acid (IAA), kinetin (Kn) and solidified with 7,000 mg/l agar. The tissues were maintained for a period of eight months by subculturing them every 5–7 weeks. Eight-month-old tissues were then transferred to MS medium supplemented with 250, 500 or 1,000 mg/l tyrosine. The tissues were tested for tyrosine-tyrosinase activity for a period of eight weeks. The cultures were grown at 27 ± 1° C, under diffused light of *ca.* 300 lux intensity. The relative humidity of the culture room was maintained at 70 ± 4%.

In order to detect tyrosine-tyrosinase activity, approximately 250 mg creamish, non-pigmented tissue(s) was taken separately into eleven vials. To the first vial (I), 4 ml of 0·1 M phosphate buffer (pH 6·8) was added which comprised the control. To the vials II, III, IV, V and VI were added 4 ml each of 0·1 M phosphate buffer containing 0·10, 0·20, 0·25, 0·50 and 1·0 mg/ml phenylalanine, respectively. All the vials with their contents were incubated at 4° C for 6–8 hr. The pigmented tissues were also treated with oxidizing agents, *e.g.*, hydrogen peroxide, potassium permanganate and potassium chlorate solutions.

The tissues grown on MS and MS + tyrosine media were harvested and extracted in 50% ethanol after every two months. The extracts as also the standard sample of tyrosine were applied separately to Whatman No. 1 chromatography paper-strips. Chromatograms were developed in *n*-butanol-acetic acid-water (250: 60: 250)[10]. Developed strips were air-dried, sprayed with 0·25% ninhydrin in distilled water, heated in an oven at 100° C for 5 min and the spots identified.

The tissues grown on MS medium first turned brown in 3–4 weeks and then black during following 7–8 weeks. The melanin saturated cells almost lost the capacity to divide. The callus tissue, therefore, had to be periodically transferred onto the fresh MS medium for its further growth after every 5–7 weeks. The callus tissues grown on MS + tyrosine medium started turning black in 24 hr and within a week the entire tissue as well as the medium turned black. The growth of the tissue was almost checked. It indicated a very high degree of tyrosinase activity in the tissues. The tissues in the control (I) and phenylalanine containing vials (VII–XI) remained unchanged, whereas the tissues in the tyrosine containing vials (II–VI) turned black. Under the present experimental conditions the phenylalanine was not converted into tyrosine. The maximum melanin production in the tissues was recorded at 0·25 mg/ml concentration of tyrosine. The dark brown or black pigment was bleached by oxidizing agents.

Paper chromatography showed the presence of tyrosine (R_f 0·4) in the tissues grown on MS and MS + tyrosine media for a period of 5–7 weeks. Beyond this period the black tissue gave rise to new tissue whose growth appeared to be of the same type as exhibited on MS medium devoid of tyrosine, *i.e.*, first creamish and then brownish. This implied the complete consumption of tyrosine from the medium during the initial culture period of 5–7 weeks. Thus, it can be concluded that callus cultures derived from different plant parts of *S. khasianum* produced melanin pigment on agar media.

Thanks are due to Dr. T. N. Khoshoo, Director, National Botanical Research Institute, Lucknow, for the facilities provided and Mr. M. S. Gorkha and Mr. J. P. Misra for technical assistance.

Tissue Culture Laboratory,
National Botanical Research Institute,
Lucknow,
December 18, 1978.

AMIN UDDIN.
H. C. CHATURVEDI.

1. Goodwin, T. W., *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London, 1976.
2. Paech, K. and Tracey, M. W., *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin, 1955.
3. Nicolaus, R. A., Piatelli, M. and Fattorsso, E., *Tetrahedron*, 1964, **20**, 1163.
4. Allport, D. C. and Bu'Lock, J. D., *J. Chem. Soc.*, 1958, **4**, 4090.
5. — and —, *Ibid.*; 1960, **1**, 654.
6. Nicolaus, R. A., *Melanins*, Hermann, Paris, 1968.
7. Chan, W. N. and Staba, E. J., *Lloydia*, 1965, **28**, 55.
8. Khanna, P. and Nag, T. N., *Curr. Sci.*, 1972, **41**, 115.
9. Murashige, T. and Skoog, F., *Physiol. Plantarum*, 1962, **15**, 473.
10. Woiwood, A. J., *Biochem. J.*, 1949, **45**, 412.

