

THE STRUCTURE AND BEHAVIOUR OF THE

CERCARIA OF SCHISTOSOMA MANSONI

VOLUME 1 (2 Vols.)

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TO SANDRA

ABSTRACT

The biology of the cercaria of *Schistosoma mansoni* has been investigated in two principal fields : firstly, the ultrastructure of the nervous system and musculature has been examined by transmission electron microscopy; secondly, the behaviour of cercariae has been observed and quantified both in the unstimulated state and in the presence of certain host-related stimuli. Linking these two perspectives is a description of the locomotory techniques of cercariae for swimming, crawling and burrowing.

Although the basic organization and fine structure of the somatic musculature is similar to that of other platyhelminths, regional specializations in the form of oral and ventral suckers appear adapted for locomotion over or through a solid substratum. Propulsive thrust during swimming is developed by a muscular tail containing a type of fast-contracting 'striated' muscle. The latter causes both tail and body to oscillate rapidly from side to side.

Various nerve cell inclusions have been identified and appear similar to those found in the nerve cells of many animal groups. Nerve endings are modified to form what appear to be unicellular sensory receptors. On comparative morphological grounds, the four types of receptor observed might be presumed to serve several sensory modes, including mechano-, chemo- and photoreception.

Cercarial attachment has been used as the principal parameter in a quantification of responses to heat and chemical stimuli and the importance of these factors in host recognition has been assessed. Simultaneous application of both types of stimulus evokes the greatest response.

LIST OF ABBREVIATIONS

A	<i>anterior</i>
AC	alimentary canal
AR	anterior retractor myofibre
AX	nerve axon
B	nerve bulb
BB	basal body
BC	body circular myofibre
BL	body longitudinal myofibre
C	circular muscle
CH	chromatin
CI	cilium
CN	central nervous system
D	desmosome
DB	dense body
DL	dense layer of fibrous zone
DP	oral sucker diaphragm
DR	dense rod
DV	dorso-ventral myofibre
E	excretory canal
EB	excretory bladder
F	furca
FC	fibrous capsule
FZ	sub-tegumentary fibrous zone
FL	posterior body flange
G	glycogen particles
GL	gland duct
H	tail 'core', the hydrostatic skeleton
HG	'head' gland

I	intercellular space
L	lateral longitudinal muscle block
LC	modified cilium of laminated receptor
M	mitochondrion
MD	mid-dorsal muscle block (longit.)
MT	microtubule
MV	mid-ventral muscle block (longit.)
N	cell nucleus
NC	caudal nerve mass
NP	electron-dense nuclear particle
NT	longitudinal nerve trunk
P	<i>posterior</i>
PC	pore of ciliated cavity
PO	postacetabular gland
PR	preacetabular gland
R	receptor
RM	radial myofibre
S	tail stem
SD	septate desmosome
SL	sarcolemma
SN	tegumentary spine
SP	sarcoplasmic tubule
SR	sarcoplasmic reticulum
ST	striation of myofibre, surface view
STT	striation of myofibre in transverse section
STL	striation of myofibre in longitudinal section
SY	synapse
T	tegument
TC	tail circular myofibre
TG	subtegumentary cell

TK	thick myofilaments
TL	tail longitudinal myofibre
TN	thin myofilaments
TR	transverse myofibre
V	large, dense vesicle
VC	small, clear visicle
VD	small, dense vesicle
VL	large, clear vesicle

GENERAL INTRODUCTION

Schistosomiasis is now one of the most significant public health problems of the tropical and sub-tropical world, the primary causative organisms being *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*. The particular subject of this investigation was the cercarial stage of *Schistosoma mansoni*.

The schistosome life cycle involves an alternation of sexual and asexual generations in vertebrate and molluscan hosts respectively. Man is the definitive host of *Schistosoma mansoni* while species of *Biomphalaria* are intermediate hosts. The succession of stages in the life cycle includes egg, miracidium, first and second stage sporocysts, cercaria, schistosomule/juvenile, adult. Schistosome adults are unusual in that they are dioecious. The significance of the cercaria is that it is this stage which actively penetrates human skin in response to host-related stimuli.

Despite the obvious importance of the cercaria as an infective stage, knowledge of its morphology and behaviour, up to 1969 when this project was initiated, was very limited. The techniques of electron microscopy had not been fully brought to bear; behavioural data had often been based on ill-defined stimuli and responses. The general approach in this project was firstly to investigate those aspects of morphology most directly related to overt behaviour (*viz* receptor systems and main locomotory musculature) and secondly to describe certain aspects of cercarial behaviour in quantitative terms. It is hoped that the development and integration of these two perspectives will contribute to a better general understanding of the biology of cercariae.

The thesis is presented in three main Sections: the first deals with cercarial morphology, the second with methods of propulsion and the third with swimming and attachment behaviour.

SECTION 1 : MORPHOLOGY

1.1 INTRODUCTION TO SECTION 1

Trematode cercariae have been classified by Luhe (1909) on the basis of general morphology but the true systematic classification is not necessarily correlated. Convergent evolution has meant that the cercariae of unrelated species have developed superficially similar general characteristics. The morphological group which includes the cercariae of *Schistosoma mansoni* is the *Furcocercariae*. These all have a well developed, forked tail and the oral sucker is frequently modified into a 'penetration organ'.

Estimates of the overall size of schistosome cercariae have varied due to different methods of killing the larvae. The use of gentle heating was a frequently used method at one time, but produced quite varied results, e.g. those of (a) Khalil (1922), (b) Gordon et al (1934) and (c) Archibald and Marshall (1932). Their average figures are given in μm :

	(a)	(b)	(c)
Body length	189	168	152
Body width	73	59	52
Tail stem length	250	211	220
Tail stem width	40	35	32
Furcae length	75	79	72
Furcae width	-	13	12

I have found that the dimensions of live, but inactive, cercariae more closely approximate to those given by Khalil. It is possible that strain differences may account for some of the variability; the above authors sampled from populations in Egypt, Sierra Leone and Sudan respectively and my material was a Puerto Rican strain.

The purpose of this section of the investigation was to study the organisation and structure of cercarial muscle and the structure of cercarial receptors, mainly by transmission electron microscopy. The most directly related studies are reviewed in more detail in the following Sections, 1.3, 1.4, 1.5.

The early detailed descriptions of trematode muscle and nerve included those of Lang (1881), Bettendorf (1897) and Zailer (1914) and, for gross morphology, some of these are still regarded as being among the most useful (Erasmus 1972). The valuable comparative text of Bullock and Horridge (1965) was to some extent based on this work. In fact, much of the general organization of platyhelminth nervous and muscle systems had been elucidated by the early years of this century; see, for example, Looss (1894), Tower (1900), Becker (1922). Significant progress beyond the resolving power of their technique had to await the development of electron microscopy (with the associated techniques for fixation, staining etc.) and also suitable histochemical techniques.

Musculature

It is naturally easier, in many instances, to establish the function of a particular muscle than it is to determine receptor function, by comparative morphology alone. There has, however, been some controversy concerning the ultrastructure of even the much-studied myofibres of vertebrates and it is only recently that the details of some invertebrate muscle types have become established. A review of papers dealing with cercarial musculature is given in Sections 1.3 and 1.4.

The ultrastructure and organization of the muscle fibres of the *Fasciola hepatica* miracidium were described by Wilson (1969a). A relatively simple system of longitudinal and circular fibres was present with some attached to one another and to adjacent tissues by means of desmosomes. Individual fibres contained arrays of myofilaments which

could be classified as thick (up to 25nm) and thin (approximately 5nm). These were arranged longitudinally but without any transverse alignment to produce striations. The variability in thickness of the thick filaments was thought to indicate that they tapered towards their ends. Small numbers of dense bodies may have been associated with the filament arrays but direct continuity was not seen.

The association of dense bodies and thin myofilaments is, however, a feature of *Schistosoma mansoni* adults (Silk and Spence, 1969b). Arrays of these dense structures were sometimes seen in close proximity within one fibre. Thick and thin filaments were 18-40nm and 5nm in diameter, respectively, and in longitudinal sections the thick filaments appeared tapered. The sarcoplasmic reticulum was not clearly defined and probably consisted of widely-spaced tubules. The general orientation of myofibres in the body wall was assumed to be circular, longitudinal and radial. Erasmus (1972) described the musculature of the strigeoid groups *Apatemon*, *Diplostomum* and *Cyathocotyle*, and filaments of 7nm and 20nm (approx) diameter were found. Dense bodies may be seen in one of his figures (Plate 1.2) and these were interpreted as being hemidesmosomes, attaching the muscle to a basement layer. A sarcoplasmic region of the muscle contained a nucleus, patches of dense endoplasmic reticulum and mitochondria.

In the planarian body, myofibres are oriented in circular, longitudinal and vertical groups (Morita, 1965). In general terms the ultrastructure of *Dugesia dorotocephala* appeared similar to the strigeid type, described above, but dense bodies were found to extend across the fibre; for this reason and because of an apparent similarity in structure to the Z-lines of vertebrate muscle, Morita named them Z-columns. Some were branched and in close contact with the sarcolemma, and others appeared to anastomose with fibrous structures of the surrounding connective tissue. Z-columns were also associated with sarcotubules

lying under the sarcolemma. Myofilaments were about 25nm and 5-6nm in diameter, but thick filaments were sometimes found up to 32nm across. A similar type of myofibre was also found in the marine turbellarian *Notoplana acticola* by MacRae (1965). A degree of conformity of muscle structure within the platyhelminth groups is suggested by the results of Lumsden and Byram (1967) who found very similar ultrastructure to that described above in adult tetraphyllid, trypanorhynch and cyclophyllid cestodes. Some very thick myofilaments were also found, however, the size range being 18-54nm, diameter. Dense bodies were generally compact rather than 'column' or bar-like, but in the neck region of *Phyllobothrium* an extensive development of the dense material was evident. Numerous bands of dense material traversed the width of the fibre; these frequently anastomosed with adjacent bands and were attached by their ends to the sarcolemma.

The studies reviewed in this section, together with many others dealing with organisms from other phyla, clearly support the contention of Prosser (1967) that the classical grouping of myofibres into striated, smooth and cardiac is now of limited value in the face of current physiological and morphological knowledge.

Nervous system

The progress in technique which has facilitated the above work on muscle structure has had a similar influence on studies of nerve tissue. The first demonstration of the presence of specific cholinesterase in helminths was that of Bueding (1952); the concentration in *Schistosoma mansoni* extracts was found to be of the same order of magnitude as in the mammalian brain. Acetylcholinesterase and choline acetylase were subsequently found in *Fasciola hepatica* by Chance and Mansour (1953). *Schistosoma mansoni* also contains a compound with similar properties to those of acetylcholine (Barker et al, 1966). Histochemical demonstration of acetylcholinesterase distribution in *Schistosoma mansoni* was provided

by Bueding et al (1967) and in *Schistosoma mansoni*, *S. haematobium* and *S. rodhaini* by Fripp (1967); the latter author found no significant interspecies variability, but Bueding et al (1967) did detect such differences in the innervation of the oral sucker between *Schistosoma mansoni* and *S. japonicum*. The schistosome nervous system consists basically of paired anterior ganglia joined dorsally and ventrally by circumoesophageal commissures. These extend forward to the oral sucker region and also posteriorly as major and minor pairs of nerve cords, to join at the posterior end of the body. These cords are linked by numerous small, transverse nerves along their entire length. Pepler (1958), Lewert and Hopkins (1965) and Panitz and Knapp (1967) were only able to find acetylcholinesterase activity in the central nerve mass of *Schistosoma mansoni* and *Fasciola hepatica* larvae but a more extensive nervous system was demonstrated in miracidia and cercariae of *Schistosoma mansoni* by Bruckner and Voge (1974). The latter paper is discussed further in Section 1.5. A monogenean system was described by Halton and Morris (1969) who used the indoxyl acetate and acetyl/butyrylthiocholine-iodide methods. They also found that some areas of the subtegumentary muscle stained strongly.

An outline of the general anatomy of the *Schistosoma mansoni* miracidium was given by Capron (1965) and a brief description of certain ultrastructural aspects of *Schistosoma mattheei* by Kinoti (1971). The most significant contribution to our knowledge of miracidial ultrastructure in general has, however, been that of Wilson, (see Wilson 1969 a, b, c, 1970, 1971, and Wilson et al, 1971). The miracidium of *Fasciola hepatica* possesses a central ganglion with peripherally placed cell bodies, and six main nerve tracts leading to the body wall. Apart from the eyespots, several distinct types of specialized nerve endings were identified (Wilson 1970) some with terminal cilia. Those lacking cilia included "lateral bulbous endings" each composed of two distinct nerve

fibres which the author suggested might not function as a transducer but perhaps have a secretory role. Internal laminated endings were tentatively ascribed a statocyst-like role while the ciliated types were associated with chemo- and mechanoreception.

One type of miracidial receptor described by Wilson consists of a cluster of cilia in a surface 'pit'; this structure is also visible under the apical filaments of *Schistosoma mattheei* (Kinoti, 1971), although the author did not describe it. Brooker (1972) has reviewed the structure of miracidial sense organs, and it would seem that the same types of organ may be found in several different species.

Wilson (1970) described several types of intracellular vesicle: small clear vesicles 30-45nm in diameter, especially numerous near synapses; small 65-75nm dense vesicles; large 165nm vesicular bodies.

Morris and Threadgold (1967), Silk and Spence (1969a) and Smith et al (1969) found unⁱciliated nerve endings in the tegument of adult *Schistosoma mansoni*. These were somewhat similar in appearance to Wilson's "lateral sheathed ciliated endings", except that, in *Schistosoma*, the tegument completely covered the protruding cilia. Morris and Threadgold thought that the schistosome receptor might detect the direction of flow of a fluid medium. Nerve cell nuclei were only found in circumoesophageal ganglia by Silk and Spence (1969a) and once again, a range of vesicle types was identified. Bruce et al (1970) studied schistosomula migration through mouse tissue and what may be a receptor cell is visible in their Fig. 18, although they did not describe it as such. A uniciliated nerve ending is present in the tegument of *Cyathocotyle bushiensis* (Erasmus, 1967) but it differs in detail from those referred to above. There is, for example, evidence of a striated rootlet system beneath the ciliary basal body.

A detailed account of the nervous system of *Dicrocoelium dendriticum* was presented by Corrales (1973) who used a silver impregnation technique. The organisation of the central system and longitudinal cords was described as well as the innervation of oral and ventral suckers, digestive system and gonads. A similarly detailed study of the aspidogastrea species *Multicotyle purvisi* was completed by Rohde (1971). A three-dimensional reconstruction of the nervous system was made from serial sections impregnated with silver. The free larva had basically the same anterior nerves as the adult, but a smaller number of commissures. Small nerve cell inclusions resembled those described by Wilson (1970) and Rohde (1972).

Lyons (1969, a,b) described the ultrastructure of sense organs in the monogenean species *Entobdella solea* and *Gyrodactylus* sp. and the general layout of the nervous system of the latter was revealed with a thiocholine technique. A comprehensive review of monogenean receptors was that of Lyons (1973). She categorized receptors into two broad groups. The first contained photoreceptors, most of which consisted of a pigment cup enclosing one or more sensory cells. There may also be a non-pigmented photoreceptor in *Entobdella soleae*. The second group contains ciliated receptor cells which occur either singly (the most common) or in groups. Both uniciliate and multiciliate cells have been found.

An acetylcholinesterase-location technique has been successfully applied to the demonstration of cestode anatomy, e.g. *Hymenolepis diminuta* and *H. nana* (Wilson and Schiller, 1969) and *Mesocestoides corti*. (Hart, 1967). In both *Hymenolepis* species, paired ganglia in the scolices give rise to a series of longitudinal nerve cords including one primary lateral pair. In addition *H. diminuta* possessed a rostellar complex of about fifteen nerve rings. Morseth (1967) described the fine structure of nerves in *Echinococcus granulosus*; a uniciliated receptor with an extensive ciliary rootlet system in the nerve bulb was present. Several kinds of vesicle

were identified in the axons of the nerve trunks. These included small, clear vesicles 25-30nm in diameter; small, dense 40-70nm vesicles; and a less-frequently seen large, dense type 110-160nm across.

Synapses were associated with accumulations of small clear vesicles.

Simple tegumentary nerve endings have also been found in *Taenia hydatigena* (Featherstone, 1972) and *Taenia pisiformis* (Morseth, 1966).

A range of nerve cell vesicles similar to the above also occurs in the planarian *Dugesia gonocephala* (Oosaki and Ishii, 1965) but the size range is slightly greater. A large dense 50-130nm type was presumed to be neurosecretory. A similar conclusion was reached by Morita and Best (1965) regarding the 40-110nm dense vesicles of *Dugesia dorotocephala* nerves. The presence of numerous receptors in the turbellarian epithelium is long established (see Gelei, 1930 and review by Hyman, 1951) and the ultrastructure of two of these has been described by MacRae (1967).

Schistosoma mansoni cercariae do not possess pigmented eye spots and so, there being no direct basis for comparison, accounts of this type of receptor will not be fully reviewed here. One comparative description of photoreceptors in trematode larvae was that of Isseroff and Cable (1968). Eakin (1965) also discussed the evolution of different types of photoreceptor cell.

The purpose of Section 1 of this investigation is to describe the ultrastructure and organization of muscle and nerve tissue in *Schistosoma mansoni* cercariae.

LABORATORY MAINTENANCE OF PARASITE

A Puerto Rican strain of *Schistosoma mansoni* was used throughout this investigation. It was obtained from Dr S R Smithers (NIMR, Mill Hill, London NW7) in the form of infected *Biomphalaria glabrata*. The snails were maintained in a constant temperature room at 27°C. The breeding colony was kept in flat, plastic trays and the infected snails in cylindrical glass tanks. The trays contained about ½ inch of water and were covered with sheets of glass; the 12 inch tanks were kept about ¾ full and were covered by inverted glass crystallising dishes. Although in the early stages, local lake water was utilized, dechlorinated tap water from a large, aerated, plastic reservoir was used subsequently.

