16. Pichinoty, F., *Arch. Microbiol.*, 1970, **71**, 116.
17. Magalhaes, L. M. S., Neyra, C. A. and Dobereiner, J., *Arch. Microbiol.*, 1978, **117**, 242.

PATENT INFECTION WITH *SCHISTOSOMA INCOGNITUM* IN WHITE MOUSE (*MUS MUSCULUS*)

M. C. AGRAWAL, NINDER PANESAR and MANORANJAN DAS
Department of Parasitology, College of Veterinary Science and Animal Husbandry, Jabalpur 482001, India.

IN a study of host susceptibility, Sinha and Srivastava[1] infected white mouse with 300 and 1000 cercariae of *Schistosoma incognitum* and did not find its eggs in the faeces of the mice. They pointed out "In white mouse, though the worms attain maturity, their size was comparatively much smaller and their eggs could not break through the wall of its intestine to be voided with the faeces". The subsequent workers[2–4] supported their view. It was therefore presumed that white mouse is an unsuitable host in which the infection remains in its latent form. For this reason, *patency* could not be taken as criterion for judging immune response in mice—*S. incognitum* model[3–6]. The results of the remark to testify this hypothesis are reported.

Twelve white mice, 4–6 weeks old, of either sex were infected each with 500–600 freshly shed cercariae of *S. incognitum* by tail immersion Method[3]. The cercariae were pooled from twenty naturally infected *Lymnaea luteola* snails which in turn were collected from a big pond in Jabalpur city. On the 30th day of infection and at biweekly intervals thereafter, the faeces of all the mice were collected together in the morning and were divided into two parts. With one part of faeces (1–2 g) routine hatching technique[4] was applied while the other part was processed by acid–ether method[7] for microscopic examination.

After 4 to 6 weeks of infection, six mice were sacrificed to assess the development of the flukes and the period for laying viable eggs. The parasites, so collected, were stained and studied to confirm species of *S. incognitum*[8].

By acid-ether method, the faeces of left over mice (six) were found to contain viable *S. incognitum* eggs on 49th day of infection (figure 1). On the 50th day of infection, the faeces from each mouse was examined

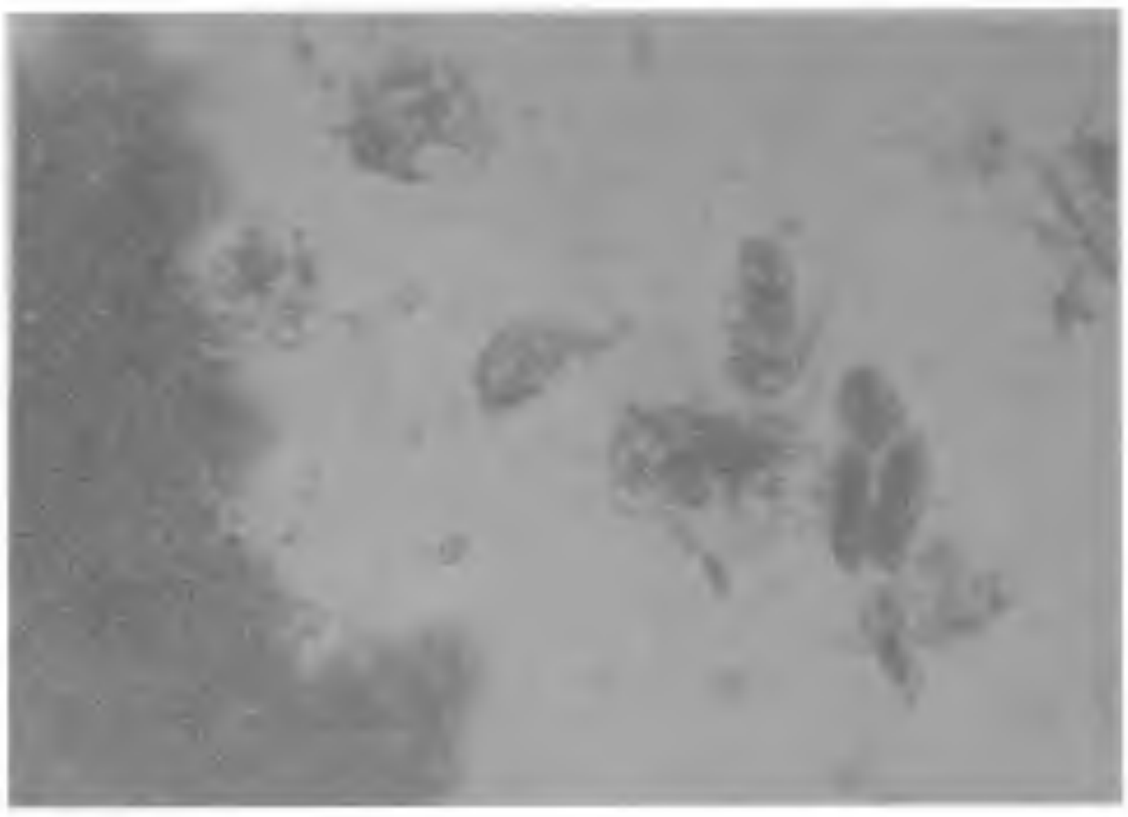

Figure 1. Viable *Schistosoma incognitum* egg in white mice faeces by acid–ether method × 100.

individually and found positive for *S. incognitum* eggs, except one mouse which turned positive on 54th day of infection. The faecal examination by hatching technique and acid–ether method on alternate days was continued till the death of the mice or sacrifice. The number of eggs varied from 300 to 1000 per g of faeces (Mean 400), and diminished in numbers before the death of the animal (table 1.)