AN APPARATUS AND METHODS OF STUDYING *IN VITRO* FEEDING OF BLOOD BY STRONGYLES OF HORSE AND THE MEASUREMENT OF THEIR PHARYNGEAL ACTIVITY

AN apparatus to study the *in vitro* feeding of *Ancylostoma caninum* in dogs has been described by Roche and Martin Torres[1]. In this study a simple modification of the above apparatus is described by means of which the *in vitro* feeding behaviour of the strongyles of the horse can be observed. An attempt has also been made to detect electrical impulses originating from the worms feeding on blood, and recording them graphically. This procedure could enable a continuous study of the pharyngeal activity of the worms for many hours and relate it to its feeding habits.

The apparatus consisted of a glass specimen bottle 6 cm high and 4 cm wide with a screw cap with a hole in its centre. A hypodermic needle of appropriate diameter was passed through a rubber male contraceptive membrane. A live nematode was held between the thumb and index finger and the tail introduced into the needle which was then drawn back through the membrane. The contraceptive membrane with the nematode attached to it was introduced into the bottle in such a way that the tail of the nematode was immersed in 9 g/l saline solution. The ring end of the contraceptive membrane was folded over the mouth of the bottle and the screw cap applied. Through the hole horse blood was introduced to completely cover the anterior end of the nematode and the hole covered with adhesive tape. The nematodes then had their anterior ends in blood and their tail and anus in saline solution (Figs. 1 and 2). The bottles were placed in an incubator at 37° C. Saline samples were obtained at intervals and examined for blood.

