tissues of young in the other litters when killed before weaning (Table 2) and at the age of 6 weeks all transmitted infection to mice by their bites except one. This one also gave negative infectivity results in all other tests but had a CFA response when killed at 13 weeks. At 8 weeks, infectivity was present in mouth swab washings of 10 out of 20 tested and at 13 weeks in 4 out of 13. Bites were infective in 1 out of 14 at 17 weeks and, in another, swabs continued to be infective up to the age of 21 weeks.

Studies were also made on 7 more females infected intramuscularly 1-3 days before conception. They all had infective litters and all the young had a viruria when weaned (Table 2).

From the age of 32 days, young in the 12 infective litters (Table 2) were killed at regular intervals and, with two exceptions, tests for infectivity were made on 1/10 suspensions of serum, salivary glands, sub-mandibular lymph nodes, kidneys and (if possible) urine. From the earliest age, pools of urine from litter-mates and, later, individual urine samples were titrated. The kidneys were titrated after 17 weeks of age (Table 3). A viraemia was detected twice only – at 4½ weeks and 8 weeks. Salivary glands were infective up to 10 weeks and the lymph nodes up to 13 weeks. Early titres of urine were > 4-0 but were rarely as high after 18 weeks. A viruria and infective kidneys persisted in some individuals up to 40 weeks.

Perpetuation of the carrier state in hamsters by lateral spread to pregnant dams

Two prenatally infected male hamsters aged 8 weeks were housed for 2 weeks with 2 normal virgin females of the same age. Both the females developed CFA responses. One had a litter 4 days after isolation from the males and two of the young
were killed when 4 days old; both had a viraemia. The 4 litter-mates were reared. At 4, 7 and 9 weeks of age, pooled urine samples from them had titres of 4-5, 5-8 and 4-0; and at 15, 21 and 28 weeks the mean titres of individual samples were 3-4, 3-6 and 2-8 respectively. At 38 weeks the individual urine titres were: trace, 1-2, 1-5 and trace.

When 10 weeks old, these 4 hamsters were placed in contact for 4 weeks with 4 normal hamsters, 3 of which, after isolation for 2 weeks, were shown by CFA tests to have been infected. If the two in utero transmissions are included, this was the fifth serial transmission of the virus in hamsters since the dam of the two original male spreaders had been infected by PTI mouse urine through a scratch. The interval between this infection and collection of the infective urine samples from the second prenatally infected litter, at the age of 38 weeks, was 12 months. This demonstrated how the infection could persist in a colony of hamsters for at least a year from the time of its introduction by contact with mice.

DISCUSSION

Besides the recent incidence of LCM in households owning a pet hamster, 48 more cases among personnel of a medical centre in the U.S.A. were recently traced to experimental hamsters on the premises (Hotchin et al. 1974; Hinman et al. 1975). Some years earlier in the U.S.A. 11 clinical cases were associated with experimental hamsters considered then to have been infected by transplants of a fibrosarcoma carrying the virus (Baum, Lewis, Rowe & Huebner, 1966; Armstrong, Fortner, Rowe & Parker, 1969). The infection of other hamsters cared for by the same staff was thought to have been lateral spread (Lewis, Rowe, Turner & Huebner, 1965). In all these reports the importance of recognizing the hamster as a potential secondary reservoir for LCM virus was stressed. Convalescence was prolonged in many patients and it was recognized that the clinical cases were but an indication of many more undiagnosed infections in which the early influenza-like symptoms did not progress to a meningitis. After evidence suggested that defects in two infants could be linked with LCM infections in the pregnant mothers (Ackermann et al. 1974), public authorities in West Germany strongly advised pregnant women to avoid all contact with hamsters.

The infection route for man was usually uncertain, a hamster bite being known in only a few cases. However, the case with which mice and hamsters can be infected through traumatized epidermis suggests that the risk of human infection through mucous membranes or skin contaminated with infective urine, or saliva, might also be high and should be guarded against by a high standard of hygiene. The bites and urine of young prenatally infected hamsters are as hazardous as those of tolerantly infected wild or pet mice. While in mice these hazards persist with a viraemia throughout life, our findings indicate that in prenatally infected hamsters the infectivity of excretions is greatly reduced by the age of 9 months. In infections contracted after weaning, the infectivity of tissues and excretions probably ceases within 2 months, as in mice infected at this age. In both species the carrier animal breeds successfully and clinical signs to suggest that it should
be culled are absent. Whereas in mice serial vertical transmissions are the infallible natural means whereby the infection is maintained, the present evidence indicates that this is unlikely in hamsters. However, it is easy to visualize the maintenance of a reservoir of carriers in successive generations of a colony by exposure of normal pregnant hamsters to infection from the urine of existing carriers. This is likely to have occurred in some infected commercial hamster colonies and a report in 1968 indicates perhaps an early source from which infection in other German colonies was derived (Petrović & Timm, 1968).

In the U.K. there is no evidence of LCM infection in hamsters, or of widespread incidence of infected mice, and human infections seem to be rare. When future cases are diagnosed, the possibility of contact with infected hamsters should be considered. It would be unfortunate, when such a source is found, if it was not eradicated before it caused proliferation of infected premises and new reservoirs of the virus in wild mice, which would be far more difficult to exterminate.

REFERENCES