The egg batches of breeding snails were collected periodically from the walls of the trays with either a broad scalpel or razor blade and then transferred to smaller dishes. Basic food consisted of boiled/dried lettuce, but young snails were given dried Sycamore leaves when available.

Infection of snails

The small intestines and livers were dissected from several mice with an infection usually over 6 weeks old. The tissue was cut into small pieces in phosphate buffer at pH8, (5.3mls 0.2M NaH₂PO₄.2H₂O + 94.7mls 0.2M Na₂HPO₄.12H₂O - diluted to 200 mls). It was homogenized, trypsin added (1mg enzyme: 10mls buffer/gut), and then incubated in a shaking water bath at 37°C for 4 hours. Two sieves, 85 and 100 mesh, were used then to separate the coarser particles and 0.85% Tyrodes saline used to wash through, (Tyrodes recipe included in Appendix 1). Washing with Tyrodes followed until the supernatant remained clear. Eggs could be stored for several weeks in saline at 5°C but were usually used immediately.

To stimulate hatching, eggs were placed into dechlorinated tap water under bright illumination for at least 1 hr. Approximately 10 miracidia were transferred into each of a series of solid watch glasses (swg) with a finely drawn out Pasteur pipette. One young snail, about 4 - 6mms in diameter was placed in each swg in a small quantity of water. When all free swimming miracidia had disappeared, the snails were transferred to the cylindrical tanks.

Infection of mice

LACA strain mice were maintained in the Departmental animal house. The method of infection was basically that of Smithers and Terry (1965), see Appendix 2.

ELECTRON MICROSCOPY

(1) Collection of cercariae

6 - 8 infected snails were placed into a beaker containing 50mls of filtered, dechlorinated tap water. The latter was drawn from a continuously aerated plastic storage tank. Adequate numbers of larvae could be obtained within 1 hr, especially if the snails had been kept in total darkness for 24-48 hrs beforehand.

A number of methods are available for the concentration of cercarial suspensions but the following was found to be the most satisfactory: firstly, the suspension was pipetted into a sintered glass filter. While the latter was held in a vertical position, most larvae swam near to the surface and the water could be drawn off from below by gentle suction. (it was, in fact, found to drain away slowly even in the absence of suction pressure). When the suspension appeared sufficiently concentrated, it was pipetted into a clean test tube prior to the next stage, fixation.

An alternative method of concentration was to spin the suspension gently in a centrifuge. The supernatant was then quickly drawn off before the larvae spread up towards the surface again. Brief cooling to 10°C prior to centrifuging inhibited larvae swimming temporarily, allowing more time for pipetting away the excess water.

(2) Fixation

A small volume of concentrated cercarial solution (less than 2 hrs after shedding started) was pipetted into a test tube. Each stage in the following fixation and dehydration schedule was carried out by adding to this tube approximately 10mls of the appropriate solution. It was then occasionally agitated so that the pellet of larvae was dispersed to ensure adequate penetration. After the necessary time interval, the

solution was pipetted off, (larvae quickly sink to the bottom of the tube after fixation, making centrifugation unnecessary) and replaced by the next solution in the sequence.

The fixation procedure was carried out in two main stages - initial immersion of larvae in buffered glutaraldehyde fixative, followed by post-fixation in buffered osmium tetroxide. The schedule is best summarized:-

1. Fix in glutaraldehyde in cacodylate buffer for 1 hr at 4°C.
2. Wash in 3 changes of buffer; each 15 mins at 4°C.
3. Leave overnight in buffer at 4°C.
4. Post-fix in osmium tetroxide in veronal acetate buffer for 4 hrs at 4°C.
5. Wash in 3 changes of buffer, 15 mins each.

Details of the preparation of fixative solutions are given in Appendix 3.

(3) Dehydration

After final washing in veronal acetate buffer, larvae were dehydrated in the following series of ethanol solutions:-

- 30% ethanol in distilled water for 15 mins at 4°C
- 50% " " " " " "
- 70% " " " " " "
- 70% " " " " " "
- 90% " " " " " "
- 90% " " " " " "
- absolute ethanol for 15 mins at 4°C
- absolute ethanol for 15 mins at room temperature.

Occasionally, specimens were left overnight before final dehydration and embedding. In this case they were left in 70% ethanol at 4°C.

Initially the second 70% ethanol stage was used for staining with uranyl acetate (1% uranyl acetate in 70% alcohol) but the results showed no improvement and so this practice was discontinued.

(4) Embedding

The embedding medium was ^{an} Araldite-Epon mixture. The ethanol in the specimens was first replaced with propylene oxide which was, in turn, replaced by the resin. The final resin medium (referred to below simple as 'resin') consisted of resin, hardener, plasticizer and accelerator in the following proportions:-

30mls Araldite (CY 212)
50mls Epon (Epikote 813)
110mls Dodeceny1 succinic anhydride (DDSA)
4mls Dibutylphthalate
6mls Benzyl dimethylamine (BDMA)

The schedule was as follows:-

1. Propylene oxide for 15 mins at room temperature.
2. Propylene oxide : resin (1 : 1) for 3 hrs at room temperature.
- * 3. Resin, overnight at 60°C.

* Larvae were transferred individually, by means of a wire loop, into the resin to eliminate clumping in the hardened block and to facilitate orientation of individual larvae for sectioning.

(5) Mounting and sectioning

Cercariae could easily be seen in the translucent, gold-coloured resin as they turned black during post-fixation. Cubes of resin, each containing one cercaria, were cut from the blocks with a scalpel or safety razor blade and the orientation of the larvae noted. The cubes were then mounted on the ends of short lengths of perspex dowel with Araldite adhesive. For longitudinal sectioning larvae were aligned at right angles to the dowel axis; for transverse sectioning alignment was parallel to this axis. The procedure was carried out under the low power of a dissecting microscope.

Final trimming with a new razor blade reduced the block face to a trapezium pyramid shape. Glass knives were prepared on an LKB knife maker and a knife reservoir made with a short piece of adhesive tape

sealed in place with wax.

Sections were cut with an LKB 4800A Ultratome. Observation of the interference colours of the sections as they floated into the reservoir allowed estimation of section thickness; from the colour scale of Peachey (1958) sections in this work were normally about 100nm thick.

A certain degree of compression of sections occurred as they were cut but this was largely alleviated by placing a small paintbrush, dipped in chloroform, close to the freshly cut ribbon in the knife reservoir. This brought about immediate and rapid extension of the compressed sections.

Ribbons were transferred to copper grids by lowering the latter on to the surface of the reservoir (using watch-makers forceps). The edge of each grid was bent slightly to make it easier for the grid to be lowered in a horizontal position. A squirrel-hair brush, with all except 3-4 hairs removed, was used occasionally to manoeuvre ribbons, or individual sections, in the reservoir. Sections adhered firmly on contact with the grid which was then placed (sections up) on filter paper to dry, before storage.

(6) Grids and support films

The grid usually used was a standard 200 mesh copper type, although later in the investigation one with hexagonally arranged bars was found to be more satisfactory. Bars of this type less frequently obscured sections from view.

Two types of support film were applied to the grids, plastic (Formvar) and evaporated carbon. Their preparation and application is described in Appendix 4.

(7) Staining

Two stains were used routinely, lead citrate and uranyl acetate. Their preparation is described in Appendix 5. Grids were placed, section-side down, onto drops of stain and were then washed in a jet of distilled water. The staining schedule was as follows (all at room temperature):-

1. lead citrate 5 mins
2. 0.04N caustic soda 1 min
3. distilled water 1 min
4. uranyl acetate 10 mins
5. distilled water 1 min.

LIGHT MICROSCOPY

Some thicker sections (240 - 280mm) were cut from the blocks prepared for electron microscopy and were examined by light microscopy.

Sections were removed from the ultratome knife reservoir with a trimmed squirrel hair paintbrush and deposited in drops of water on glass microscope slides. The water was positioned over etched marks on the reverse side of the glass, this facilitated relocation of the sections after drying.

The slides were then flooded with stain, warmed gently, and washed in distilled water before DPX mountant was applied. Optimum contrast was obtained using 1% toluidene blue in 1% borax.

Whole mounts of larvae were also prepared. Firstly it was hoped that the orientation of tail myofibres might be observed, in whole, un-sectioned specimens. Secondly, an experiment to localize acetylcholinesterase activity was carried out using whole larvae. An indication of myofibre orientation was, in fact, obtained from the latter preparations.

Acetylcholinesterase localization

The technique is based on that of Bueding et al (1967). Details of the preparation of stock and substrate solutions are given in Appendix 6.

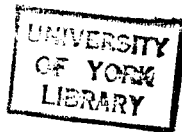
Cercariae were fixed by immersion in ice-cold formaldehyde for 10 mins. This was carried out in a centrifuge tube so that the centrifuge method of passing larvae through a series of treatments could be used. The schedule after fixation was as follows:-

1. Wash in several changes of distilled water.
2. Stock solution, 4 hours.
3. Substrate solution, 1 hour.
4. Wash in 3 changes of sodium sulphate solution (saturated at room temperature).

5. 70% ethanol saturated with hydrogen sulphide, 1 minute.
6. 50% ethanol, 3 minutes.
7. distilled water, 1 minute.
8. 30% acetic acid, 20 minutes.
9. Mount on slides for observation.

The sites of ACHE activity should become visible because of a localized deposition of cupric sulphide. Any ACHE present on the larvae hydrolyses the substrate to form copper thiocholine and acetic acid. The hydrogen sulphide brings about the conversion to cupric sulphide, which precipitates.

The technique met with only limited success in locating internal nerve tissue but surface structures could be distinguished and the results compared with those obtained by transmission electron microscopy.



Publications

Some of the results included in the following sub-sections have previously been published. Copies of the two papers concerned are included in Appendix 7.

INTRODUCTION

There have been few detailed descriptions of cercarial somatic musculature, this being due, perhaps, to a focussing of attention on the more highly modified tail muscles. Somatic fibres can conveniently be classified as those associated with (a) the body wall (b) the suckers and (c) other internal organs.

The organization of muscle in the body wall of most species of cercaria conforms to a relatively simple pattern. There is an outer circular layer, oriented at right angles to the long axis of the body, which is composed of numerous, closely-packed fibres. These overlie a basically similar layer of longitudinally-oriented fibres. See, for example, Cort (1919), Price (1931), Neuhaus (1952) and Inatomi et al (1970).

In addition to the body wall network, regionally specialized muscle systems occur in the form of oral and ventral suckers. Both radial and circular fibres are present with their associated nuclei and other organelles but the suckers are largely isolated from the surrounding organs by a thin layer of fibrous tissue (Hockley, 1970) which is continuous with a subtegumentary fibrous zone. Dorso-ventral retractor muscles may also be present, attached at one end to the ventral sucker 'capsule' and at the other to the dorsal body wall (Wesenberg-Lund, 1934). It is the adhesive properties of the suckers together with the high degree of flexibility conferred by the body wall muscles which facilitates the attachment and crawling behaviour of species such as *Schistosoma mansoni*. A more detailed description of these locomotory movements is given in Section 2.

Ebrahimzadeh and Kraft (1971) and Dorsey and Stirewalt (1971) illustrated the close association of muscle fibres and penetration gland ducts. The latter authors concluded that these fibres function in the control of secretion by the glands. Ebrahimzadeh and Kraft (1969) have also described the myofibres surrounding different regions of the alimentary canal.

Lumsden and Foor (1968) compared the ultra-structure of somatic and caudal muscles in *Heterobilharzia americana*, showing that the cytological organization of somatic fibres was similar to that in cestodes (Lumsden and Byram, 1967) and turbellaria (MacRae, 1965). *Heterobilharzia* somatic myofibres contained both thick and thin myofilaments the latter of which were continuous with scattered dense bodies. However, neither the latter nor the thick filaments were aligned transversely and so no striations were apparent. Cysternae of the sarcoplasmic reticulum were present around the periphery of the contractile elements, in close proximity to, but not in direct contact with, the plasmalemma. MacRae (1965) hypothesized that in turbellaria the association of sarcoplasmic reticulum and plasmalemma may be the functional equivalent of a T-system and Lumsden and Foor (1968) suggested that this might also be the case in schistosomes.

The purpose of this chapter is to describe the somatic musculature of *Schistosoma mansoni*.

RESULTS

Body wall muscle. This consists of an outer, circular and an inner, longitudinal layer of myofibres lying beneath the tegument. It is separated from the latter by a layer of interwoven fibres (the appearance of which is described later). Figs. 1, 2 illustrate the size and location of body wall myofibres in relation to other tissues. The transverse section in Fig.1 was taken from the posterior end of the body where the postacetabular glands can be seen occupying a major part of the body volume.

The sarcoplasmic regions of circular and longitudinal fibres are innermost, as in the cercarial tail. They contain nuclei and some of the mitochondria (Figs.3, 4, 5). The nuclei, with their clumped distribution of chromatin may also contain clusters of small 60-80nm electron-opaque particles (Figs. 5, 6).

There appear to be numerous points of attachment between adjacent tissues/cells in the body wall. These are the highly electron-dense desmosomes a number of which are indicated in Fig.4.

The contractile regions of circular fibres are about 0.4 μ m to 0.55 μ m deep and 2.0 μ m to 3.0 μ m in width. Although immediately adjacent fibres are generally of a similar width, those near the anterior or posterior ends of the body are usually narrower than those of the central region, (Figs.4, 7, 8). Both thick and thin myofilaments occur, the diameters of the former occasionally reaching 30nm although they are normally less than this. They may also be up to 7 μ m long and the thin : thick filament ratio is about 12 : 1, (Figs. 4, 9, 10).

The sarcoplasmic reticulum is only occasionally in evidence in transverse sections (see Fig. 4) but can more readily be seen in longitudinal

sections as a widely-spaced system of tubules lying beneath the sarcolemma and closely investing the contractile regions of the fibres.

The subtegumentary fibrous zone, lying between the tegument and the circular muscle is about $0.02\mu\text{m}$ thick. It is composed mainly of randomly oriented fibres but there is also a continuous dense layer adjacent to the basal membrane of the tegument (Fig. 4, 8, 10, 11).

The general form of *longitudinal fibre* contractile regions is similar to the above. In transverse section they may vary in size from $1.5\mu\text{m}$ by $0.5\mu\text{m}$ to $5.95\mu\text{m}$ by $0.75\mu\text{m}$, the former size occurring near the anterior end of the body, (Figs. 12, 13), and the larger fibres were generally posterior. Variability could be significant, however, between the fibres seen in any one transverse section, e.g. see Fig. 14.

The total number of longitudinal fibres visible in transverse sections is not constant along the length of the body. Although there are no more than 20 at the posterior end, there may be 30 or more anteriorly, (Figs. 1, 14). Adjacent fibres are usually packed closely together as in the circular muscle but they may become separated by the longitudinal nerve cords or other tissues, (Figs. 2, 14).

The slightly oblique transverse section in Figs. 15-17 demonstrates a gradual change in the shape of longitudinal fibres towards their posterior insertions in the tail/body junction. The fibres indicated by asterisks have been sectioned nearer their insertions than those of the opposite side and appear more rounded. The myofibre contents also change posteriorly from a complement of thick and thin filaments comparable to that of the circular fibres to one of almost entirely thin filaments. The latter region is, perhaps, more clearly shown in longitudinal sections, (Figs. 18, 19, 20). It surrounds the excretory bladder and occupies

most of the space between the latter organ and the tegument in the tail/body junction. The muscle insertions are just posterior to the bladder and appear to be closely associated with the anterior insertions of caudal longitudinal fibres, (Fig. 18).

The form and extent of the sarcoplasmic reticulum appear to be comparable to those of the circular muscle.

In view of previous descriptions of cercarial smooth muscle, in which thin filaments were found to be continuous with scattered 'dense bodies', a similar pattern was expected in *Schistosoma mansoni*. Figs. 3, 5, 6, 8 show these structures but they do not invariably stain prominently. They can appear, however, in transverse sections of smooth fibres as extended rods attached at one end to the sarcolemma, (Figs. 5, 21).

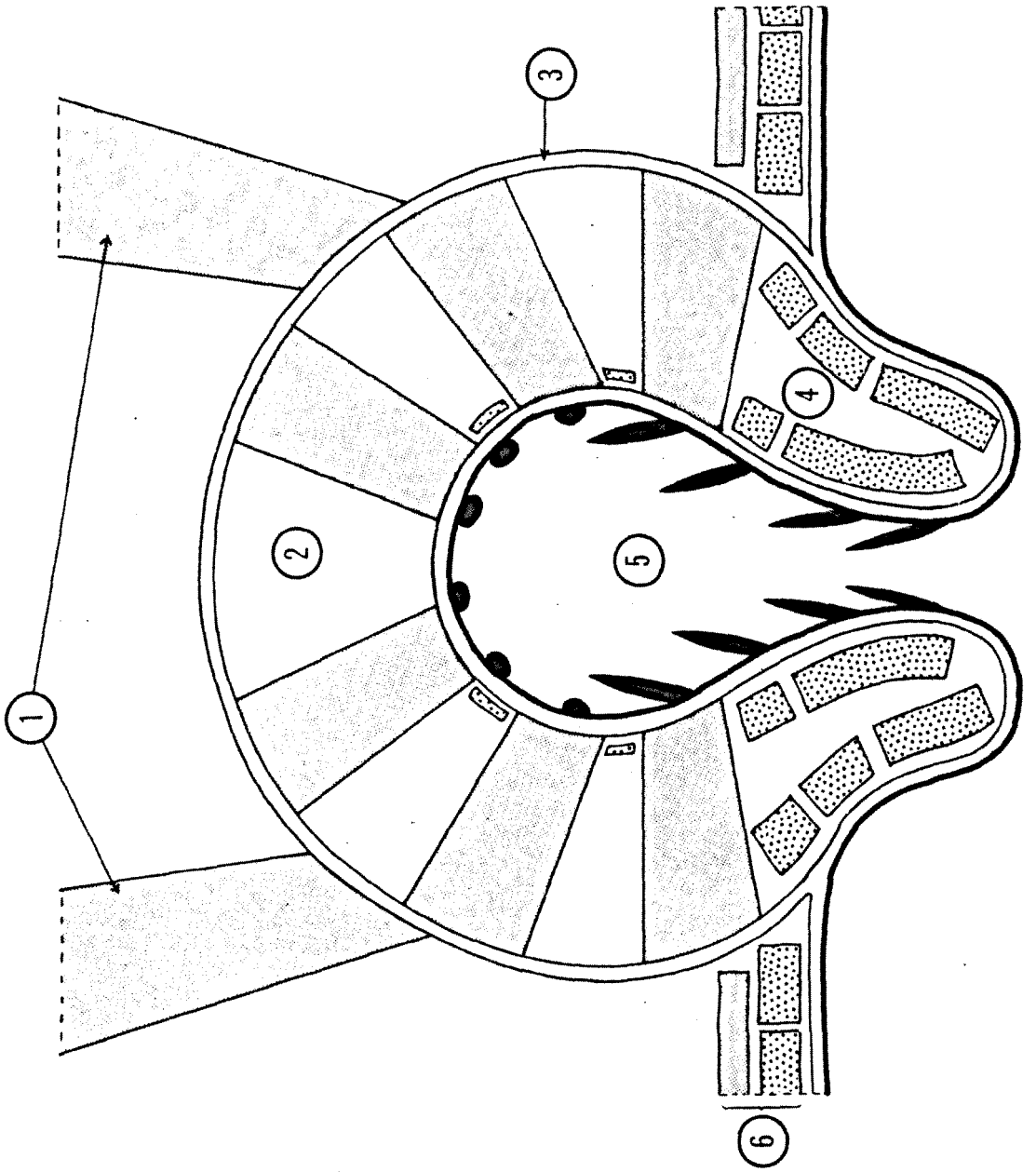
The ventral sucker is roughly spherical in shape and is packed with smooth muscle, (Figs. 22-25 and Text Fig. 1). The organ is enclosed by a layer of fibrous tissue (the fibrous capsule) which is continuous with the subtegumentary fibrous zone. It is assumed, however, that this capsule will be perforated in one or more places by nerve axons which for example must link the somatic nerve cords to the ciliated nerve endings of the sucker cavity.