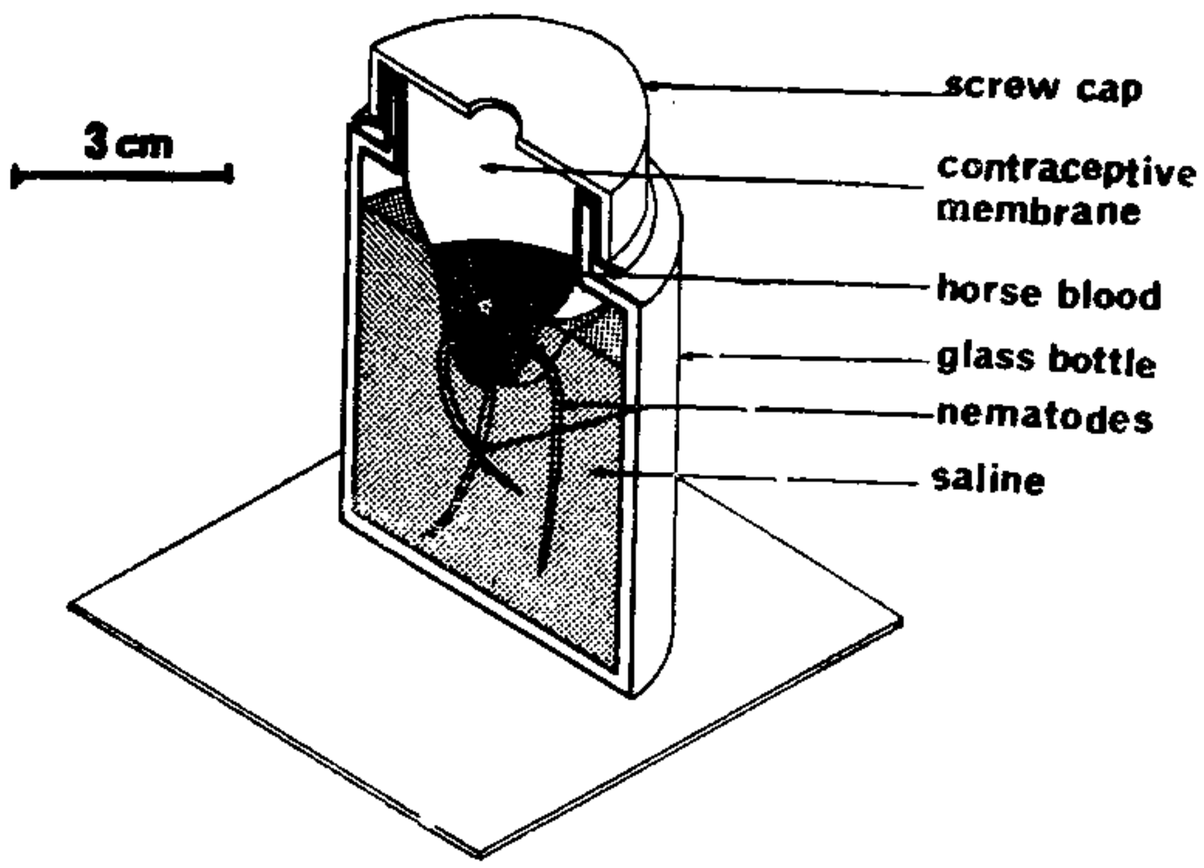

FIG. 1. Sectional isometric drawing of the apparatus, showing the position of the nematodes with the anterior end in the blood and tail in the saline.

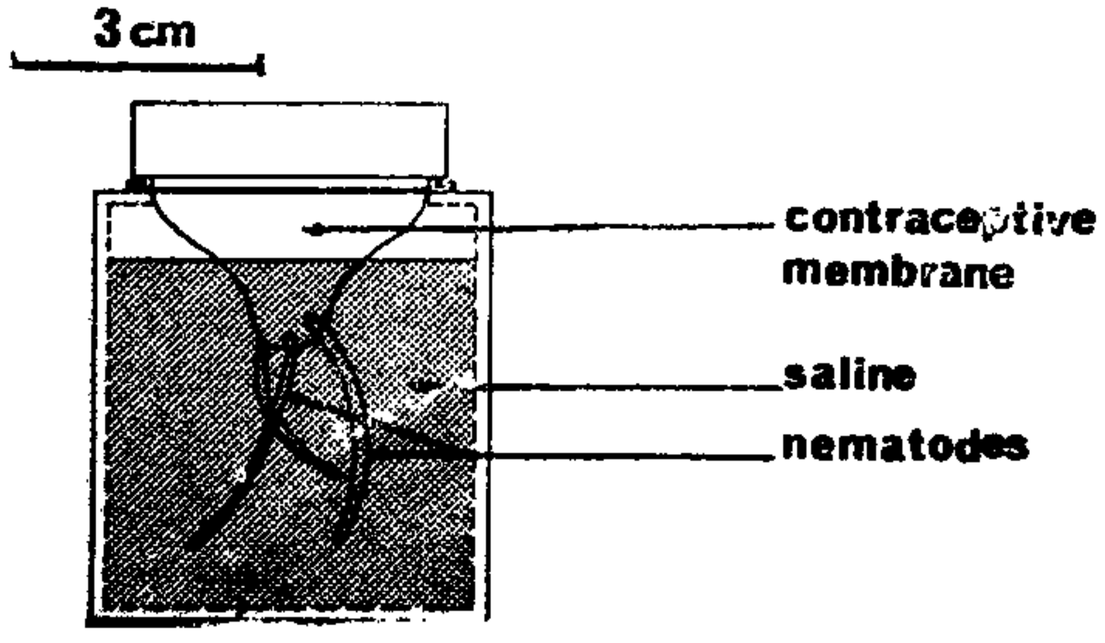

FIG. 2. Side elevation of the apparatus showing the contraceptive membrane and the tail end of nematodes in saline.

For the measurement of pharyngeal activity *in vitro*, the nematodes were placed in the apparatus, two platinum electrodes were introduced, one into the saline chamber and the other into the blood chamber. The electrodes were connected to a EEG and Polygraph Data Recording system, Grass Model 79 D (Grass Instrument Co., Quincy, Mass., U.S.A.). A wide band filter on the preamplifier was used and for the recording a 35 H_z filter was used on the last stage of amplification. Amplitude was 200 uV. The apparatus containing the nematode was kept in a water bath at 37° C. The pulses observed were recorded by the ink recorder. Observations were made on *Strongylus vulgaris* and *A. caninum*. Both nematode species were kept in the apparatus for 1–4 hrs in an incubator before recordings were made.

Live specimens of species in the genus *Cylicocyclus* incubated in the apparatus lived for 12–24 hrs and no erythrocytes or haemoglobin was detected in the saline chamber. Species of *S. vulgaris*, *S. edentatus* and *S. equinus* remained alive for 24 hrs. The nematodes started to pass blood through the anus into the saline chamber 6 hrs after incubation. The contents of the saline chamber revealed intact erythrocytes and also showed traces of blood by the benzidine test for iron using test strips of 'Hemastix'. Similar results were obtained with *A. caninum* which fed on dog blood in the same manner.

