5. Beauchamp, C. O. and Fridovich, I., *Biochem. Biophys. Acta*, 1973, **317**, 50.
6. Ahuja, B. S., Ushakiran and Sudershan, *Indian J. Biochem. Biophys.*, 1980, **17**, 77.
7. Mishra, H. P. and Fridovich, I., *J. Biol. Chem.*, 1972, **247**, 3170.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
9. Beauchamp, C. O. and Fridovich, I., *Anal. Biochem.*, 1971, **44**, 276.
10. Weisiger, R. A. and Fridovich, I., *J. Biol. Chem.*, 1973, **248**, 4793.
11. Christopher Jackson, Jane Dench, Anthony L. Moore, Barry Halliwell Christine. H., Foyer and David O. Hall, *Euro. J. Biochem.*, 1979, **91**, 339.
12. Walter D. Bonner, J. R., *Methods in enzymology*, Academic Press, New York, 1967, **10**, 126.
13. Hodgson, E. K. and Fridovich, I., *Biochemistry*, 1975, **14**, 5294.
14. Tyler, D. D., *Biochem. J.*, 1975, **147**, 493.
15. Arron, G. P., Henry, L., Palmer, J. M. and Hall, D. O., *Biochem. Soc. Trans.*, 1976, **4**, 618.

MOUSE MODEL FOR EXPERIMENTAL HEPATIC AMOEBIASIS

R. K. SRIVASTAVA, PRADEEP DAS AND S. R. DAS
Central Drug Research Institute, Lucknow 226 007, India.

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 mouse (25 to 30 g), a small opening was made in the upper abdominal region (keeping the animal under nembutol anesthetia) 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[1], (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 abscss 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 upto 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.

TABLE 1

Results of intra-hepatic inoculation of hamster and mouse with PK and H-39 strains of E. histolytica

	No. infected	Remarks°
Hamster:		
Strain H-39	6 (3.5)	3 died on the 3rd day. 3 killed on the 4th day.
PK	6 (3)	2 died on the 3rd day. 4 killed on the 4th day.
Mouse:		
Strain H-39	0 (0.0)	No mortality
PK	0 (0.0)	No mortality

° 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.

TABLE 2

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

	No. of animals infected			
	Hamster		Mouse	
	H-39	PK	H-39	PK
I Passage	10 (3.5)	8 (3)	6 (2)	4 (2)
II Passage	10 (4)	10 (3.5)	8 (3)	6 (2.5)
III Passage	10 (4)	10 (4)	10 (3.5)	10 (3.0)

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

Figure 2. Amoebic liver abscess of mouse.

Figure 3. Mouse livers showing different grades of lesions.

average of 3.5 grade lesion, which rose upto 4 grade in the next liver passage (table 2). Initially 40% infection with 2 grade lesion was produced in mouse liver inoculated with a piece of abscessed liver produced in hamster by PK-amoebae. 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 respectively (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 leucocytes. 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[2] achieved little success in infecting inbred mouse intracaecally with *E. histolytica*. Westphal[3] reported successful caecal infection of mice with *E. histolytica* using a diet rich in carbohydrate and vitamins. The caeca 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 Jarumilinta[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 maintain 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