The sucker can be broadly divided into two regions. The first of these surround the opening of the sucker cavity, forming the 'lips' of the sucker. It contains several myofibres which encircle the opening (see label 4, Text Fig. 1). The second region contains an array of thicker myofibres which are radially arranged, i.e. join the inner (cavity) wall to the outer (capsule) wall (see label 2, Text Fig. 1). Myofibres lying within the capsule evidently do not comprise the whole functional system, however. Further smooth fibres connect the outer surface of the capsule to the dorsal body wall (Figs. 26, 27).

TEXT FIG.1

Diagrammatic reconstruction of the ventral sucker, in saggital section.

1. Myofibres connecting the ventral sucker capsule to the dorsal body wall.
2. Region of radially arranged myofibres. Nuclei and mitochondria are packed in between contractile elements.
3. Fibrous capsule, continuous with subtegumentary fibrous zone.
4. Lips of sucker containing myofibres which encircle the opening to the sucker cavity.
5. Sucker cavity, lined by prominent tegumental spines which are inwardly-directed.
6. Outer, circular and inner, longitudinal myofibres of the body wall.



The oral sucker is, in *Schistosoma*, far more complex than the ventral sucker. The alternative name of 'anterior organ' might be more appropriate but as 'oral sucker' has become generally accepted I shall continue to use it for this report. A diagrammatic reconstruction of the oral sucker region is presented in Text Fig.2 as an aid to interpretation of the electron micrographs.

A fibrous partition, continuous with the subtegumentary fibrous zone, separates the tissues of the sucker from those of the main body compartment (Figs. 28-30) and is perforated by gland ducts, alimentary canal and nerves. As this partition is closely associated with variously oriented myofibres I shall refer to the resulting general structure as the *oral sucker diaphragm*. Arranged on the posterior surface of the diaphragm fibrous layer are radial myofibres, (Figs. 28-30). Comparison of different transverse sections indicates that these fibres retain their orientation, (e.g. compare Figs. 31, 32). In this feature they differ from myofibres on the anterior face of the diaphragm which appear longitudinally aligned at the periphery of the diaphragm but almost circular in the central region, (Figs. 32, 33).

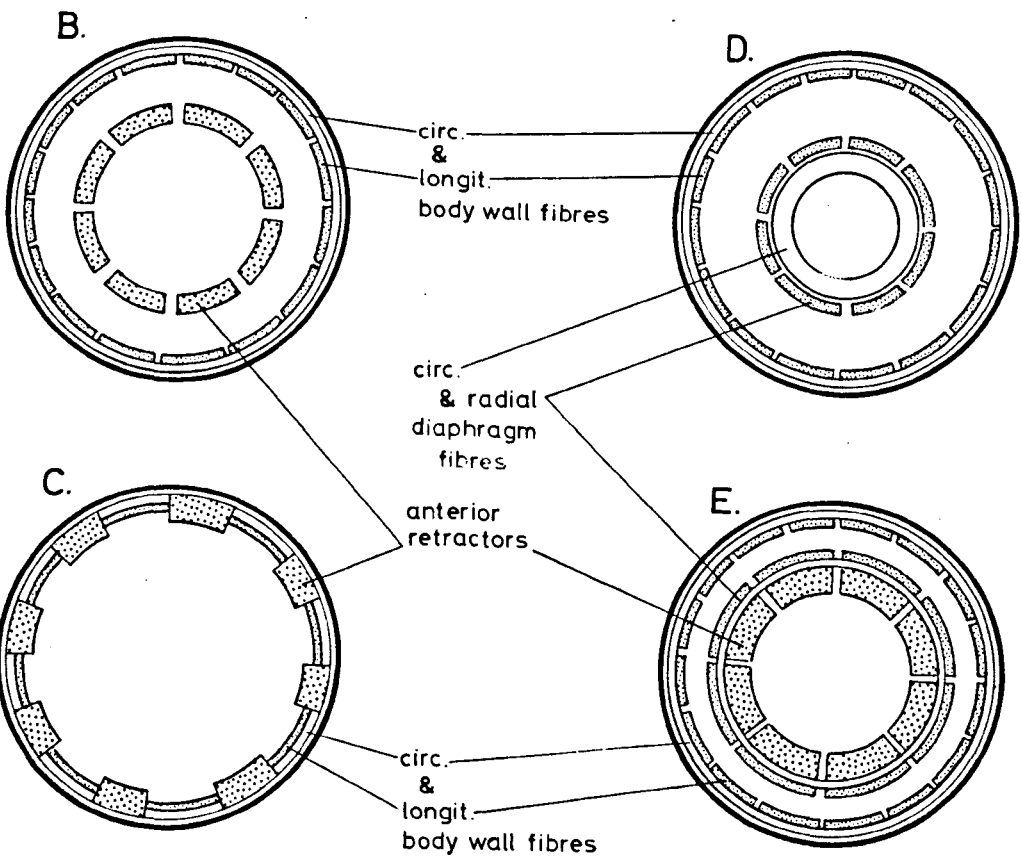
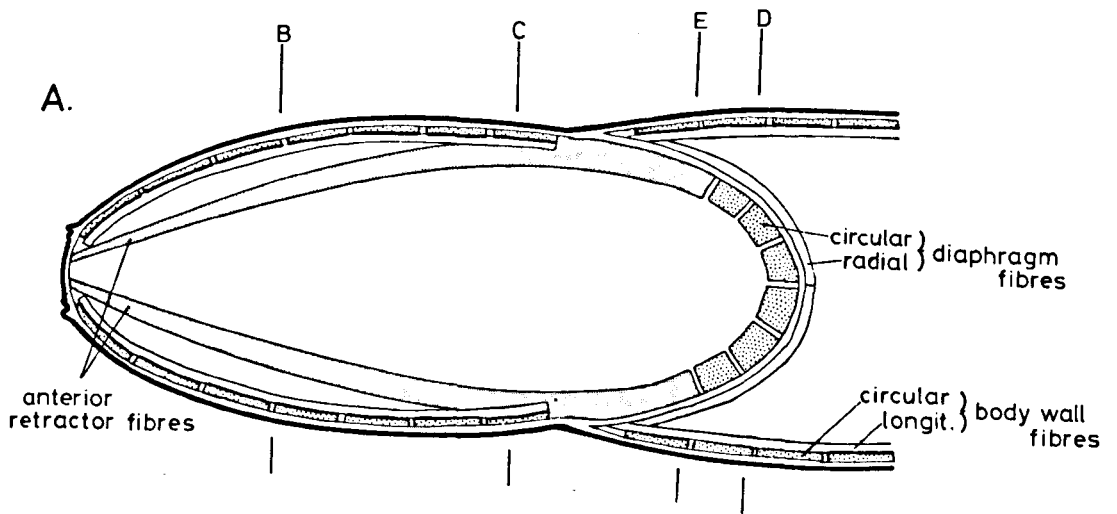
In the region of the join between diaphragm and body wall, an additional set of longitudinal myofibres appears to interdigitate with those of the body wall. They displace inwards towards the anterior end of the sucker, (Figs. 28, 31). These same fibres extend across the periphery of the diaphragm; Fig.31 shows a slightly oblique transverse section of this region, and the positions occupied by these fibres may be seen both within the diaphragm and adjacent to body wall fibres. See 'anterior retractor fibres', in Text Fig. 2.

The longitudinal fibres of the oral sucker region possess a higher concentration of mitochondria than the more posterior body wall musculature, (Figs. 31, 33).

TEXT FIG.2

Diagrammatic reconstruction of the musculature in the oral sucker region, shown in both saggital section and in transverse sections.

- A. Saggital section of oral sucker region to show relative positions of body wall myofibres and those of the diaphragm and anterior retractors. Although the latter are shown in longitudinal section, they are shaded for clarity. The locations of the following transverse sections are indicated as B, C, D and E.
- B. Transverse section in central region of sucker.
- C. Transverse section just anterior to the periphery of the diaphragm. Note the interdigitation of anterior retractor and body wall myofibres.
- D. Transverse section of posterior end of sucker, which passes through the prominent, circular myofibres of the diaphragm.
- E. Transverse section near the periphery of the diaphragm showing the anterior retractors adjacent to the diaphragm radial myofibres.



Apart from the above-described sheets of muscle tissue there are present strips of muscle close to the oral disc, (Fig.34) aligned approximately longitudinally. Further such fibres are also present between the capsule and the body wall, (Fig. 29).

Other tissues present within the oral sucker area include the 'head' gland and its ducts, (Figs. 29, 31, 34); paired pre- and post acetabular gland ducts, (Figs. 31, 35); the alimentary canal, (Figs. 33, 36); and a number of distinct types of tegumentary and internal nerve endings with their associated axons. The nerve tissue is described in Section 1.5.

Muscle associated with other internal organs

Muscle tissue associated with the gland cells is most prominent near to the point of entry of the gland ducts into the oral sucker, (Figs. 32, 33) and it consists primarily of circularly oriented fibres. It is in evidence to at least some extent, however, over most of the length of the glands including the funduses, but is absent from the extreme oral ends of the ducts.

A feature which may be related functionally with these myofibres is the presence of numerous longitudinal microtubules in the walls of the ducts and funduses, (Fig. 37).

Both longitudinal and circular myofibres are associated with the alimentary canal (Figs. 32, 33, 36) and others, of varied orientation with the excretory bladder (Figs. 20, 38).

DISCUSSION

In general layout the body wall musculature in *Schistosoma mansoni* conforms to that found in other trematode species, no diagonal or spiral fibres being found in the body wall. The plasticity conferred by this basically simple arrangement is, however, considerable and may be clearly seen in ciné films produced during the course of this investigation. Although the relative degree of development of longitudinal and circular fibres in the body, tail stem and furcae varies somewhat, each of these parts is capable of extension and contraction as well as flexure in any direction; these movements normally occur in combination.

In a theoretical consideration of the operation of muscle systems in acoelomate animals, "body fluid" is often used to denote, in a general way, fluids or deformable tissues of diverse origin which act as a hydrostatic skeleton. Chapman (1958) described the latter as "a fluid mechanism which in one way or another provides the means by which elements can be antagonized". One of the principal distinctions between hydrostatic and hard skeletons is the fact that individual muscles in a hydrostatic system are not antagonized by other, specific muscles but, during contraction, become opposed to all the other muscles of the system. The efficiency of this antagonism, together with the nervous co-ordination required for operation were probably influential factors in the evolution of a circular/longitudinal plan in so many animal groups. Very different arrangements have, nevertheless, evolved (e.g. in the nematodes).

The flexibility conferred by the hydrostatic skeleton must necessarily be related to a connective tissue structure able to accommodate this (sometimes considerable) change in shape. The fibrous components of connective tissue are often inextensible individually, however. In

many species a geodetic pattern of left- and right-handed helices of fibres is present. Changes in shape of the animal are accompanied by changes in the angles of these fibres relative to the longitudinal axis; the limits of extensibility are approached as these angles approach zero. The subtegumentary fibrous zone described here for *Schistosoma mansoni* cercariae probably has this function, although clear alignment of fibres was not usually found; a network of randomly oriented fibres might still function in the above way (described in detail by Cowey, 1952) but perhaps less efficiently than a highly ordered system. The presence of similar fibrous layers around both oral and ventral suckers in cercariae further suggests a mechanical role. Desmosomes were also often seen to link fibrous tissue to adjacent myofibres.

There was some variation in the number of longitudinal myofibres visible in transverse sections; there appeared to be fewer fibres posteriorly. A change in myofibre cross-sectional area is, to some extent, correlated, the largest being seen in and posterior to the ventral sucker region. The smaller areas were generally recorded at the anterior end of the body. Considerable variation in this parameter was, however, found even within individual sections. It is possible therefore that at least some fibres are branched.

An interesting correlation is that between the dimensions of circular and longitudinal body wall myofibres (as reported here) and the periodicity of nerves in the peripheral nerve net reported to be present by Bruckner and Voge (1974). The widths of longitudinal myofibres seen in electron micrographs lie within the range 1.0 μ m to 3.7 μ m, the mean being 2.4 μ m. The corresponding figures for circular myofibres are 0.8 - 2.9 μ m with a mean of 1.6 μ m. Bruckner and Voge estimated the distance between longitudinal elements of the 'nerve net' at 3-4 μ m and between circular fibres as 1.5-2.0 μ m. The correlation between these two sets of figures implies that there is close to being one nerve for every body

wall myofibre. In addition, the general orientation of the posterior longitudinal muscle, illustrated diagrammatically by Capron (1965) for *Schistosoma haematobium*, bears a close resemblance to the lines of deposited stain in the light micrographs of Bruckner and Voge (1974). This might imply that the nerve tracts follow the lines of the myofibres - or the intercellular space between fibres. It should also be noted that Halton and Morris (1969) found that parts of the subtegumentary muscle of *Diclidophora merlangi* (Monogenea) stained strongly in a cholinesterase preparation. The 'quantity' of subtegumentary nervous tissue, implied by the results of Bruckner and Voge, has not been identified in electron micrographs. This may be a reflection of the difficulty in identifying axons, which are relatively undifferentiated in form, unless they contain one or more types of vesicle, or microtubules. Areas, larger than would be predicted from Bruckner and Voge's results, apparently not containing nerve tissue, are often found at the ultrastructural level. Until confirmatory evidence is available, either from electron micrographs or from the application of appropriate histochemical methods to *sectioned* larvae, I must remain sceptical of the existence of the peripheral nerve net in *Schistosoma*. If further studies also indicate a lack of closely-spaced subtegumentary nerves, alternative explanations of the ACHE results would have to be considered. One such possibility is that the superficial periodicity produced by this technique might be due to a deposition of stain between the body wall myofibres.

Distinct from the cercarial body wall musculature are a group of branched myofibres extending from the dorsal side of the ventral sucker capsule to the dorsal body wall. Dorso-ventral muscles in other animal groups are thought to bring about flattening of the body during swimming or crawling movements (e.g. the Hirudinea). Chapman (1958) considered that the body curvature required for 'looping' movements would be difficult

in the absence of flattening. However, the region of maximum curvature in crawling schistosome cercariae is between the ventral and oral suckers, and relatively little body flattening occurs as the two suckers are brought together. As the ventral insertions of the dorso-ventral fibres are applied to the ventral sucker capsule itself it is more likely that they are functionally associated with the sucker musculature. Cercariae left to 'age' in an experimental vessel eventually stop swimming and sink to the bottom. Although still showing internal activity, e.g. of flame cells, they have presumably exhausted their limited energy reserves (but note the discussion of this point on p. 163). 'Exhausted' larvae lie with their ventral suckers protruding clearly beyond the level of the body wall; this suggests that active muscular contraction is required to hold the sucker in a retracted position and that during relaxation it tends to protrude. Similarly - placed dorso-ventral muscles have been identified in a number of other species, e.g. by Neuhaus (1952) and Wesenberg-Lund (1934).

It is possible that these dorso-ventral fibres also function in increasing the efficiency with which the ventral sucker adheres to the substratum. The radial muscles of the sucker (see label 2, Text Fig.1) probably contract after the lips (see label 4) have been applied to the substratum. This would have the effect of reducing the pressure in the sucker cavity, so long as a good 'seal' is maintained at the periphery of the lips and consequently establishing the sucker's attachment. The circular muscles of the lips could contract to pinch a segment of tissue (or other soft substratum); the inwardly-directed spines of the lips and cavity would reinforce attachment to this segment. The efficiency of the radial fibres would depend on a firm, unyielding point of attachment at the outer periphery of the sucker capsule; the dorso-ventral fibres might therefore contract at the same time as the sucker radials to achieve this. The fact that cercariae can adhere firmly to

smooth surfaces (e.g. glass) suggests that the dorso-ventral/ radial suction system is quite effective even without the operation of circular muscles and spines as described above. Mucus secretion also probably has an important role in adhesion to solid surfaces.