The pulses recorded (Figs. 3 and 4) were in the form of two types of waves, one slow and the other fast.

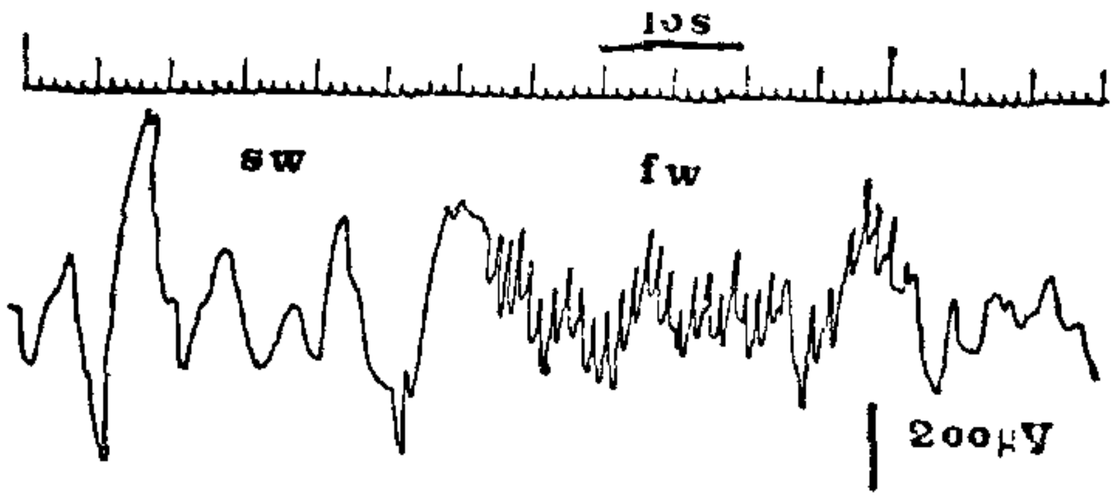

FIG. 3. *In vitro* observations on the impulses produced by *Strongylus vulgaris*, feeding on blood through a rubber membrane.

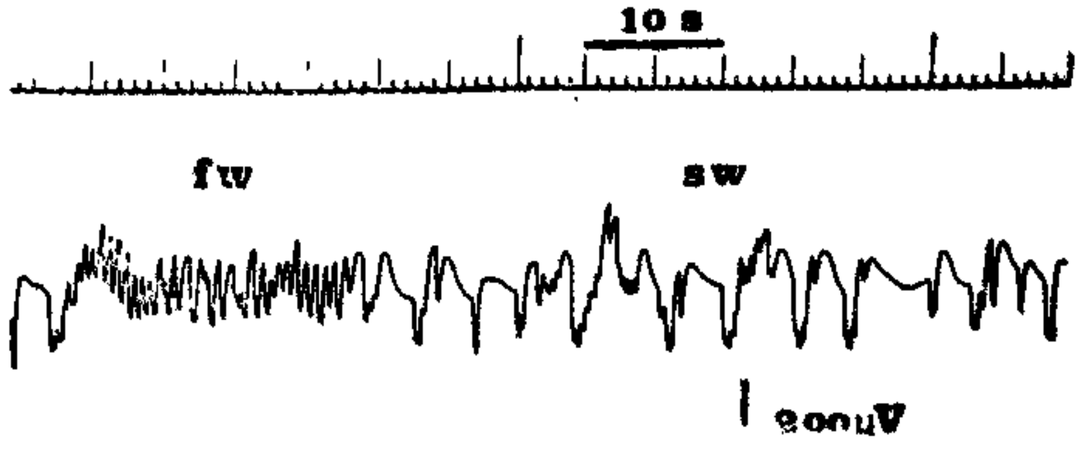

FIG. 4. *In vitro* observations on the impulses produced by *Ancylostoma caninum* feeding on blood through a rubber membrane.