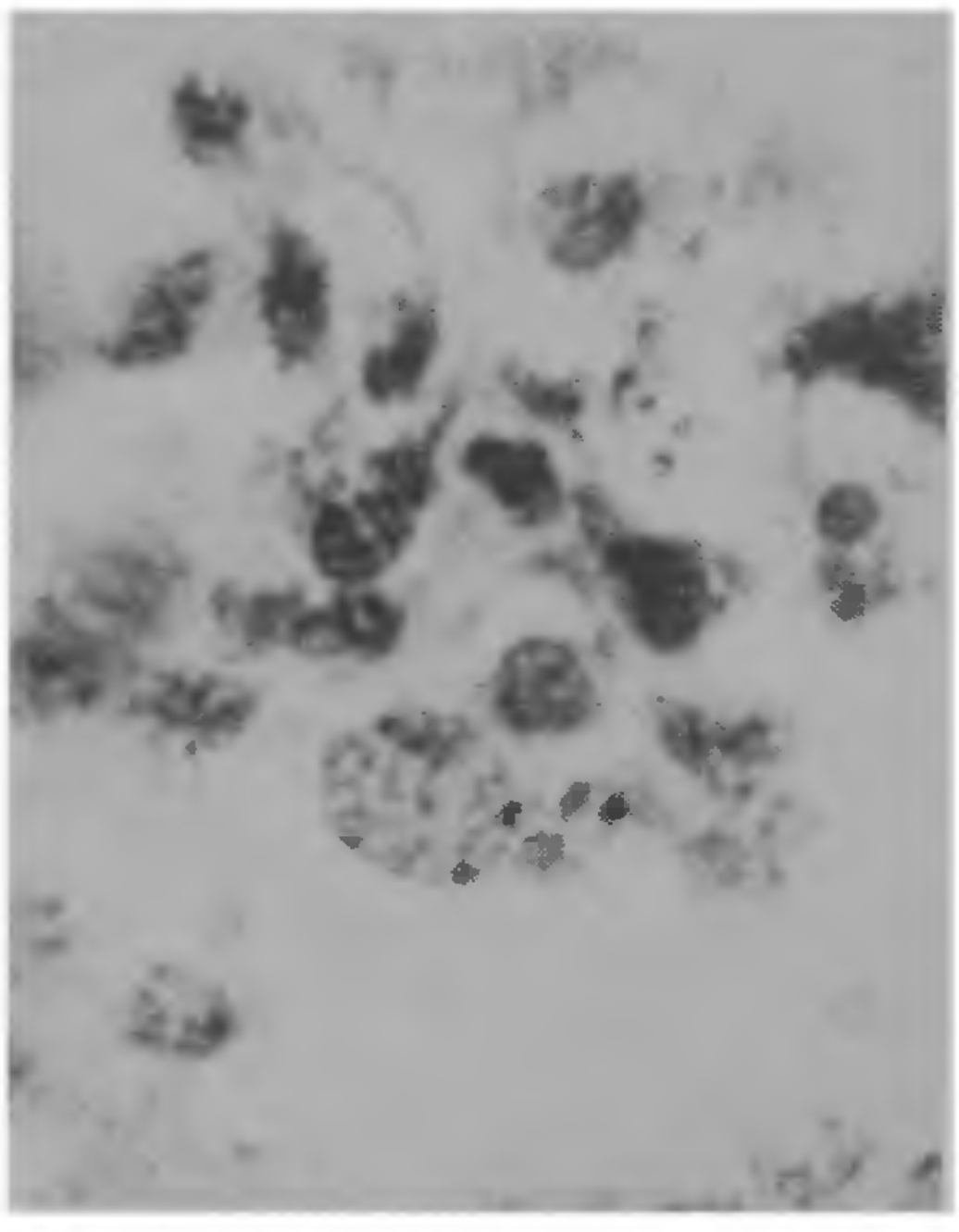

Figure 4. Histopathological preparation of infected mouse liver showing trophozoites and necrosis of the liver tissue (× 600).

1. Dutta, G. P., *Proc. Indian Nat. Sci. Acad.*, 1970, **B36**, 99.
2. Neal, R. A. and Harris, H. G., *Protozool.*, 1977, **3**, 197.
3. Westphal, A., *Z. Tropenmed Parasit.*, 1970, **21**, 220.
4. Woolfe, G., Everest, R. P., Williams, G. A. A. and Wilmshurst, E. C., *Trans. R. Soc. Trop. Med. Hyg.*, 1967, **61**, 427.
5. Jarumilinta, R., *Ann. Trop. Med. Parasitol.*, 1966, **60**, 139.

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

P. BHARGAVAN AND C. N. MOHANAN
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 differt scapo prominenti floribus 3–6, floribus parvioribus, labiis simplicibus et parvioribus, columna brevioribus.

Porpax Chandrasekharanii Bhargavan et Mohanan sp. nov.