Dönges (1964) found paired myofibres between the oral sucker capsule and lateral body wall of *Posthodiplostomum cuticola*. The presence of similar fibres in schistosomes is suggested by the results here. The additional longitudinal myofibres within the oral sucker, underlying the body wall, may be involved in the retraction of the anterior end of the body with its battery of ciliated receptors. Contraction of the diaphragm musculature would tend to make this structure less convex, hence compressing anterior tissue. This could therefore be part of the mechanism for extending the anterior end of the body, with the longitudinal retractors antagonizing the diaphragm fibres. The main musculature of the 'oral sucker' is therefore very unlike a sucker as such. It appears more like a system for extending and thrusting the anterior end of the body forward, (by contraction of the diaphragm and circular body wall fibres), then increasing the overall cross-sectional area (by contraction of the anterior retractors and longitudinal body wall fibres). This mode of operation might be an integral part of burrowing locomotion as discussed in Section 2.

The cytological organization of body myofibres is similar to that previously described in *Heterobilharzia americana* by Lumsden and Foor (1968). Thick and thin filaments are present, the latter appearing to be continuous with electron-dense structures, or dense bodies, which may have the form of rods attached to the sarcolemma and extending into the fibre. The more compact structures found may also represent the same feature, but they frequently did not stain as strongly. The attachment of filaments to dense bodies, which are in turn attached to the sarcolemma,

presumably provides the means whereby the contractile force developed at the filaments is transmitted to the fibre surface. Many desmosomes are present linking adjacent body wall tissues and especially numerous are the connections between myofibres and the subtegumentary fibrous zone.

1.4 CAUDAL MUSCULATURE

INTRODUCTION

The structure and function of cercarial tail musculature have been discussed in many previous reports. Some of the earlier of these, however, included only brief reference to the organization of muscle tissue, e.g. Cort's (1919) description of *Schistosoma japonicum* and Archibald and Marshall's in 1932 of *S. mansoni*. A basic plan had been established by that time, though, of an outer circular and an inner longitudinal layer of muscle fibres situated immediately beneath the cuticle.

Slightly more detail was presented by Price (1931) for *Schistosomatium douthitti*. Describing the tail, she said that "Six or more rows of large cells which have the form of muscle cells lie beneath the cuticula of the tail stem. The body of each cell is drawn out anteriorly and posteriorly into a long fibre which is attached to the inner surface of the cuticula. These cells are situated obliquely to the longitudinal axis and each has a conspicuous nucleus."

This oblique orientation of longitudinal muscle fibres was also inferred for some of the Danish species illustrated by Wesenberg-Lund (1934). In an account of the causative organisms of dermatitis in New Zealand, MacFarlane (1949) said "Beneath its spinous (tail) cuticle is a herringbone arrangement of muscle cells. Each has a large nucleus and the cytoplasm extends into two long processes There are four sets of about twenty-five of these cells." By "herringbone arrangement" MacFarlane was referring to the fact that the anterior ends of the cells were situated laterally while their posterior insertions were close to the mid-dorsal or mid-ventral line of the tail. Just such an arrangement was later described by Neuhaus (1952) in *Trichobilharzia szidati*.

Striated tail muscle was not found by the above authors although it had been found in other species, e.g. *Cercaria equitator* by Sinitzin (1911) and in *Cercaria nassa* by Martin (1945). Pearson (1956) was able to describe not only the transverse striations of longitudinal muscle cells in the tails of two species of *Alaria* but also small, transverse muscle fibres in the furcae. The longitudinal fibres were found to be arranged in four groups, dorso- and ventrolaterally, with about twenty bipolar cells in each group. They lay parallel to the tail axis. Olivier (1940) had described a similar arrangement for the cercaria of *Apharyngostrigea pipientis*. Pearson (1961) again found four main groups of longitudinal muscle in *Neodiplostomum intermedium*, there being about twenty eight fibres per group in 'herringbone' orientation. These muscles also extended along the lateral faces of the furcae. The cell bodies containing nuclei lay centrally in the tail. The outer, circular fibres were, however, said to be anucleate. Dönges' (1964) description of *Posthodiplostomum cuticola* indicated a basically similar pattern for this species, except that monopolar, longitudinal fibres were involved. Both Dönges (1964) and Pearson (1961) illustrated a line of small, regularly-spaced, lateral nuclei but, whereas Dönges assumes that they are within circular muscle cells, Pearson associates them with lateral nerves. It may be that both authors are correct for their own species. Alternatively it may be that examination of these cells by electron microscopy is required to resolve the difference of opinion.

The development of electron microscopy has, of course, greatly facilitated study in greater depth of the organization of muscle fibres and of their subcellular constituents. Early fixation technique, frequently consisting of the use of O_3O_4 alone, did not, however, produce satisfactory results. For example, Kruidenier and Vatter (1958) were not able to find thin myofilaments in *Schistosoma mansoni* or

Tetrapapillatrema concavocorpa. Similar deficiencies were evident in the preparations of Cardell and Philpott (1960) of the tail of *Himasthla quissetensis*. The latter authors also postulated that the striated longitudinal muscle of *Himasthla quissetensis* formed a complete layer beneath the circular muscle, the myofibres running spirally around the tail. Chapman (1973), however, concluded that the longitudinal muscle in *Himasthla quissetensis* was arranged in four main blocks, as in *Alaria* (Pearson, 1956), with the fibres of each block extending the full length of the tail, parallel to the tail axis.

Lumsden and Foor (1968) compared the ultrastructure of body and tail muscle in *Heterobilharzia americana* cercariae. While the body fibres did not differ significantly from those found previously in adult platyhelminths transverse striations in the longitudinal, caudal fibres were found which the authors attributed to a regular arrangement of 'dense bodies' associated with the thin myofilaments. The striated fibres were also said to be positioned obliquely to the longitudinal axis of the tail, but a reconstruction of muscle organization was not attempted. Nuttman (1974) did propose such a reconstruction of *Schistosoma mansoni* musculature which indicated a 'herringbone' arrangement in this species. Striations were shown to be due to an interdigitation of dense bodies (or rods) and tubules of the sarcoplasmic reticulum.

Other previous authors have attributed the striations to the dense bodies alone, e.g. Chapman (1973) in *Himasthla quissetensis* and *Cryptocotyle lingua*, or to tubules of sarcoplasmic reticulum alone, e.g. Kruidenier and Vatter (1958). Pearson (1956) compares those of *Alaria* spp with the Z-lines of vertebrate striated muscle and this conclusion also seemed to be assumed by Inatomi et al (1970) for *Schistosoma japonicum*. No clear indication of cross-banding was found in the tail of *Parorchis acanthus* by Rees (1971) who also said that longitudinal myofibres took a "straight course along the length of the tail."

The following is an account of the caudal musculature of *Schistosoma
mansoni*:

RESULTS

A layer of subtegumentary muscle is present along the entire length of the tail stem. The fibres are closely-spaced and arranged in outer, circular and inner, longitudinal layers. The contractile regions of the circular fibres each measure approximately $1.40\mu\text{m} \times 0.24\mu\text{m}$ in transverse section and are consistently smaller in this respect than corresponding fibres in the body (Figs. 39, 40). The orientation of body and tail circular fibres is similar in that both lie at right angles to the longitudinal axis. There appears to be minimal intercellular space between adjacent tail fibres which are attached to the subtegumentary fibrous zone by means of desmosomes (Figs. 40, 41).

There is no regular, transverse striation in the circular fibres to be compared with that of some longitudinal fibres. As might be expected, the myofilaments are rather longer than those of the striated muscle, the myofilament complement of non-striated tail fibres being basically similar to that of body fibres. (Figs. 40, 41).

Nuclei have only infrequently been seen associated with circular fibres and occur in cell bodies lying in the tail core beneath the longitudinal, striated fibres (Fig. 42).

There are six separate blocks of longitudinal muscle in the tail stem, lying immediately beneath the circular muscle layer. They do not pass directly through the tail base into the body: the excretory bladder is situated centrally at the tail/body junction and is partially enclosed by the terminal region of body longitudinal muscle which contains, at this point, almost exclusively thin myofilaments. Caudal longitudinal fibres are attached to the inside of the posterior end of the resulting

'cylinder' of thin filaments (Figs. 18-20, 43-45).

Mid-dorsal and mid-ventral blocks are relatively small, each comprising two closely-adjacent fibres which are round anteriorly but become flattened dorsoventrally towards the distal end of the tail (Fig.46). No evidence of regular, transverse striation was found in these mid-dorsal and ventral myofibres, (Figs. 40, 47).

Figs. 48-53, showing transverse sections of the distal end of the tail stem, indicate a different fibre pattern : here, the pairs of mid-dorsal and mid-ventral fibres have separated and the lateral, longitudinal blocks are absent. Oblique myofibres are visible and may represent the circular muscle (Fig.53).

Throughout the greater part of the tail stem, the exception being the distal (approx.) 10%, the four lateral muscle blocks are seen. These are actually in the dorsolateral and ventrolateral positions, on each side. Each block appears in transverse sections to consist of up to five fibres (Figs. 54-57) although this may be reduced to three near the anterior end.

The longitudinal muscle in species of furcocercariae, however, does not necessarily lie parallel to the longitudinal axis of the tail. In *Schistosoma* these fibres may be seen by light microscopy (of whole mounts) to lie at an angle of approximately $10-12^{\circ}$ to the longitudinal axis (Figs. 58, 59). The anterior extremity of each fibre is situated laterally and its most posterior insertion is close to the mid-line. Also seen in the same plane of focus as the elements of this 'herring-bone' pattern of fibres is a series of transverse lines, with an approximately $1\mu\text{m}$ periodicity. It was thought originally that this might have been caused by circular muscle fibres, but because their plane of focus and their distribution matched those of the longitudinal

fibres it was concluded that they were a structural feature of the latter. The fine structure of these striations is described below.

A complete lateral muscle block therefore contains many more fibres than any one transverse section, e.g. Figs. 55, 56, might suggest. Where a fibre is flanked on both sides by others it appears U-shaped in cross-section. The corresponding appearance of the terminal regions of a fibre is L-shaped, eventually tapering rapidly (Fig. 55).

These figures illustrate the two clearly defined regions within each fibre: the contractile region lies near the periphery of the tail and is sheathed by a fenestrated sarcoplasmic reticulum. An inner sarcoplasmic region (or cell body) contains a nucleus and other organelles. The structure of the contractile region will be described first.

Lateral longitudinal fibres are distinctive in their structural periodicity, transverse striations occurring at 0.8 - 1.0 μ m intervals. These may be seen in whole mounts or sections examined under the light microscope as well as in material examined by electron microscopy. Figs. 60, 61 are light micrographs on which the striations are indicated by the arrows. The striations of adjacent fibres are often aligned across the width of each muscle block and traverse approximately at right angles to the fibre axis (Figs. 62, 63). The latter axis does not appear parallel to the long axis of the tail.

Closer examination of the muscle, in longitudinal section, reveals that each striation is composed of a row of electron-dense bodies associated with a series of small, membrane-bound areas. From these sections alone, the latter could represent either oval vesicles or tubules in cross-section (Figs. 64, 65). Transverse sections of the same fibres show that the latter is the correct interpretation and that tubular branches of the sarcoplasmic reticulum interdigitate with the dense bodies which are themselves elongate (Figs. 66, 67). As the plane of the section in

Fig.68 does not pass only through the centre of a striation, the arrangement of filaments between striations may also be seen. The centre of a striation is indicated by the asterisk and both dense bodies and sarcoplasmic tubules are present. To the right of this, immediately adjacent to the striation, only tubules and thin myofilaments (in TS) are seen. Most of the sarcomere area, however, contains both thick and thin myofilaments. There are up to 10 or 12 of the latter surrounding each thick filament although this ratio varies slightly. (Figs.66-68).

Thick filaments are much shorter than those of the smooth fibres, never exceeding the length of a single sarcomere. The thin filaments appear to be continuous with the dense bodies (or dense rods) which are attached, at least in part to the sarcolemma (Fig.69). In both longitudinal and transverse sections the sarcoplasmic reticulum is seen to enclose the contractile region of the fibre as a double envelope which is perforated by numerous holes. This envelope is absent from the area immediately overlying the 'free ends' of the transverse sarcoplasmic tubules.

Fig.62 shows several striated fibres in a longitudinal section of a lateral muscle block and appears to illustrate branching of the contractile region (but see p. 55).

The inner, sarcoplasmic regions of the longitudinal fibres contribute much of the volume of the tail core (the hydrostatic skeleton) as can be seen in Fig.70. An irregularly-shaped nucleus is associated with each fibre. A clumped distribution of chromatin is seen within (Figs. 56, 60), this being more electron dense than the nuclear ground substance. Clusters of the small electron opaque particles (recorded above in other nuclei) are also seen. Each particle is about 60-80nm across, and invariably appears roughly spherical in shape, regardless of the plane of sectioning (Figs. 41, 42).

Accumulations of what are assumed to be glycogen granules are found. These may be closely associated with nuclei, with the contractile region, or lying free in the largely structureless sarcoplasmic regions (Figs. 70-73). The mitochondria of striated fibres are larger and more numerous than in other cercarial tissues and are well endowed with cristae. Most mitochondria occurred in or near the 'troughs' formed by the inner surfaces of the contractile regions (Figs. 55, 74, 75). Groups of cristae were sometimes found to be oriented towards adjacent myofibre membranes.

The sarcolemmal surface is not smooth but to some extent interdigitates with the sarcolemma of the adjacent fibres (Figs. 69, 70).

Furcae

The two furcae appear somewhat flattened laterally and myofibres are not grouped into discrete blocks but are more widely spaced. ^{No} / No furcal fibres are striated.

The contractile regions of furcal longitudinal fibres may be up to $2.3\mu\text{m}$ x $0.8\mu\text{m}$ in transverse section on the posterior side of each furca, but are smaller anteriorly (Figs. 76-80).

Circular fibres are very fine, often being only $0.5\mu\text{m}$ in diameter but, once again, are similar in basic structure to non-striated muscle elsewhere in the tail and body.

In addition to circular and longitudinal fibres there exist narrow, transverse fibres which join anterior and posterior sides of the furcae (Figs. 82, 83). A particularly large cluster of dense particles can be seen in a nucleus associated with these fibres in Fig. 77.

As in the tail stem, much of the furcal 'core' is a largely structureless matrix, but towards their distal ends the volume of the excretory canals assumes a greater significance relative to the total furcal volume (Fig. 84). The distal tip of the tail is seen in Fig. 85.

The precise organization of myofibres at the tail/furcae junction has not been determined. It is possible that the mid-dorsal and ventral muscle blocks of the tail stem are continuous with longitudinal fibres in the furcae but, as described above, striated fibres in the stem do not extend as far as this junction. Fig.85 shows that fibres are present at the distal tip of the tail stem, between the bases of the two furcae. This could indicate that the fibres on the posterior sides of the two furcae are continuous. Contraction of these muscles would then flex the furcae posteriorly.

Axons have not yet been traced across the tail/body junction, although nerve tissue has been identified immediately anterior to the bladder and a bilobed nerve mass is situated posteriorly. Axons have been shown to link ciliated nerve endings in the tegument to the sub-muscular nervous system (see Section 1.5) but a positive identification of a neuromuscular junction has not yet been made. The juxtaposition of nerve and muscle cell membranes (e.g. in Fig.21) is suggestive of functional contact, however.

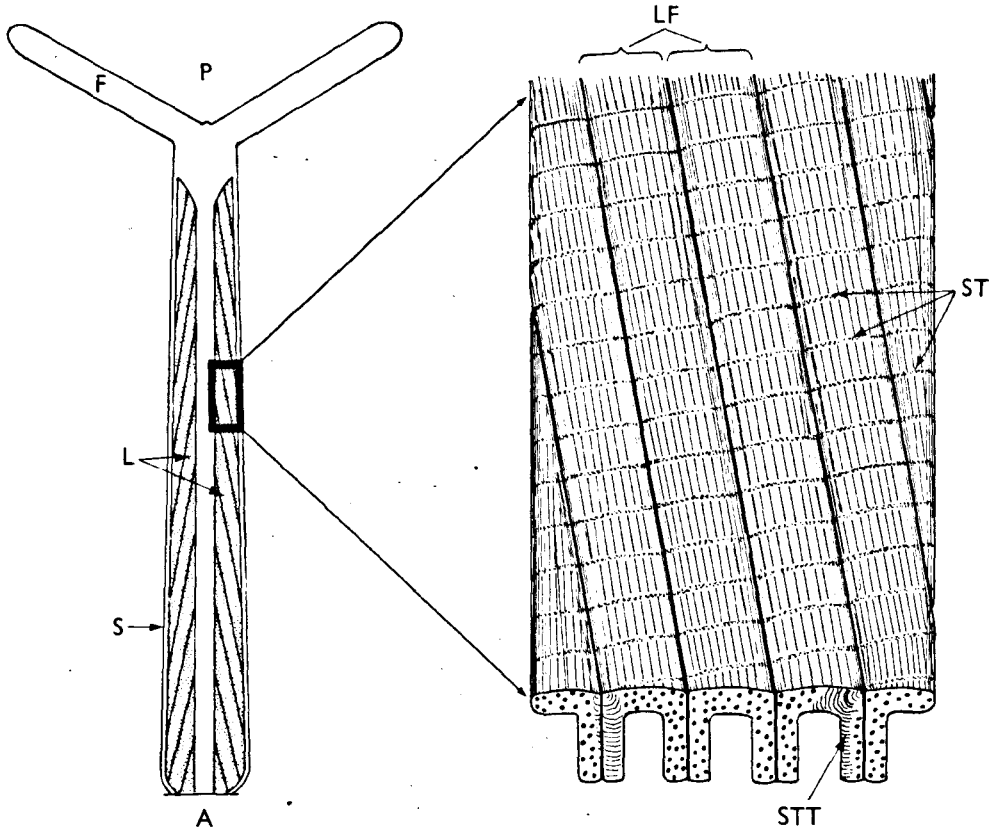
Diagrammatic representations of the tail musculature are presented in Text Figs. 3 and 4.

