MOUSE MODEL FOR EXPERIMENTAL HEPATIC AMOEBIASIS

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Golden hamsters are generally used as a model for experimental hepatic amoebiasis. There are certain disadvantages in this model. Hamster liver is very much susceptible to amoebic infection and death of animals invariably occurs within 4-6 days with the liver abscess. Hamster does not serve as an ideal host for antiamoebic drug screening and for immunoprophylaxis studies. Breeding of this animal under laboratory conditions is rather difficult. A mouse model for hepatic amoebiasis described here was found suitable for studies on experimental amoebiasis.

Two virulent strains of Entamoeba histolytica PK and H-39, isolated from "carriers" and acute cases respectively, were used. Liver abscess was first produced in the liver of golden hamster by intra-hepatic inoculation of trophozoites. For production of liver abscess in mice (25 to 30 g), a small opening was made in the upper abdominal region (keeping the animal under nembutal anesthesia) and a small piece of necrotic tissue from the abscessed hamster liver was placed on the mouse liver just below the sternum with a sterile forceps. The mouse was killed after 7 days and the grade of lesion was assessed as described by Dutta, (0 = normal; 1 = tiny superficial lesion; 2 = 5-15% of liver with lesions; 3 = 25% of the liver showing lesion and 4 = more than 25% of the liver showing lesions). Once the hepatic abscess was developed in mouse liver, more mice were inoculated by the small pieces of the necrotic liver tissues. The strains of E. histolytica were maintained in the animal by liver to liver passage. Smear preparation of the necrotic and abscessed liver piece was made to observe active E. histolytica trophozoites and culturing of the tissue was made in Boeck & Drbohlav medium. Histopathological preparations were made to confirm the pathology of the amoebic infection.

Both H-39 and PK strains of E. histolytica are highly virulent to hamster liver. They produced an average of 3.5 and 3 grades of liver lesions respectively by intra-hepatic inoculation of trophozoites (figure 1); but they failed to infect mouse liver by a similar route of inoculation (table 1). In the case of H-39 cent per cent liver abscess with 3.5 grade of lesion was observed by infected liver piece inoculation method. By liver to liver passage, the grade of lesion was increased upto 4.0 (table 2). Mouse liver inoculated with a piece of abscessed hamster liver, initially produced 60% infection with 2 grade liver lesion, but after the liver to liver passage in mouse with infected liver piece inoculation method, the rate of infection went up to 100% with an average of 3.5 grade liver lesion (table 2; figure 2).

Figure 1. Amoebic liver abscess of hamster. (a) control liver. (b) abscessed liver.
Results of intra-hepatic inoculation of hamster and mouse with PK and H-39 strains of *E. histolytica*

<table>
<thead>
<tr>
<th></th>
<th>No. infected</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hamster:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain H-39</td>
<td>6 (3.5)</td>
<td>3 died on the 3rd day. 3 killed on the 4th day.</td>
</tr>
<tr>
<td>PK</td>
<td>6 (3)</td>
<td>2 died on the 3rd day. 4 killed on the 4th day.</td>
</tr>
<tr>
<td><strong>Mouse:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain H-39</td>
<td>0 (0.0)</td>
<td>No mortality</td>
</tr>
<tr>
<td>PK</td>
<td>0 (0.0)</td>
<td>No mortality</td>
</tr>
</tbody>
</table>

*Animals which died on the 3rd day showed 4 grade lesions in the liver. In each case the number of animals inoculated is 6. Figures in brackets refer to grade lesion average of 6 animals.*

Results of infection of mouse liver with piece of necrotic liver tissue taken from infected liver with amoebic abscess, caused by PK and H-39 strains of *E. histolytica*

<table>
<thead>
<tr>
<th></th>
<th>No. of animals infected</th>
<th></th>
<th>Hamster</th>
<th>Mouse</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H-39</td>
<td>PK</td>
</tr>
<tr>
<td>I Passage</td>
<td>10 (3.5)</td>
<td>8 (3)</td>
<td>6 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>II Passage</td>
<td>10 (4)</td>
<td>10 (3.5)</td>
<td>8 (3)</td>
<td>6 (2.5)</td>
</tr>
<tr>
<td>III Passage</td>
<td>10 (4)</td>
<td>10 (3.5)</td>
<td>10 (3)</td>
<td>10 (3.0)</td>
</tr>
</tbody>
</table>

The number of animals inoculated in all cases is 10. Figures in brackets refer to the average lesion grade. In all cases the abscessed liver piece of Hamster was used initially.