The miracidia could not be detected by the routine hatching technique when applied with the faecal samples. According to Lagrange[9], eggs of *S. mansoni* also failed to hatch in the faeces of the albino mouse. Nevertheless, the intestinal washing, taken during postmortem, showed large number of the miracidia, in the present studies.

Two main reasons are responsible for failure of previous workers[1–4] in detecting *S. incognitum* eggs in white mouse faeces. First, they started examining the faeces on 30th day of infection to find the eggs within a week as it happens in pigs. Secondly, hatching technique was relied upon more to detect the infection and no specific method like acid-ether was applied for faecal examination. Naturally, the early reports of absence of eggs in the faeces also deterred the workers to believe otherwise. In the present studies, acid-ether technique was used to a greater extent with the careful examination of all the faecal sediment; the examination was continued for a long period inspite of early negativeness of the faeces. However, existence of a new strain of *S. incognitum* can not be ruled out as the snails were collected from a new pond.

The authors thank ICMR, New Delhi for financial support.

Table 1 *Schistosoma incognitum worm recovery from white mice along with the egg out put in the faeces. Faecal exam was started on 30th day.*

No. of cercariae (*S. incognitum*)	Time in days before sacrifice	*Schistosoma incognitum* Recovery Male	Female	Turned positive (in days)	Faecal Egg load Min.	Max. (eggs per g)
500	36	145	23	-ve	—	—
600	36	153	69	-ve	—	—
500	40	36	8	-ve	—	—
600	30	180	36	-ve	—	—
500	41	30	31	-ve	—	—
600	32	181	34	-ve	—	—
500	65	16	3	54	300	600
600	56	57	8	49	300	800
500	73*	2	1	49	300	600
600	56	83	10	49	300	1000
600	63	31	7	49	300	800
500	71*	2	1	49	300	800

Died. Worm recovery was not by perfusion technique.

2 April 1985

1. Sinha, P. K. and Srivastava, H. D., *Indian Vet. J.*, 1965, **42**, 664.
2. Ahluwalia, S. S., Ph.D. Thesis. Agra University, Agra, 1968.
3. Nikhale, S. G., M. V. Sc. Thesis. J. N. Agriculture University, Jabalpur, 1972.
4. Agrawal, M. C., Ph.D. Thesis, J. N. Agriculture University, Jabalpur, 1978.
5. Borkakoty, M., M. V. Sc. Thesis. J. N. Agriculture University, Jabalpur, 1975.
6. Shrivastava, H. O. P., Ph.D. Thesis. J. N. Agriculture University, Jabalpur, 1977.
7. Graig, C. F. and Faust, E. C., *Clinical Parasitology*, Lea and Febiger, Philadelphia, 1951, p. 887.
8. Sinha, P. K. and Srivastava, H. D., *Parasitology*, 1956, **46**, 91.
9. Lagrange, E., *Annls. Soc. Belge. Med. Trop.*, 1964, **44**, 477.

ANTHER CULTURE OF *CATHARANTHUS ROSEUS* L.—DEVELOPMENT OF POLLEN EMBRYOIDS

LEELA GEORGE

Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

CATHARANTHUS ROSEUS L is a small herbaceous subshrub growing wild and also cultivated because of its medicinal value. Vincristine and vinblastine now extracted commercially from the leaves of *C. roseus*, are used in the treatment of leukaemia and Hodgkin's disease and also other types of cancer. In recent years, the technique of anther culture is being increasingly used for rapid isolation of homozygous recombinants in several plants of economic importance[1]. Anther culture of *C. roseus* has been initiated for rearing the haploids and isolating the superior lines with increased alkaloid content. The following is a brief account of the results obtained.

Flower buds at the uninucleate stage of pollen development (bud size 0.3–0.5 cm) collected from vigorously growing plants were surface-sterilized and the anthers were cultured aseptically on MS[2] basal medium alone or MS medium containing various auxins, cytokinins and other growth adjuvants in varying concentrations and combinations. Solid as well as liquid media containing 8% sucrose were used for culturing the anthers. For cold treatment prior to culture the buds were stored at 10 C for 0, 3, 7 and 14 days. Elevated temperature treatment was given by incubating the cultured anthers at 30 C for 0, 3, 7, 14 and 21 days in a BOD incubator before transferring them to 22±2 C in dark. For each experiment a minimum of 240 anthers were used and over 11,000 anthers were cultured in all.

A few anthers were examined at regular intervals from each treatment. Most of the anthers turned brown in 6-8 weeks. Addition of activated charcoal into the medium did not prevent their browning. All the anthers were observed cytologically after 8 weeks