TEXT FIG.3

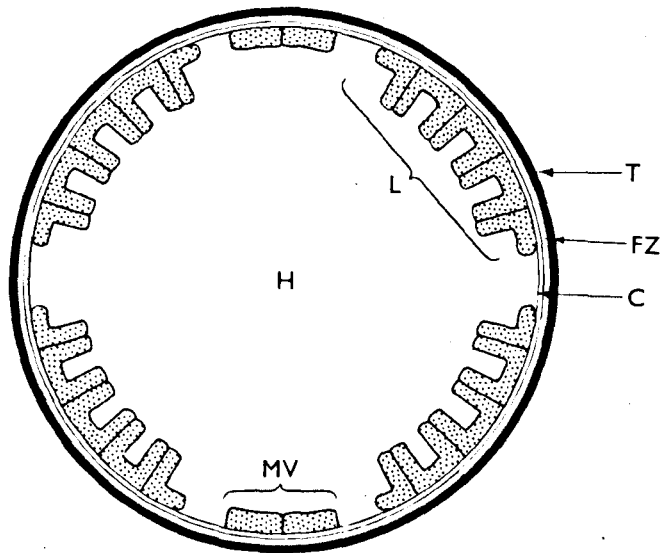
Diagrammatic reconstruction of the organization of the tail stem musculature.

- A. Dorsal view of tail showing the location of dorsolateral muscle blocks and the orientation of the myofibres. Not all myofibres are shown, for clarity. Also shown is an enlarged segment of one of these blocks; this relates the appearance of the block surface to its appearance in transverse section. Only the contractile region of the block is drawn.
- B. Transverse section of the central region of the tail stem. The sarcoplasmic regions of myofibres, the excretory system etc. are omitted. All four lateral blocks may be seen, together with the mid-dorsal and mid-ventral (non-striated) blocks.

A



B



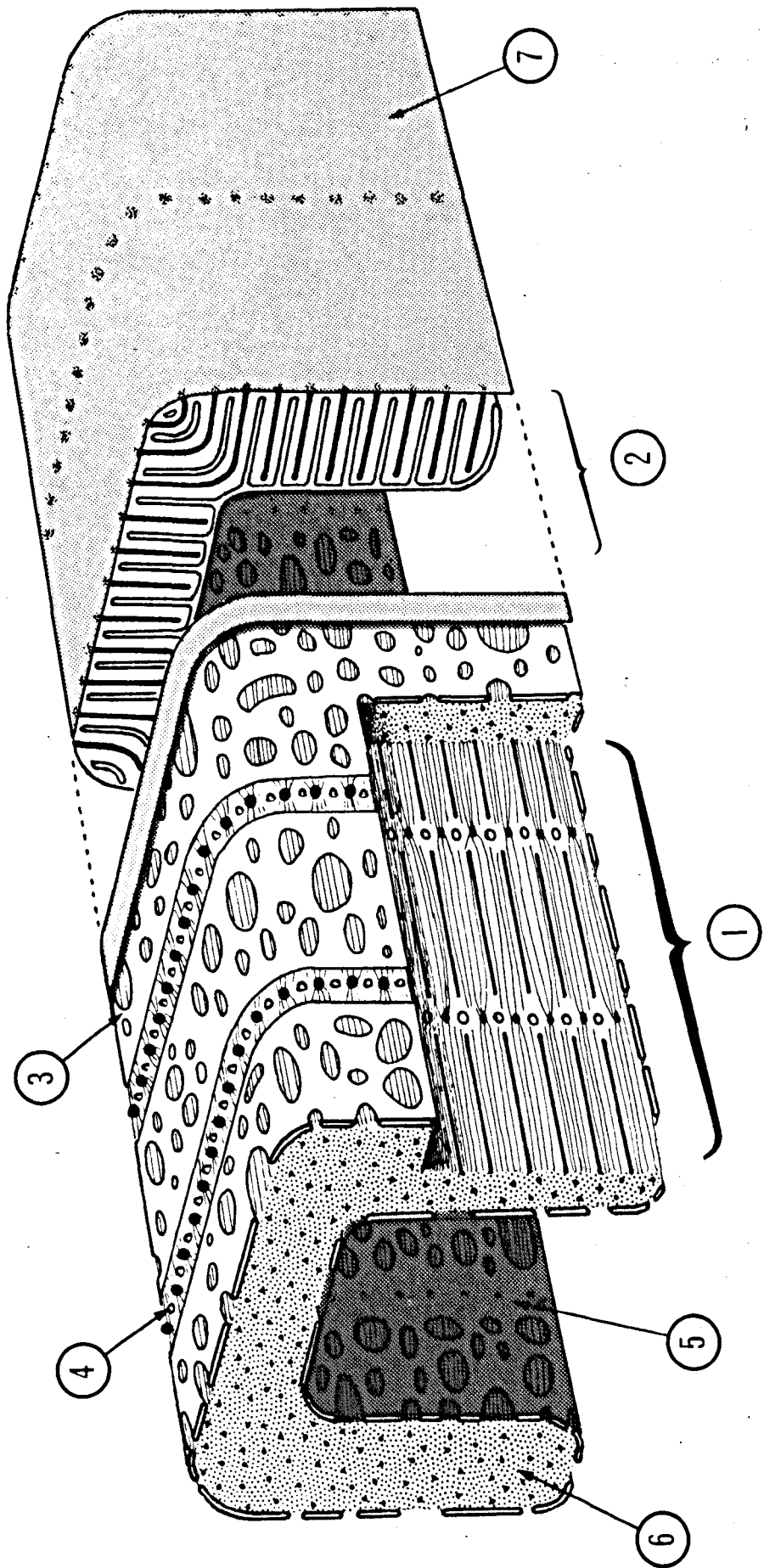
TEXT FIG.4

Ultrastructure of a striated myofibre contractile region.

1. Section removed from side of fibre to show sarcomere in LS.

Note

- (a) sections of sarcoplasmic reticulum tubules and dense rods.
 - (b) full length of thick and thin filaments, the latter being continuous with the dense rods.
2. Section of fibre removed to reveal striation in TS. Dense rods, attached at one end to the sarcolemma, interdigitate with tubules of the sarcoplasmic reticulum (SR).
 3. Surface of fenestrated SR, sarcolemma omitted.
 4. Striation - not covered by SR on outer surface of fibre. Rods attach to the inner surface of the sarcolemma and tips of SR tubules lie adjacent to the sarcolemma.
 5. Note complete covering of striation by SR on inner aspect of fibre. Free ends of dense rods just visible beneath SR.
 6. TS in region between striations showing thick filaments surrounded by thin. Note also the SR in TS.
 7. Sarcolemma covers entire fibre. Underlying striation visible.



DISCUSSION

Previous descriptions of caudal musculature have shown that although the same basic layers are always present, the orientation of longitudinal myofibres may vary between species.

Cardell and Philpott (1960) have claimed that, in *Himasthla quissetensis*, a continuous sheet of longitudinal muscle lay beneath the tegument and that the fibres were oriented spirally around the tail. Their three-dimensional reconstruction of tail structure has been used in at least two recent textbooks, Erasmus (1972), Meglitsch (1972). However, the evidence they presented did not support the structure proposed. In his more recent interpretation, Chapman (1973) found that the longitudinal muscle in *Himasthla secunda* had a greater cross-sectional area dorso- and ventro-laterally to produce four main muscle blocks and that there was no lateral longitudinal muscle at all. He also found this same general pattern in *Cryptocotyle lingua*. Although in their diagrammatic representation of longitudinal muscle Cardell and Philpott show it as a complete layer around the circumference of the tail, the electron micrograph in their Fig.3 shows the four-block organization. This can be seen much more clearly in Chapman's Plate 1A.

Chapman, however, further proposed that each myofibre within each muscle block could be traced along the length of the tail, but it is difficult to reconcile this with the variable number of myofibres visible in the serial transverse sections presented. Could it be that, as in *Posthodiplostomum* or *Schistosoma*, *Himasthla* provides another example of longitudinal fibres making an acute angle with the long axis of the tail such that, although all transverse sections would still indicate approximately the same number of fibres per section, the same fibres are not necessarily seen? In addition, exact orientation of the tail during longitudinal sectioning would preclude similarly exact orientation of

the fibres within. Unusual fibre patterns (e.g. in Chapman's Plate 2A) might be explained in the above way.

Lumsden and Foor (1968), in their description of *Heterobilharzia americana*, described the axial musculature as being positioned obliquely to the tail axis. These authors also suggested that myofibres were branched (see their Fig. 7). Fig. 62 of this work appears to show the same feature in *Schistosoma mansoni* but branching is not considered to be responsible. What appear to be branches are, in fact, the two sides of the one fibre contractile region which is U-shaped in transverse section.

The presence of four main dorso- and ventro-lateral muscle blocks appears, therefore, to be common to *Neodiplostomum intermedium*, *Posthodiplostomum cuticola*, *Himasthla secunda*, *Cryptocotyle lingua* and *Schistosoma mansoni*, but species (and/or the opinion of respective authors) differ as to the precise arrangement of myofibres within each block.

The present work, prefaced by Nuttman (1974), is the first in which a dual system of axial and oblique longitudinal fibres has been proposed: lateral blocks consist of oblique fibres while the mid-dorsal and mid-ventral blocks each consist of a pair of non-striated, axial fibres, extending the full length of the tail.

The suggestion has been made that circular tail muscle may be anucleate (e.g. Pearson, 1961) but, if so, this cannot apply to all species. As shown in this report, nuclei may be seen in association with the circular muscle fibres of *Schistosoma mansoni*. This association was, however, seen less frequently than might be expected in transverse sections examined by transmission electron microscopy. The possibility exists, therefore, of the association of each nucleus with a number of circular fibres.

Whenever they have been identified, the cell bodies of circular muscles occurred beneath the longitudinal fibres contributing, together with the cell bodies of the latter, to the hydrostatic skeleton - the tail core. The large 'caudal bodies' described by Pearson (1961) in *Neodiplostomum* were not found in *Schistosoma mansoni*, much of the volume of the tail core being accounted for by the muscle cells described.

The appearance of the muscle cell body (or sarcoplasmic) regions contrasts with that of the subtegumentary and glandular cells of the body. Large areas are seen to be devoid of granular cytoplasm or membranous organelles etc. A layer of relatively large mitochondria is invariably applied to the inner surfaces of longitudinal fibres. An interesting point to note is that cristae have sometimes been found aligned at right angles to the mitochondrial outer membrane at the point of contact with the adjacent fibre. This may be coincidental, or it may be an adaptation for facilitating the release of high energy molecules from the mitochondria to the fibre. Future work could perhaps include an examination of mitochondria from various locations in the tail and a statistical analysis of the angular orientation of cristae with respect to the adjacent fibre membranes.

Granules which were assumed to be glycogen were found in all muscle cells, but especially in those of the lateral tail blocks. Distribution was usually somewhat scattered, large accumulations of granules being rare and often close to cell nuclei.

The rapid, lateral vibration of the tail responsible for locomotion is presumably due to the contraction of the main (striated) muscle blocks on the left and right sides alternately. Rotation about the longitudinal axis exhibited by swimming *Schistosoma mansoni* cercariae might be explained by slight differences in the degree, or timing, of tension developed in the two blocks of one side. In their analysis of tail

movement by high-speed cinematography, Graefe et al (1967) did not mention this rotation. Detailed analysis of the movements of the tail stem and furcae during swimming would be necessary to help explain the absence of striated fibres in *Schistosoma mansoni* furcae but their existence in *Neodiplostomum intermedium* and *Posthodiplostomum cuticola*. The mechanisms of cercarial propulsion are discussed further in Section 2.

This study has demonstrated that, in *Schistosoma*, striations are absent from all fibres except those of the lateral tail stem blocks. It has previously been assumed that these striations are due either to transverse arrays of electron-dense material or to tubules of the sarcoplasmic reticulum. In *Schistosoma* they are due to the presence of regular transverse arrays of sarcoplasmic tubules which are continuous with the fenestrated reticulum enclosing the longitudinal fibres. The tubules interdigitate with transversely-extended dense bodies, or rods, which are continuous with the thin myofilaments, and which are attached at one end to the sarcolemma. Dense bodies are the functional equivalent, therefore, of the Z-disc of vertebrate muscle. Franzini-Armstrong and Porter (1964) considered that Z-discs might be important in the co-ordination of myofilament movement.

It is clear that the type of striated muscle described here is, in some ways, intermediate in structure between non-striated muscle and the striated type found in, for example, insects and vertebrates. In the latter, a transverse tubule (T) system has evolved which consists of invaginations of the sarcolemma. The tubules are closely associated with cisternae of the sarcoplasmic reticulum so that, in transverse section, the so-called triads may be seen. *Schistosoma* does not possess a T-system, although the sarcoplasmic tubules are in many places closely associated with the sarcolemma. The small size of myofibres in the cercarial tail may make the evolution of a T-system unnecessary.

In a recent study of *Parorchis acanthus* (Rees, 1971) there was no comparable demonstration of striated myofibres. This could presumably be related to the slow rate of oscillation of the tail of this larva (about 3 per sec) compared with that of the other species discussed.

In addition to longitudinal and circular fibres, the furcae in *Schistosoma* contain numerous, small transverse fibres which may be related to the normally flattened appearance of the furcae. During normal (tail-first) swimming, the furcae are spread out laterally and the flattening would, therefore, have the effect of increasing the lateral surface area and presumably also the thrust. The presence of transverse furcal fibres has previously been reported in other furco-cercariae such as those of *Alaria* spp (Pearson, 1956) and *Posthodiplostomum cuticola* (Dönges, 1964).

A complete investigation of behaviour inevitably progresses to a consideration of the process of attachment to and penetration of the primary host's skin surface. A characteristic feature of this process is that the cercaria sheds its tail. This is in fact an early sign that the metamorphosis from water-tolerant cercaria to saline-tolerant schistosomulum has begun. Very little is known, however, of the physiological or even morphological aspects of tail-shedding. The morphology described in this work is that of a free-swimming cercaria, which has not been subjected to mechanical, chemical or other stimuli which would cause shedding. All sections were cut from 'complete' individuals. The longitudinal body musculature has been shown to terminate in the region of the excretory bladder and these posterior insertions are attached to the longitudinal tail fibres. Separation evidently occurs at the level of the excretory bladder during tail-shedding, but whether the exact line of fracture occurs through this

junction of body/tail muscle fibre or alternatively through the body fibres themselves is not yet known. The latter would appear to be a possibility in view of the unusual myofilament content of the posterior ends of these fibres. (They contain almost exclusively thin myofilaments.) As it is possible to stimulate tail shedding in vitro, fixation of cercariae at intervals after stimulation could provide the material necessary to elucidate these problems.

1.5 NERVOUS SYSTEM

INTRODUCTION

Classical works on the trematodes, such as Bettendorf (1897), notwithstanding, in many early studies of the cercaria of digenetic trematodes the nervous tissue appears to have received only a perfunctory treatment. See, for example, Cort (1919) or Price (1931). Slightly more detail was given by Neuhaus (1952) for *Trichobilharzia szidati*, including a brief description of presumed sensory structures.

Several authors have since found that AgNO_3 staining may be used for mapping tegumentary nerve endings. Schistosome species have been examined in this way by Wagner (1961), Capron (1965), Richard (1971) and Short and Cartrett (1973). The latter authors, however, not only provided quantitative information on the extent and frequency of variation in the patterns of these structures, but also gave a detailed report on the anterior tip of the cercaria. This had previously been found difficult due to the concentration of structures in this region and to the close proximity of similarly-staining gland openings. Authors using the AgNO_3 technique to examine other species include Seitner (1951), Ginetsinskaya and Dobrovolskii (1963a) Heyneman and Umathevy (1966), Lie (1966), Chapman and Wilson (1970) and Mohandas (1971). These nerve endings do not always appear uniform, however, even within one species.

Wagner (1961), Lie (1966), Mohandas (1971) and Short & Cartrett (1973) have all commented on the different types of structures appearing after AgNO_3 treatment. It is appropriate at this point to discuss the varied terminology associated with the structures in question - e.g. 'integumentary papillae' (Lie 1966), 'argentophilic cuticular structures' (Heyneman & Umathevy 1966), 'argentophilic papillae' (Short & Cartrett 1973). From electron microscope studies have emerged 'integumentary sense

receptors' (Matricon - Gondran 1971) and 'ciliated nerve endings' (Nuttman 1971). Divergence from the earlier general use of 'papillae' was probably due, at least partially, to the discovery that not all types actually protruded beyond the tegument surface as 'papillae' implies. This led to the use of generalized terms which still did not imply a direct commitment on the part of the authors as to the *function* of these structures - hence 'argentophilic structure' as an analogue of the electron microscopists' 'osmiophilic structure'.

The application of transmission and scanning electron microscopy to this field has considerably enhanced the case for simply using the term 'receptor'. It is now clear that these structures are very similar in appearance to known receptors in other animal groups and that they are continuous with underlying nerve cells. I, therefore, propose to use the term 'receptor' here, as have Erasmus (1967), Morris (1971) and Matricon - Gondran (1971).

Descriptions of cercarial receptors at the ultra-structural level appeared at first to be a by-product of investigations of the tegument itself, e.g. Erasmus (1967) Morris (1971). Both of these authors described a bulb-like expansion of the nerve termination with a single cilium protruding through the tegument. Similar structures appear to be common in cercariae and many may be present on any one individual, e.g. see Bibby & Rees (1971), Chapman and Wilson (1970), Dixon and Mercer (1965), Robson and Erasmus (1970), Nuttman (1971), Kjøie (1971), Matricon-Gondran (1971). More complex receptors, still derived from single nerve cells but possessing several cilia occur, for example, in *Schistosoma mansoni* cercariae (Morris 1971; Nuttman 1971) and in *Echinostoma paraensei* (Matricon-Gondran, 1971). In some instances, receptors have been visualized by scanning electron microscopy, e.g. Robson and Erasmus (1970, Bibby and Rees (1971), Kjøie (1971) and Short and Cartrett (1973). Cilia nearly always appear to be distorted during

this procedure, but some surface structure can be seen and the location of receptors can be correlated with light microscopy data.

Considerably less is known of the fine structure and organization of non-sensory neurons. Dixon and Mercer (1965) studied the nervous system of *Fasciola hepatica* cercariae; they identified several different types of intracellular vesicle and what they considered to be both nerve-nerve and nerve-muscle junctions.