The duration and the amplitude of the various deflections and intervals were very irregular in both *S. vulgaris* and *A. caninum*. Throughout these observations, the worms moved their posterior end in saline chamber. The slower waves depicted in Figs. 3 and 4 were clearly related to their body movements as the apparatus was positioned in such a way that both the posterior end of the worm and the ink recordings could be observed side-by-side. No evidence was obtained to show that the fast waves were related to pharyngeal activity.

The advantage of this apparatus over that used in an earlier study[1] was its simplicity of design and ready accessibility of the components such as the contraceptive membrane. Using this apparatus it was possible to demonstrate that species of cyathostomid nematodes on one hand and *Strongylus* spp., and *A. caninum* on the other, display a different feeding behaviour *in vitro*, in that the former did not ingest sufficient blood, for the blood to pass from its anus.

The study of electrical impulses emanating from worms feeding on blood through a rubber membrane did not provide any satisfying results but it cast some doubt on the validity of the interpretations offered in an earlier study[2] where this technique was used in a study of the feeding of *A. caninum*. It was not possible to show that pharyngeal pulses were responsible for electrical impulses in the apparatus used and it was considered that the slow waves were due to the change in capacitance of the system as the nematode moved relative to the recording electrode and the faster waves were probably due to the action potential of muscles both in the body wall and the pharynx.

The authors are grateful to Dr. Cross, for his help in conducting the *in vitro* pharyngeal activity experiments at the Department of Physiology, University of Queensland, Brisbane, Australia.

Department of Parasitology,
University of Queensland,
St. Lucia, Brisbane, Australia,
March 6, 1978.

S. ABDUL RAHMAN.*
A. H. WADDELL.

* Present address: Department of Parasitology, Veterinary College, Hebbal, Bangalore, 560 024, India.

1. Roche, M. and Martinez Torres, C., *Expl. Parasit.*, 1960, 9, 250.

2. —, — and Macpherson, L., *Science*, 1962, p. 148,

EFFECT OF ENDOSULFAN ON THE MID-GUT EPITHELIUM OF THE ADULT *ODONTOPUS VARICORNIS* (DIST.) (HEMIPTERA : PYRRHOCORIDAE)

Introduction

HISTOPATHOLOGICAL studies have shown that destructions like shedding of cytoplasm and chromatin clumping in the cells of the alimentary canal of insects are caused by organophosphorus and organochlorus insecticides[1-3]. The present work reports the effect of the insecticide, endosulfan on the mid-gut epithelium and its secretory activity in the adult *Odontopus varicornis*.

Materials and Methods

Specimens of *O. varicornis*, collected from the vicinity of Annamalainagar, were reared as described earlier[4]. The insecticide, endosulfan (Technical grade obtained from M/s. Bharat Pulverising Mills Pvt. Ltd., Bombay), was dissolved and diluted with acetone for the treatment. Median lethal doses were experimentally ascertained in terms of micrograms of insecticide per gram weight of the insect, as suggested by Nayar *et al.*[5] and the value of LD_{50} was found to be 0·002143 μg/g.

Adult male insects of the same age group were kept inside the refrigerator for a few minutes to slow down their activities. Each insect was, then, injected with 0·075 ml of LD_{50} doses of endosulfan, into the intersegmental thoracic region with a micrometer syringe fitted with a 26 gauge needle. These insects have become moribund in about 3 to 4 hours after injection and their alimentary canals were vivisected in insect saline solution. The different mid-gut regions of the alimentary canal of endosulfan treated insects, acetone treated insects and non-treated insects were, then, fixed in Bouin's fluid. The permanent slides were made following the paraffin embedding procedure. Serial sections of 6 to 8 μ thickness were deparaffinised and stained in Heidenhain's hematoxylin, using eosin as counterstain.

Observations

The folded mid-gut epithelium with distinct cell boundaries of acetone treated and normal insects (Figs. 1, 3, 5 and 7) show, under endosulfan treated condition, signs of degeneration, shrinkage and indistinct cell boundaries in all mid-gut regions (Figs. 2, 6 and 8) excepting the second mid-gut which is characterized by hypertrophy of cells (Fig. 4). The epithelium of third mid-gut region appears to have been seriously affected by endosulfan as it stands separated from the muscular layers (Fig. 6).

Further, the cytoplasm of the mid-gut epithelial cells show sparse distribution of cytoplasmic granules (Figs. 2, 4, 6 and 8). The secretory product, which is usually present in the lumen of first, second and third