Abscessed liver piece of PK strain of *E. histolytica* from hamster liver, produced 80% infection with an average of 3.0 grade lesion initially, but after the second liver passage, there was 100% infection with an
average of 3.5 grade lesion, which rose up to 4 grade in
the next liver passage (table 2). Initially 40% infection
with 2 grade lesion was produced in mouse liver inocu-
lated with a piece of abscessed liver produced in hams-
ter by PK-amoebe. Liver to liver passage in mouse, the
infection rate increased from 60 to 100% and the
grade of lesion also changed from 2.5 to 3.0 respec-
tively (table 2; figure 3).

Histopathological preparation (figure 4) revealed
typical amoebic lesions in the liver with an abscess
cavity and trophozoites in the liver tissue. The lumen
was filled with necrotic material mixed with fluid, red
blood cells and leukocytes. Smear preparations of the
infected liver tissue showed active trophozoites under
microscope (figure 4). Portion of infected liver when
inoculated in culture medium gave rise to positive
*E. histolytica* cultures. Neal and Harris\(^3\) achieved
little success in infecting inbred mouse intraceccally
with *E. histolytica*. Westphal\(^1\) reported successful
caecal infection of mice with *E. histolytica* using a diet rich
in carbohydrate and vitamins. The caecum of infected mice
showed pin-head-sized ulceration containing amoebae. Woolfe et al\(^4\)
produced liver abscess in mice by using selective strain of *E. histolytical* by repeated
passage through mouse liver. For mouse liver infection
they used the "gelatine sponge" method of Jarumili-
lanta\(^5\) and got cent per cent infection. The main
advantage of the present method is that as many as

10–15 mice can be easily infected from a single infected
liver. This method of infection is being employed for *in
vivo* screening of antiamoebic drugs. The method also
ensures that the amoebae used for inoculation main-
tain their virulence.

We wish to thank Dr. Nitya Nand, for his keen
interest in the study, and also to ICMR, for research
grant to RKS for the study.

26 April 1982

B36, 99.
197.
220.
4. Woolfe, G., Everest, R. P., Williams, G. A. A. and
60, 139.

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**PORPAX CHANDRASEKHARANII**

BHARGAVAN ET MOHANAN—A NEW
SPECIES OF ORCHID FROM SILENT
VALLEY

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Botanical Survey of India, Coimbatore 641 003,
India

*Porpax* Lindl. is a small genus found only in the
Mainland of Asia\(^1-5\) It differs from its close relative
*Eria* Lindl.in having the sepals jointed into a tube and
having a very short pseudobulb which is wider than
long. *Porpax* Lindl.consists of 10 species\(^6\) of which 6
are known earlier from India. *Porpax jerdoniana*
(Wight) Rolfe and *P. reticulata* Lindl. are the two
species reported from South India\(^7-10\).

While on an exploration to Silent Valley a curious
population of *Porpax* was located. Comparing with
other known species of the genus, the plants were
found to possess distinct features and is therefore
described here as a new species. The plants were growing
on lichen covered tree trunks in moist shady places.

*Porpax chandrasekharanii* Bhargavan et C. N.
Mohanan sp. nov.

*Porpax elwesii* (Reichb.f.) Kraze.,affinis sed differ-
t scapo prominenti floribus 3-6, floribus parvioribus,
labiis simplicibus et parvioribus, columna brevioribus.

*Porpax Chendrasekharanii* Bhargavan et Mohanan
sp. nov.

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**Figure 4.** Histopathological preparation of infected
mouse liver showing trophozoites and necrosis of
the liver tissue (x 600).