Rees (1967) described the central ganglion of *Parorchis acanthus* as being composed of a central, axonal neuropile enclosed by a peripheral cell rind. This general pattern had, in fact, been identified in *Orientobilharzia dattai* by Dutt and Srivastava (1962) by light microscopy who said that "Lying on the surface of the nerve masses are numerous nuclei The centre is non-cellular and appears to be fibrous".

The above techniques are not entirely suitable for mapping the course of nerve tissue. Lewert and Hopkins (1965) applied histochemical methods (acetylcholinesterase localization) using *Schistosoma mansoni* but only a general picture of a central nerve mass and longitudinal cords was obtained. Short and Cartrett (1973) have reported a lack of success in plotting internal nerve tissue in the same species. Success was finally achieved by Bruckner and Voge (1974) who altered the surface permeability properties of cercariae prior to incubation for acetylcholinesterase activity. Histochemical methods were also used with some success by Jennings and Le Flore (1972) on *Himasthla quissetensis* and *Zoogonus lassius*.

Bruckner and Voge (1974) commented that surface papillae were also highlighted by the acetylcholinesterase technique and that nerves could be seen connecting them to underlying nerve cords. They did not, however, discuss in detail the locations of this 'surface activity'.

Some information in this respect is presented here and an attempt is made to correlate the varied results to date from scanning/transmission electron microscopy, AgNO₃ staining and acetylcholinesterase localization in order that a composite view of the cercarial nervous system may be developed.

The aim of this section of the investigation was to describe the ultrastructure and general layout of sensory receptors and nerves, in both tail and body of the *Schistosoma mansoni* cercaria.

RESULTS

The central nervous system consists of a bilobed nerve mass lying between the gland funduses and the oral sucker. From it arise three main pairs of longitudinal nerve trunks situated dorsally, laterally and ventrally. The latter, however, are the most prominent, (Figs. 14, 31, 86, 87). An anteriorly situated nerve is seen in Fig. 88.

The only other major accumulation of nerve tissue occurs in the base of the tail stem and consists of a bilobed ganglion which extends across the width of the tail between mid-dorsal and ventral muscle blocks, (Figs. 89-91). A median nerve cord from this ganglion lies immediately beneath each of the above muscle blocks, running the full length of the tail stem to the furcae, (Figs. 91, 92).

Closer examination of the central nervous system reveals a central mass of unmyelinated axons, the neuropile, surrounded by a layer containing many cell bodies, the cell rind, (Figs. 14, 93). Some cell bodies are also found in the caudal ganglion but this structure is largely axonal in content, (Figs. 88, 89, 94). Synapses are not confined to ganglia as they are also found in the longitudinal nerve cords of both body and tail, (Figs. 95, 96). They may be distinctive in appearance, adjacent V-shaped areas on the 'connecting' neurons being strongly osmiophilic. The axoplasm close to these junctions is normally packed with small, spherical vesicles. These may be classified into two main types (on the basis of their appearance in electron micrographs only): small, dense vesicles 40nm to 80nm in diameter with a less dense periphery and small clear vesicles about 20-30nm in diameter. An asymmetrical distribution about the V-junction is normal. Dense and clear types are mixed within individual axons in these figures and this is generally the case in the nervous system. Both are widely distributed in the

axons of central and peripheral systems but usually at a lower density, (Figs. 92, 93, 97, 98), than is evident at synapses. Occasionally, however, high concentrations are found apparently not associated with a V-junction, (Figs. 99, 100).

It is emphasised that the distinctive V-junctions may well not be the only functional synapses in the cercarial system but that they are the only easily-recognized type.

Although not easily identified when viewed in transverse section, numerous 20nm microtubules are present in the axoplasm. They are more clearly seen when axons are sectioned longitudinally, (Fig. 98) when tubules can be more easily distinguished from small, clear vesicles. Aggregations of larger vesicles, usually electron dense and up to 330nm across, were found in the earlier stages of the investigation and seemed to be associated with muscle rather than nerve tissue. Clear continuity between the regions containing the vesicles and adjacent sarcoplasm was not at that stage convincingly demonstrated, however, (Fig.101). Similar vesicles within what was thought to be nerve tissue were then found and so these large dense vesicles are tentatively categorized as nerve cell inclusions, (Figs. 20, 102-105). They are often associated with a smaller, clear type of vesicle and the latter may be seen in several of these figures.

The large 60nm to 160nm electron-lucent vesicles found, (Figs.102, 105) are a feature of many specialized nerve endings (see below) in the cercarial tegument. These nerve endings are generally assumed to have a sensory function and so will be referred to as 'receptors' here.

Receptors are of a number of distinct morphological types, but all appear to possess one or more cilia or at least evidence of ciliary basal bodies. The following nomenclature will be used:

- 1) unsheathed uniciliate receptor
- 2) sheathed uniciliate receptor
- 3) ciliated cavity receptor
- 4) laminated receptor

Details of their ultrastructure, together with some information on their distribution, have been obtained by transmission electron microscopy and are presented below. Surface structures which are assumed to correspond to these receptors are evident in the acetylcholinesterase preparations, description of which follows the E.M. results.

1) UNSHEATHED UNICILIATE RECEPTORS These have a wide distribution over the cercarial body and tail. Each consists of a bulbous nerve ending 1.0 - 1.5 μ m across, possessing a single projecting cilium, situated in the tegument frequently in a small cavity, (Figs. 106, 107). The tegument is raised to form a papilla to enclose the nerve bulb which lies completely distal to the sub-tegumentary fibrous zone. An axon extends from each bulb through the fibrous zone and body wall musculature to the underlying nerve cord. Microtubules in the axon may extend into the cytoplasm of the bulb, (Figs. 107, 108).

The point of attachment between nerve cell and tegument appears as a collar of osmiophilic material, lying under the tegument plasmalemma, opposing a similarly dense structure in the nerve bulb, (Figs. 106, 108). The space between the two tissues contains a less dense material which may show a structural periodicity (Fig. 106). This intercellular linking complex has been called a septate desmosome.

A further osmiophilic ring occurs beneath the desmosome, adjacent to the nerve cell plasmalemma and may be continuous with other dense material in the bulb, (Fig. 106, 108).

The ciliary basal body is composed of several different elements.

The largest is basically cylindrical, the dense wall being continuous with the peripheral microtubules of the cilium, (Fig. 109). A dense plate is situated centrally, at the level of the cell surface and, distal to this, is a second, smaller body continuous with the central ciliary tubules. Further elements are also seen radiating from the outside of the basal body near the plasmalemma of the distal end of the bulb, (Figs. 107, 109). These appear to link with the peripheral desmosome material.

The cilium itself protrudes far beyond the tegument surface and overlying pericercarial envelope (up to 7 μ m has been observed). Microtubules in the axoneme probably conform to a pattern of 9 peripheral pairs surrounding 2 single tubules that are centrally placed. On one occasion a cilium of this type was seen to be clearly associated with a further group of microtubules, (Figs. 110-111).

Apart from the structures associated with cilium and desmosome, described above, the nerve bulb may contain large clear vesicles as well as microtubules, lying in a granular cytoplasm often showing clear areas, (Figs. 106, 109, 112). Continuity with underlying nerve tissue is illustrated in Fig. 113.

For comparison of ciliary structure, Fig. 114 shows the cilia of a cercarial flame cell.

2) SHEATHED UNICILIATE RECEPTORS This type of receptor is only found at the anterior end of the oral sucker adjacent to the openings of the gland cell ducts - a region known as the oral disc, (Figs. 115-117). Cilia are relatively shorter than those of unsheathed receptors, only 1 - 2 μ m being recorded from micrographs, and they are partially enclosed by tegumental sheaths; only the distal tips of the cilia are therefore

directly exposed to the exterior.

Nerve bulbs are similar in size and contents to those of unsheathed receptors although some may contain a high concentration of large clear vesicles extending into the axon, (Figs. 118, 119). Up to five adjacent receptors of this type have been found in single sections, (Fig. 12) and axons extend to underlying nerve tissue (Figs. 117, 121).

3) CILIATED CAVITY RECEPTORS Cavity receptors are only present on the cercarial body. More specifically, isolated receptors have been identified (in transmission electron micrographs) in the following locations: on the ventral side of the body, posterior to the ventral sucker; roughly dorsal to the ventral sucker; along the line of the lateral nerve cords; and finally around the anterior end of the body, where they appear most numerous. They invariably lie close to underlying nerves.

Each nerve bulb is rounded and flask-shaped, up to $1.5\mu\text{m}$ across and derived from a single nerve cell, (Figs. 122-125). Unlike both types of unciliated receptor, ciliated cavities lie largely beneath the tegument, subtegumentary fibrous zone and, indeed, the circular body wall myofibres as well.

The 'neck' of each bulb is attached to the surrounding tegument by means of a septate desmosome. Through the centre of the desmosome rings is a $0.2\mu\text{m}$ pore (approx.) opening into a cavity packed with small, dense vesicles 10nm to 50nm in diameter with a less electron dense periphery. Most are at the higher end of the size range. Projecting into this cavity from the bulb wall are five or six cilia, $200 - 250\text{nm}$ across and at least $1\mu\text{m}$ long. Peripheral ciliary microtubules are continuous with cylindrical basal bodies lying in the bulb wall, (Figs. 126, 127).

The pattern of axoneme microtubules appears to vary slightly - the maximum number recorded here is 9 peripheral pairs plus 1 central tubule, (Fig. 127, 128). It seems likely, though, that the 'full' complement of 9 + 2 is present close to the basal body. Figs. 128, 129 show a pair of cavities from the left and right sides of the anterior end of the body.

The most common cytoplasmic inclusions in these receptors are large, clear vesicles, 50-100nm in diameter, usually spherical in shape. These are sometimes also present in large numbers in the axon leading to the bulb, (Figs. 122-129).

In the tegument of the oral sucker region there were occasionally found lateral complexes of ciliated cavities and unsheathed receptors, (Figs. 130, 131). These figures show two sections through the same complex. The sheathed receptors of the apical disc also lie close to a cavity receptor (Fig. 132).

Occasionally, a multiciliated cell, resembling a cavity receptor, was found (Fig.133). However, some of its cilia projected through the pore to the exterior. Fig.134 probably shows the cilia of this type of cell adjacent to the cilium of a uniciliate receptor.

4) LAMINATED RECEPTORS These are the only type of internal receptor identified so far. Two are situated laterally, very close to the oral disc (Fig.135). The nerve bulb consists of a thin-walled, roughly oval sac. Into the central cavity protrude at least 4 (and possibly 5 or 6) laminated structures, each of which is attached to the bulb wall by a short stalk. The latter contains what could be a basal body. Distal to this, in the club-like centre of each laminated structure, are what appear to be microtubules, sectioned obliquely. Some small, dense particles lie scattered in the bulb cavity.

These four types of receptor are illustrated diagrammatically in Text Fig.5. Their location in relation to the tegument and body wall myofibres may be compared. Also included are the main types of vesicle found in cercarial nerve cells.

Acetylcholinesterase localization

The extent to which larvae were stained by the AChE technique was quite variable. A diffuse internal reaction was often seen (Figs. 136, 137) corresponding in location to central nerve ganglia and main nerve tracts but dark deposits were only regularly seen at the surface. The appearance of the reaction could, in fact, be broadly classified as either straw-coloured ('pale') or reddish-brown ('dark'). Although both were usually present on the body, only the former occurred on the tail. Specific surface locations often appearing dark included the extreme anterior tip, the cavity of the ventral sucker and the posterior end of the body, (most larvae lost their tails during the preparation). This result was not always obtained: e.g. Fig. 138 shows a larva in which the anterior tip has stained darkly, but the posterior has not.

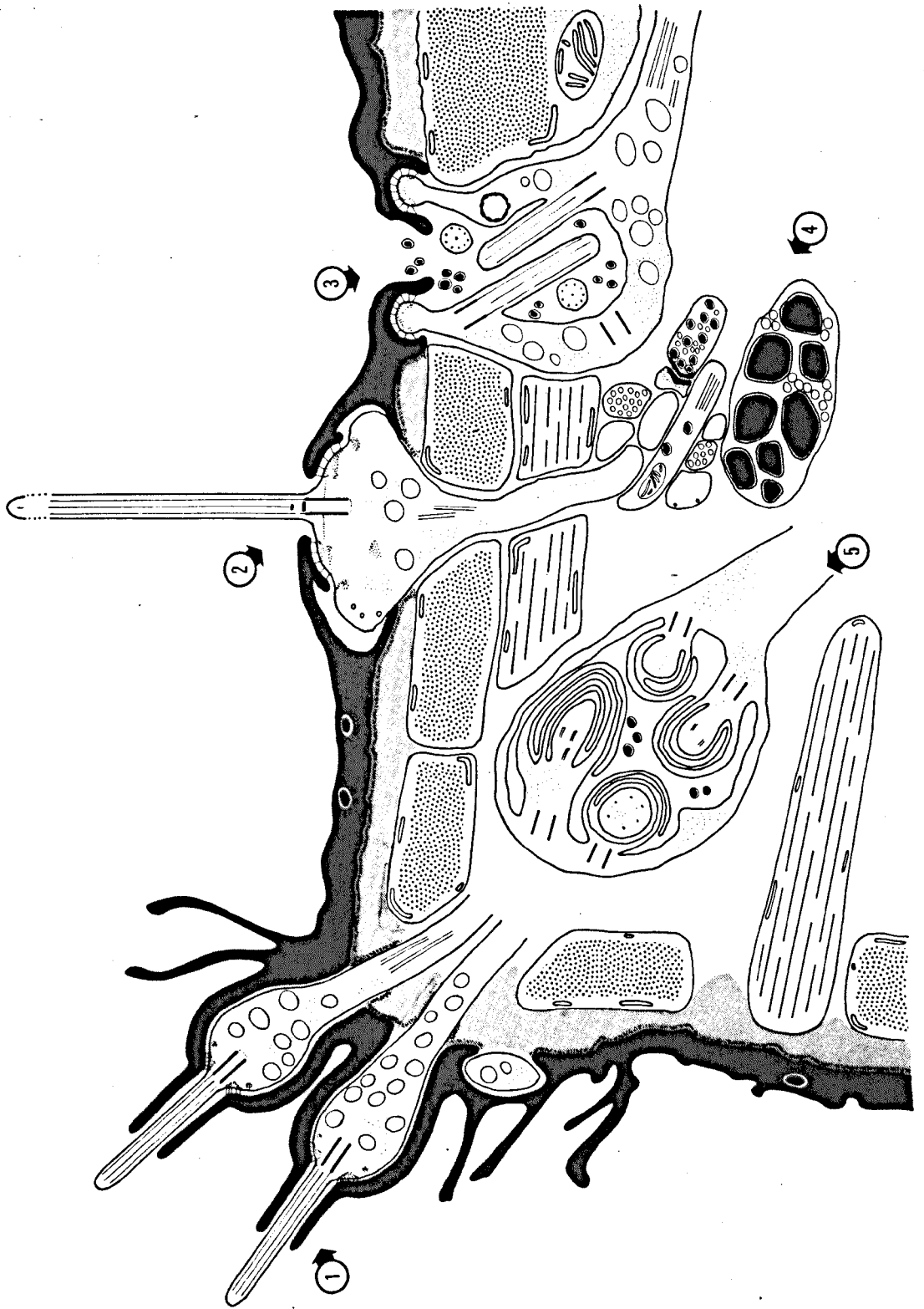
Surface reactions could often be associated with a protruding receptor (Figs. 138-140). The latter usually appeared pale, although some attracted a heavy deposit which obscured surface structure. Dark staining was often obtained adjacent to and beneath receptors, particularly in certain specific locations.

In order to identify these surface locations clearly I shall use the nomenclature of Short and Cartrett (1973) which is based on AgNO_3 staining of surface receptors. Text Fig.6 is based on their map of receptor locations and is included to clarify the following description. This is a list of the locations which, in many specimens, I have found

TEXT FIG.5

Diagram to show the four main types of receptor found in cercariae.

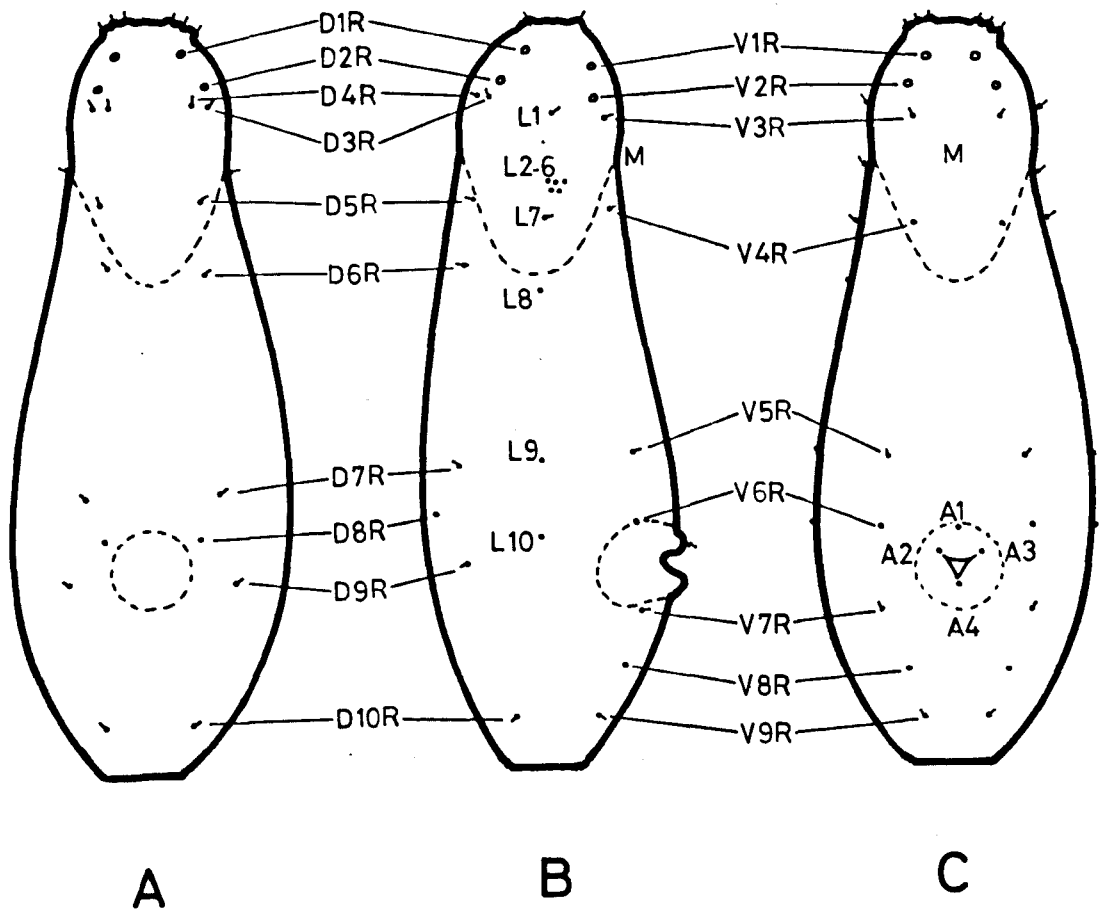
1. Sheathed, uniciliate receptor, only found at the anterior tip of the body adjacent to the openings of the gland ducts.
2. Unsheathed, uniciliate receptor. The most common type, found scattered over body, tail stem and furcae.
3. Ciliated cavity receptor. Relatively small number, only found on body surface.
4. Group of nerve axons, containing the types of vesicle described in the text.
5. Laminated receptor. So far, only found near the anterior end of the body. An *internal* receptor with no direct contact with the exterior.



TEXT FIG.6

Map of receptor locations on cercarial body. Outlines of oral and ventral sucker are included. Redrawn from Short and Cartrett (1973).

- A. Dorsal view
- B. Lateral view
- C. Ventral view



to be heavily stained:

- (a) L2 - L7 (The "neck-groups" of Short and Cartrett). This is most clearly seen in Fig.139. The left hand group appears to have stained more strongly than the right. Separate receptors are seen protruding on both sides.
- (b) D6. Seen in Figs. 138, 140, this receptor was sometimes located slightly posterior to the standard position mapped by Short and Cartrett.
- (c) D7 - D9. The most consistent of the heavily staining regions, (Figs. 140-142); sometimes it appeared as one large unit, but a single receptor could occasionally be distinguished separately.
- (d) D10 and V8-V9. A more variable response, one or both of these areas staining darkly in some specimens (Fig.143) and lightly in others (Fig.144). The distribution of active sites near to the ventral sucker was too varied for a definitive map to be prepared. Compare, for example, the patterns lateral to the sucker in Figs. 144, 145. Fig. 144 is a ventro-lateral view of the body and it can be seen that the pattern of activity differs on each side of the sucker. A negative result which may, nevertheless, be significant is that heavy staining was not normally found along the mid-lateral line, (L8, 9, 10).

The protruding ventral sucker of several specimens demonstrated clearly the presence of receptors on this organ, (Figs. 141, 146). Details of the anterior tip of the body were never clear, though receptors may just be visible in profile in Fig. 139.

In some specimens, the number of active sites on a particular area of the body surface was less than might be expected from the 'standard' map. In others, supernumary (or possibly 'misplaced') sites were found. These

are seen ventrally (Fig. 138) between V4 - V5 and dorsally (Fig.142) between D6 - D7.

Pale, highlighted points were found scattered along the length of the dorsal and ventral sides of the tail stem and on the posterior face of the furcae; the extent of the deposit per location varied to some degree. The tips of the furcae were nearly always stained but, surprisingly, the base of the (shed) tails only rarely so. The total number of active sites on each tail was fairly constant but differences in the details of their location were usual. See Figs. 147-152.

DISCUSSION

A composite description of the cercarial nervous system must be based on the results of a number of different techniques, for example AgNO₃ staining and the ACHE localization at the light microscope level, as well as electron microscopy. The aim of this discussion is to correlate the results of these techniques in order to present such a composite picture. Surprisingly, this has only recently become possible for *Schistosoma mansoni* and is largely due to the development of suitable histochemical techniques, to ultrastructural studies, and to a systematic study of the distribution of surface receptors.

It is presumed that part of the cercarial surface (probably the neutral polysaccharide coat, Stirewalt and Walters, 1973) is relatively impermeable to the substrates used for ACHE localization. Incubation of untreated cercariae may give some indication of centrally placed nerve tissue but no detail of nerve cords is visible (Lewert and Hopkins, 1965; Fripp, 1967). Bruckner and Voge (1974) injected cercariae into the peritoneal cavity of mice and incubated them, on recovery, 3 hours later; this process led to the shedding of the surface coat. The following account of the general layout of nerve tissue is based largely on their results.

A bilateral nerve mass occurs near the centre of the body linked by a transverse commissure. This is continuous with thick longitudinal cords (their medial trunks) extending posteriorly to join near the tail-body junction. These expand to form a complex near the ventral sucker. There is also a more laterally placed longitudinal cord extending for most of the length of the body (their lateral trunks). Anterior ganglia occurred within the oral sucker, associated with both longitudinal lateral cords and transverse elements. Overlying these tracts is a subtegumental network of regularly-spaced circular and

longitudinal nerves.

It has not, however, been possible to corroborate this account, (see comments on p. 38). In addition, transverse sections from the central body region appear to contain six longitudinal nerve cords, not four as suggested by Bruckner and Voge; the dorsal pair were not identified by the ACHE technique. It may be that the narrow dorsal cords do not extend very far from the central nerve ring. Rees (1967) illustrated the ventral and lateral cords of *Parorchis acanthus* extending to the posterior end of the body with a dorsal pair not distinguishable as far back as the ventral sucker. Bruckner and Voge (1974) did not suggest any explanation for the numerous 'dense spots' associated with their nerve net (see their Fig.17). Larger reactive regions on the surface were associated with tegumentary "papillae".

The central nervous system is seen by transmission electron microscopy (this report) to consist of a peripheral layer of cell bodies enclosing a central fibrous core. This type of distribution was also noted by Rees (1967) in *Parorchis*.

Some caudal esterase activity was noted by Fripp (1967) and it has been shown that dorsal and ventral median nerve cords extend the length of the tail stem beneath the sub-tegmental muscle layer, (Nuttman 1971, 1974, this report). These are joined at the tail base by a dorso-ventrally oriented commissure. The tail base nerve mass contains a small number of cell nuclei and is largely axonal in content. Nerves have been traced linking surface receptors to these nerve cords.

The bilaterally symmetrical nervous system of the cercarial body appears to conform to an expected pattern. The apparently central rather than anterior site of the major nerve mass and commissure is accounted for by the extensive development of the oral sucker region in this species.

The particularly heavy ACHE response in the ventral sucker region requires explanation. It could be that the active crawling behaviour characteristic of stimulated cercariae (see Sections 2, 3) makes necessary a co-ordinating complex at this location. On the other hand, not only are the movements of the oral sucker region far more complex than those of the ventral sucker but the anterior sensory apparatus is also more extensive and varied. Despite this apparently greater sensory and motor requirement, the anterior ganglia appear less extensive than the posterior. Perhaps the balance is redressed by anterior functions being coordinated, in part, by the main central ganglia; the presence of a prominent nerve cord between the latter and the oral sucker would suggest this. In addition, the posterior ganglion may also play a part in the initiation or coordination of tail movement. Ablation studies, although perhaps crude by the standards of the electrophysiologist, could provide some of the answers to these questions.

Such an experiment was initiated during this investigation, but due to technical problems was not developed. The apparatus used was a Hadron 513 Biolaser with a theoretical minimum beam diameter in $1\mu\text{m}$. It was generously loaned for a trial period by the company. Unfortunately, the equipment proved faulty and a replacement was not readily available. This, or a comparable experimental arrangement could at some time form the basis of the above ablation work.

Transmission electron microscopy has supplied most of the fine structural detail of nerve cells, with some additional information available in Stereoscan micrographs. Communication between cells occurs at synapses which are identified by their characteristic accumulations of axonal vesicles and the dense staining properties of the apposed membranes. Synapses occur not only in the main ganglia but also in the nerve cords. (Perhaps the small dense spots visible in Bruckner

and Voge's preparations could be synaptic groups). The distinctive synapses described here are similar to those of many other animal species. It should not be assumed, however, that they represent the only points of information flow between cells. Other less distinctive junctions may be present. One such category could be the cercarial neuromuscular junction; although on many occasions axons have been found to lie very close to a sarcolemmal membrane, no electron dense regions were seen and there was no involution of the sarcolemma as occurs in vertebrate junctions. Junctions were not found by Dixon and Mercer (1965) in *Fasciola hepatica*, but in fact are reported in *Schistosoma mansoni* by Robson (pers. comm.).

The two types of vesicle associated with synapses also occur elsewhere in nerve cells but at a lower density. This would be consistent with the theory that some, at least, are manufactured in the nerve cell body and then transported to axon terminals. Vesicles appear to correspond to the type 'a' and 'b' vesicles of Dixon and Mercer (1965). The small, dense vesicles found here within cavity receptors are very similar to those found elsewhere in nerve cells, but are on average slightly smaller. This resemblance may be deceptive, however, as there is no biochemical or other type of evidence to link the two. Dixon and Mercer (1965) proposed that their type 'd' vesicles were neurosecretory. It is interesting to note their comment that "Cells containing typical granules have been noted in sites apparently not within the cerebral ganglia and which we could not identify as nervous". This type might correspond to the large, dense vesicles described here, as it was not always easy to associate them with nerve tissue. Robson (pers. comm.) has said that he considered the large dense vesicles to be contained within muscle cells. The fourth type of vesicle identified in cercarial nerves is the large, clear vesicle. These are particularly common in cavity receptors and in some unciliated receptors. The clear vesicles seen in

the receptors of other species probably have a similar function, but it is not yet known what this function may be. From comparison with other animal species, the small clear vesicles are assumed to contain a neurotransmitter substance which is released into the synaptic clefts during depolarization of the presynaptic membrane. The small dense vesicles have been interpreted as containing catecholamines.

In the morphologically less-specialized axons, other intra-cellular components include microtubules and occasional mitochondria in the clear axoplasm. The cell bodies contain relatively little granular cytoplasm and their nuclei stain slightly less-strongly than muscle nuclei. The difficulty in reconciling the transmission EM results with those of Brucker and Vogt (1974) may be due partially to the difficulty in identifying axons which may contain few if any of the above features in individual sections.

A number of types of specialized nerve endings are identifiable by electron microscopy. Three types were described by Nuttman (1971) and a fourth may be added. These are uniciliate unsheathed, uniciliate sheathed, ciliated cavities, and internal laminated receptors. The unsheathed uniciliate type was the most common and occurred on both body and tail. The multiciliate types, however, were found only on the body. It is possible that a fifth type could eventually be added to the list, as Short and Cartrett (1973) found an elevated non-ciliated structure in the neck region of *Schistosoma mansoni* cercariae. These were identified by scanning electron microscopy only, there being no other information available.

Uniciliated nerve bulbs are the most common receptor structures in a number of cercarial species. Most authors consider their most likely function to be mechanoreception, e.g. Dixon and Mercer (1975), Chapman and Wilson (1970). It may be premature, however, to label all cells of this

general appearance as mechanoreceptors. Laverack (1968) has described an unsheathed, uniciliate, chemoreceptive cell on the sensory hillock of gemmiform pedicellariae in *Echinus*. Apart from modification of the basal body-rootlet system it appeared identical to nearby mechanoreceptors. The presence of a projecting cilium is not a prerequisite in chemoreceptive cells, the Pacinian corpuscle nerve in vertebrates being an obvious illustration of this. In *Helix* statocysts, sensory cells have basal bodies but no ciliary shafts; it may be that the basal body area is more important in transduction of the stimulus than the ciliary shaft - the latter perhaps functions as an amplifier rather than a transducer. Further evidence implicating the basal body system in transduction was provided by Tucker (1967) who removed 99% of the cilia from the olfactory bulb cells of the box turtle by a process which left the basal bodies intact and the cells still chemoreceptive. Even if the basal body does function in this case as a transducer, this cannot be a universal feature. The olfactory bipolar cells in elasmobranchs possess neither ciliary shafts nor basal bodies, (Reese and Brightman, 1970).

If ciliated cavities act as mechanoreceptors, then it must be in a different way: they might be 'squash' receptors, responding to general compression of the surrounding tissue. There is apparently direct continuity between the cavity and the exterior, however, so the possibility of a chemoreceptive function cannot be ruled out. The results of behavioural experiments (e.g. MacInnis, 1969; Clegg, 1969; this report) strongly suggest that cercariae are able to distinguish between different chemical stimuli. The internal, laminated structure could not function in this way, however. The 'squash' reception function might apply or, more speculatively, photoreception. The latter possibility is mentioned in view of the marked response of cercariae to a sudden reduction in light intensity.

The technique of AgNO_3 staining has been applied by a number of authors to plot the distribution of surface receptors; the most comprehensive account to date is that of Short and Cartrett (1973). Bruckner and Voge (1974) remarked that surface structures stained for ACHE but did not attempt to map their distribution. My results correlate well with Short and Cartrett's map, occasional variance being accounted for by the natural population variability for this feature. The nomenclature of Short and Cartrett is used here for denoting receptor location.

The most common pattern included 76 body receptors and 50 caudal receptors, all of the latter being of the unciliated type. On the body, receptors are usually situated symmetrically about the mid-line and occur on both suckers. There are two groups of up to seven sheathed receptors at the anterior tip, each group arranged in a crescent, close to the openings of the gland ducts. Up to 22 of the body surface receptors may be ciliated cavities: Morris (1971) found at least two at the anterior end, an observation which was confirmed here. Short and Cartrett (1973) considered that their AgNO_3 - reactive sites D1, 2 and V1, 2 corresponded to the same structures. These authors found that "non-ciliated papillae" occurred in locations V6, 8, D8, L6, 8, 9, 10 and thought that they might also be cavities. They were, however, unable to find any trace of L8, 9, 10 by scanning EM. This study has confirmed that cavities do occur laterally in (or near to) the L9-10 region, at L8 and L6, as well as dorsally at D8 and ventrally at V8. In view of this degree of correlation it seems probable that the non-ciliated AgNO_3 - reactive sites in the cercarial tegument are, indeed, ciliated cavities.

The sensory complex described here is probably the "neck group" of Short and Cartrett (1974) and a mixture of receptor types is clearly visible in electron micrographs. The latter authors found that some of the neck

group cilia were considerably shorter than cilia found elsewhere. It is possible that their "short cilia" are in fact the tips of cilia projecting from cavities, as described in this Section.

At one time it appeared to be generally accepted that parasitic organisms were morphologically and functionally regressive in comparison with related 'free-living' species and this appraisal was often specifically related to neural and locomotor characteristics. This appears somewhat surprising if an objective view of parasitism is taken and especially if those species with complex life cycles are considered. In general terms, larval forms serve a number of basically important functions: for example, dispersion, maturation and location of the adult environment. Parasite larvae can frequently be associated directly with these functions, with the addition in some groups of asexual reproduction. The observed behaviour of trematode larvae is reviewed in Section 3. Their responses have often implied the existence of receptors adapted to different sensory modes and they (collectively) possess the coordinating and effector systems for a variety of locomotory techniques, both ciliary and muscular. Similarly, many adult parasites appear to have a 'preferred site' in their host, but the mechanisms of site location in endoparasites are largely unknown. Active site selection by the migrating parasite, on the basis of chemical and mechanical information is, however, often the unavoidable conclusion.

The platyhelminths as a group may be used to illustrate a number of important steps in animal evolution; the development of locomotory technique is an example. Miracidia and oncomiracidia swim in much the same manner as the ciliate protozoa (except that the trematodes cannot reverse), muscular activity playing no part in providing propulsive thrust. Turbellarians use both cilia and (slow) muscles; the cestodes and post-miracidial stages of trematodes use muscle alone, but body movements are, again, relatively slow. Many cercariae, however,

possess a prominent, muscular, locomotory organ, the tail, which in some species contains a type of striated or 'fast' muscle. The cercaria of *Schistosoma mansoni* falls into this latter category.

In principle the body wall musculature of worm-like animals could consist of even randomly-oriented fibres. This system would be capable of the same changes in shape as one in which fibres were oriented in circular and longitudinal layers only. The random system would, however, be very uneconomic, especially in terms of the complexity of nervous coordination required. The main advantage of a circular/longitudinal plan is that coordination of activity can be accomplished with a relatively simpler nervous system. That trematodes basically conform to this very common muscle plan has been long established. In addition, some stages in the life cycle may possess regionally differentiated muscle systems, the oral and ventral suckers, and these may be modified to perform different functions. The *Schistosoma mansoni* cercaria has an oral sucker containing a number of different types of tissue and has been termed an 'anterior organ' by some authors, reflecting its size and complexity. The ventral sucker in this species is smaller, almost entirely muscular and has the primary function of adhesion. The range of locomotory movements exhibited are described more fully in Section 2.

Although the gross morphology of the main cercarial effector systems was determined many years ago, knowledge of cytological detail did not improve significantly until the advent of electron microscopy. The demonstration of myofilament systems in vertebrate striated muscle (by electron microscopy and X-ray diffraction) explaining the periodicity observed by light microscopy, led inevitably to the development of new theories of contraction - notably the sliding filament theory of Huxley and his group (see Huxley, 1957; Huxley, 1972). Most authors

have subsequently tried to fit observed muscle structure in vertebrates and invertebrates to this scheme. Opinion has been divided, however, regarding the types and numbers of myofilaments in vertebrate 'smooth' fibres, the issue mainly in dispute being the disposition of myosin. Shoenberg (1969a) for example, was unable to demonstrate thick filaments resembling myosin in guinea pig *Taenia coli* muscle, but such filaments were found in fresh homogenates of smooth muscle in the presence of Ca^{++} , Mg^{++} and ATP (Shoenberg, 1969b). Cook and Fay (1972) suggested that conditions supporting normal contractile activity might be necessary for thick filaments to be demonstrated in large numbers. (The varied density distribution of these filaments is still to be explained, though).

The observed structure of vertebrate smooth muscle cannot be applied generally to other animal groups. There is, rather, a spectrum of morphological types, of which vertebrate smooth muscle is a part. Muscle diversity is also reflected in the recorded time constants: contraction times may vary 50-fold and relaxation times 10,000-fold, (Prosser, 1967). It is interesting to note, however, that the range of maximum force is only 30-fold. Prosser (1967) presented a classification which included cercarial muscle in an "intermediate category". In this, striated muscle is formed on one side, or in one part, of the fibre, while a distinct sarcoplasmic region contains a nucleus, mitochondria etc. In all of the ultrastructural studies of platyhelminths reviewed in this section, 'smooth' myofibres were clearly seen to consist of two sizes of filament, the total range of diameters being relatively uniform at 5 - 7nm for thin filaments but varying from 18-54nm for thick filaments. My results suggest that the thin filaments of cercarial muscle are continuous with areas of highly electron-dense material, here referred to as dense bodies. In non-striated cercarial muscle these may be situated centrally in the fibre, surrounded by filaments, or at the

fibre edge, attached to the sarcolemma. In the latter case they sometimes appear as highly localized structures and groups of closely adjacent dense bodies are sometimes seen. This is similar to the situation in adult schistosomes (Silk and Spence, 1969b). Dense bodies may also have the form of transverse bars, extending from the sarcolemma into the filament mass, roughly perpendicular to the fibre axis. MacRae (1963, 1965) found a very similar pattern in marine and fresh water turbellaria. She speculated that dense bodies may have originated by local differentiation of the sarcolemma, those in the centre of the fibre having detached during the course of fibre evolution. Transverse bars are particularly prominent in the vertical muscles of the 'head' of *Dugesia dorotocephala* (Morita, 1965) and at their attachment to the sarcolemma are apposed by fibrous material in the external connective tissue. A system of transverse bars, to which thin filaments were attached, was described in the barnacle, *Balanus nutilus*, by Hoyle and McAlear (1963). In rabbit arteriole smooth muscle cells, dense bodies form bar-like structures regularly distributed and projecting inwards from the sarcolemma (Rhodin, 1967).

Both Morita (1965) and MacRae (1965) compared the dense bodies of invertebrate fibres with the Z-discs of vertebrate fibres and Needham and Shoenberg (1967) have suggested a functional correlation between the dense bodies of vertebrate smooth muscle and the Z-disc. The superficial resemblance between dense body structure and that of desmosomes may have a definite basis. Franzini-Armstrong and Porter (1964) thought that dense bodies might have evolved from a type of desmosome. It is generally accepted that desmosomes have a mechanical function, providing strong points of attachment between adjacent cell membranes and so from this point of view would provide suitable anchorage points for elements of a contractile system.

The length of thin filaments in cercarial striated muscle has not been determined. No pale bands are visible in the central region of sarcomeres which might suggest discontinuity of the thin filaments (as in vertebrate striated muscle) but attachment of both ends of thin filaments to the dense rods would rule out a simple sliding filament theory of contraction; the thin filaments would have to shorten during contraction, perhaps by coiling. There is a strong case for the preparation of very thin sections of this tissue to establish more precisely the nature and extent of thin filaments. This could be carried out on muscle fixed under different conditions, e.g. stretched, relaxed, contracted and the different filament patterns compared.

Normally, no direct contact between adjacent dense bodies is seen (MacRae, 1965) but a degree of continuity is sometimes found in cercarial muscle, giving a branched appearance in transverse sections. This is very similar to the "branched bands" of some cestode muscle (Lumsden and Byram, 1967).

The sarcoplasmic reticulum of most non-striated muscle (including that of cercariae) is relatively sparse. This contrasts with the extensive system of cercarial striated fibres. It is probable that the functions of a transverse tubule (T) system are served by the transverse sarcoplasmic (S) tubules described in this report. No true T-system has yet been found in platyhelminth muscle. In the cercarial system, excitation of the plasmalemma would influence the reticulum first of all at the distal ends of the S-tubules which in turn traverse the contractile region. The problem of initiating the simultaneous contraction of a packed group of myofibrils does not exist in cercariae; each muscle cell may be considered as one fibre containing one fibril. The longitudinal myofibres in the cercarial tail normally occur in layers only one cell thick - i.e. there is no packing in depth. Providing

that this simple system exists, together with an S-tubule system, there may be no significant advantage in possessing a true T-system, continuous with the sarcolemma. It seems, however, that for more than a few oscillations per second to occur, an S-tubule system must be present: this is presumably related to the efficient transfer of Ca^{++} between the sarcoplasmic reticulum and filament area during excitation-contraction coupling. Although cercarial myofilaments normally lie parallel to the fibre axis and perpendicular to the dense rods (when the latter are present) this is not the case in all animal groups. Hirumi et al (1971) described a type of nematode fibre in which the filaments were oriented in many directions within each cell and were attached to plate-like dense bodies, the "Z-plates".

The longitudinal tail fibres of *Schistosoma mansoni* cercariae are not aligned parallel to the long axis of the tail but together form what is best described as a 'herringbone' pattern. It is not yet possible to correlate the occurrence of this type of fibre pattern with swimming technique in other species; there are too few published studies giving sufficient information either concerning muscle structure or swimming technique.

Examples of the close apposition of myofibre membranes and vesicle-packed nerves have been found in *Schistosoma* and in other platyhelminths, but special adaptations such as involuted post-junctional membranes (as in vertebrate neuromuscular junctions) have not been described.

Evidence has been obtained which suggests that acetylcholine (ACH) is present in schistosome adults as an inhibitory neurotransmitter, while 5-hydroxytryptamine (5HT) may be stimulatory. Histochemical techniques were used by Bennet and Bueding (1971) to demonstrate the distribution of 5HT and catecholamines (CA) in schistosomes. CA was found in the central ganglia, commissure and main nerve cords. From the latter arose similarly-reacting nerves which anastomosed to form an extensive network

throughout the body. The distribution was very similar to that previously shown for acetylcholinesterase (ACHE) by Bueding et al (1967). However, as it is generally supposed that individual nerve cells produce predominantly (if not exclusively) one transmitter substance (e.g. ACH or 5HT), the cells visualized by the different techniques may not be the same. ACHE localization was also recently achieved in schistosome cercariae by Bruckner and Voge (1974) but a finer resolution of nerve fibres was claimed (distance between regularly-disposed fibres as little as 1.5 μ m). Bennett and Bueding (1971) found 5HT localized in structures near the central ganglia and commissure and in widely-distributed 'granules'. In the gynecophoric canal, reactive surface structures, connected to underlying fibres, were thought to be the ciliated receptors described in EM studies (e.g. Morris and Threadgold, 1967; Smith et al, 1969a). Considerable depletion of the 5HT 'stores' was achieved by incubation with reserpine.

It is likely that the adult transmitters will also be present in cercariae. In view of the potential for significantly depleting the accumulations of these compounds (e.g. 5HT above) these techniques might usefully be combined with an EM survey. The frequency and appearance of the various nerve cell vesicles could then be compared after different treatments. It seems possible, for example, that the clusters of large dense vesicles could be the 5HT stores of Bennett and Bueding but, as yet, there is no positive evidence as to the function of these structures. There is evidence that not only are 5HT, ACH and noradrenaline transmitters in the vertebrate central nervous system but that other compounds such as dopamine, glycine, γ - aminobutyric acid and glutamic acid are as well.

Dopamine, 5HT, ACH and noradrenaline have all been found in planarian nervous systems (Welsh and King, 1970). It is therefore possible that

at least some of these other compounds might eventually be shown to operate in helminths as well.

The small clear and small dense vesicles described here in cercarial axons may contain these transmitters. However, if it is assumed that these two types of vesicle contain different transmitters, then their presence in the same axons (demonstrated here) is inconsistent with the theory that each nerve cell can only manufacture one type of transmitter. Therefore, either the theory does not apply to these organisms or, alternatively, one or other vesicle type does not contain a transmitter.

There is considerable scope, then, for future studies in correlating histochemical and ultrastructural information so that a more complete understanding of trematode nerve function may be developed. Until recently, there has been little or no experimental backing for the assumptions made about the functions of the nerve cell inclusions discussed. This lack of experimental evidence is also prominent in studies of trematode sense organs.

The small size of these organs, together with the difficulty in locating them accurately in live animals, makes direct recording of electrical activity extremely difficult. There are no reports, to date, of direct recording from trematode receptors and hence no direct evidence as to their function. Function therefore has to be implied from ultrastructural and behavioural data alone. A first step in this field might consist of recordings taken from central ganglion cells or main nerve cords, the positions of which are now known with some certainty.

The basic layout of platyhelminth nervous systems has been established for some considerable time (e.g. see Micoletzky, 1907; Tower, 1900; Looss 1894). Some of the more primitive systems in the phylum are found in certain acoel and allocoel turbellarians (Meglitsch, 1972) and consist primarily of a fine subepidermal plexus, which is more

concentrated anteriorly, near the statocyst. Although the subepidermal plexus may be retained in the more advanced turbellaria, the main nerve layer becomes a sub-muscular plexus. An anterior pair of ganglia becomes associated with a pair of ventral, longitudinal nerve trunks. Serially arranged ring commissures develop between the trunks. A prominent central nervous system has been described in a number of trematode and cestode species, basically following this plan.

A general evolutionary pattern is, therefore, established in the platyhelminths (including the parasitic species) which is retained in the nervous system of the higher invertebrates of the annelid-arthropod line : this includes progressive cephalization; more varied and more numerous receptors; higher degree of integration of developing muscle systems and of sensory input.

There are two main fields of study which have, in recent years, helped to dispel the notion of parasites having a very limited sensory capability. The *behaviour* of parasites has implied the existence of a wide range of receptors and *ultrastructural* studies have produced an extensive catalogue of cells and organs. The most common type of receptor in trematodes appears to consist of an expansion of a nerve axon terminal, the nerve bulb, from which a cilium projects to the exterior. Most authors consider that these uniciliate cells respond primarily to mechanical stimuli and I would concur that some at least of the cercarial cells probably act in this way. The observation that unstimulated cercariae immediately cease swimming movements when the furcae strike the surface film (see Section 2) suggests that receptors might exist at the distal end of the tail. This stimulus could then be fed back negatively to the tail muscle stimulating system. A line of 5 - 6 uniciliate receptors are indeed present along the posterior surface of the furcae. Their cilia project about 8µm or more beyond

the tegument and so must be subjected to considerable lateral force during oscillation of the tail. When the furcae strike the surface film, this force must increase suddenly. Morris and Threadgold (1967) speculated that the uniciliate cells of schistosome adults detect the direction of flow of a fluid medium, although no polarization of nerve cell structure is visible at the electron microscope level.

Directionality of mechanoreceptors may also be related to modifications of accessory structures: this is seen, for example, in the hair plate sensillum of the honey bee where a radially symmetrical nerve cell is associated with an asymmetrical joint at the base of the hair, (Thurm, 1965). There is, however, no clear evidence of asymmetry in the tegumental sheaths surrounding trematode receptors. There still remains the possibility that an asymmetrical basal body structure might function in this way.

Compound uniciliate receptors of the type described by Lyons (1969b, 1972) were not found in *Schistosoma*, but the "neck group" has been shown to consist of both uniciliate and multiciliate nerves. The structure of multiciliate cells depends upon both species and larval stage. Whereas in schistosome cercariae the cilia project into a cavity with a small pore to the exterior, in *Fasciola hepatica* miracidia and *Entobdella* oncomiracidia they project from the floor of an open pit. A ciliated cavity very similar in appearance to that in *Schistosoma* is present in the rhinophore of *Nautilus*, (Barber and Wright, 1969a) and it seems probable that its function is chemosensory. Ciliated cavities have not yet been recorded in adult schistosomes and it is possible that they disappear during maturation. However, there has been no systematic search for them in adults and, in view of their relatively small number, it is still possible they remain in the adult. They are certainly present, for a short time at least, in the schistosomulum (Bruce et al, 1970; Robson, pers. comm.). Neither is it known whether the other

ciliated nerve cells of the cercaria develop into the known adult receptors or whether the cercarial receptors are lost and new ones developed during maturation. There is, in fact, very little published information available on the ultrastructure of development in juvenile trematodes (with the possible exception of tegumentary structure).

Michaels (1969) has provided a strong indication that linearly arranged receptors involved in mating are present in adult schistosomes. There is a case for a preliminary histochemical study to locate these receptors, to be followed by the preparation of thin sections of the specific regions so labelled.

The oncomiracidium of *Entobdella soleae* possesses a pair of large, complex cells which are presumed to be sensory (Lyons, 1972). They contained the elements of at least 18 cilia and a group of lamellated structures each of which measured about 1µm in diameter and consisted of up to 13 layers. Lyons considered their probable function to be photoreception. Similar structures are found in the ciliary eyes of certain molluscs, e.g. *Cardium edule* (Barber and Wright, 1969b) in the feeding tentacle eyes of the annelid *Branchionma vesiculosum* (Krasne and Lawrence, 1966) and in the ctenophore *Pleurobrachia pileus* (Horridge, 1964). Photoreceptive cells characteristically possess an enlarged membrane surface area, in the form of surface folds or internal lamellae etc., and this is thought to support an array of photolabile molecules. It seems possible that the internal lamellated cell of schistosome cercariae could also be a photoreceptor. It is much simpler, however, than some of the lamellated structures reviewed above; perhaps it represents a 'primitive' type from which the other, more complex, structures have evolved?

No other structure has yet been found in *Schistosoma* cercariae which might account for their rapid swimming response to a reduction in light

intensity, i.e. a 'shadow response'. Ciliary eyes in molluscs, producing "off" responses to light were described by Land (1968) and Barber and Wright (1969b). Lyons has suggested that, at least in *Entobdella*, these ciliary organs are more likely to mediate a photoperiodic response, such as diurnal hatching, than a shadow response.

I have not described, or reviewed in detail, the pigmented eyes of trematodes as there is no direct basis for comparison with *Schistosoma*. It should be noted, however, that some species possess both pigmented eyes and lamellated cells and so their relative importance (if indeed they do respond to the same stimulus mode) should be considered when more experimental evidence is available.

In many accounts of sense organ structure, the complement of ciliary microtubules has not been recorded, but a 'full' complement of 9 + 2 is present in some, e.g. compound receptors of *Entobdella*, uniciliate receptors of schistosome cercariae. Mechanoreceptive cilia with the 9 + 2 pattern are present in the ctenophore *Leucothea multicornis* (Horridge, 1965) and in the honey bee hair plate sensillum (Thurm, 1965), but other patterns may be present in mechanoreceptors. Chemoreceptors seem to be somewhat more variable in form: olfactory cilia in the frog (Reese, 1965) contain a 9 + 2 pattern proximally but most of the cilium displays a modified pattern, including 9(single) + 2, 8(single) + 2, 8 + 1. In *Schistosoma* the maximum number found in the cilia of ciliated cavities was 9 + 1 but it was often less than this. Another feature common to the schistosome cavity receptors and to chemoreceptors in other species is the variance from a circular cross-section of cilia. Motile cilia are normally circular, as are most mechanoreceptors. This again implies a chemoreceptive rather than mechanoreceptive function in cavity receptors. A recent review of the structure of cilia in sense organs is that of Barber (1974).

Morphological studies of both nervous and muscular systems in cercariae have benefited from the collation of results from the various technical approaches described, e.g. light microscopy and classical staining, AgNO₃ staining, various histochemical techniques; transmission and scanning electron microscopy; behavioural studies (see Section 3).

There is still much speculation concerning the *functioning* of various parts of the system, but it remains to be seen whether the technical difficulties of electrophysiology or ablation can be overcome in the near future.

SECTION 2 : PROPULSION

2.1 INTRODUCTION TO SECTION 2

There are many references in the literature to methods of propulsion in cercariae, but most are only superficial observations made either as part of a more general study of the species involved or of a different aspect of cercarial behaviour, e.g. Cort (1919), Miller (1928), Price (1931), Bevelander (1933).

The diversity of form and habits of cercariae would suggest similarly diverse locomotory technique. The cercarial tail, often containing conspicuous musculature is a characteristic feature but may be of very different form in different species. Cable (1965) and Erasmus (1972) have commented on this diversity and warned against a taxonomy based on tail morphology alone. In some species, the tail is considerably reduced and does not play a major part in locomotion; see, for example, some of the illustrations of Wesenberg-Lund (1934). In others, a complex three-dimensional waveform is described by a long, muscular tail. Analyses of these movements have been presented by Neuhaus (1953a), Graefe et al (1967), Rees (1971), Graefe and Burkert (1972) and Chapman and Wilson (1973).

Graefe et al studied the swimming movements of *Schistosoma mansoni* by high-speed cinematography, (500-800 frames per second). Cercariae were found to swim either tail-first with the furcae spread out laterally, or body-first with the furcae close together and in line with the tail stem. During this latter mode, the body was seen to lengthen by 30-40% and the tail-body junction appeared relatively inflexible. Waves of muscular contraction in a lateral plane brought about a typical undulatory type of locomotion. Tail-first swimming was more complex; the body and tail oscillated about two fixed points, or nodes, one in the region of the ventral sucker, the other near the distal

end of the tail stem. Furcae were held roughly at right angles to the distal tail axis during the entire movement cycle and the authors interpreted this as implying a 'rowing' action which supplied the main locomotory thrust. Both swimming modes were described in terms of two-dimensional movements only.

Rees (1971) described the locomotory movements of *Parorchis acanthus* which swam body foremost by means of ventral flexures of the body and tail. Apparently the upward, recovery stroke did not produce any significant thrust, making the overall swimming movement appear spasmodic. One complete movement took about a third of a second at 22°C. Cercariae sometimes shed their tails prematurely and, when this happened, neither the body nor the tail segments were capable of independent swimming. The author attributed this to a loss of "balance" in the body and loss of "nerve supply" to the tail. Locomotion over a solid substratum was also seen, but the movements were not analysed. This 'creeping' or looping action, involving the use of the body suckers, has been reported for many species.

The most comprehensively illustrated study of cercarial locomotion was that of Chapman and Wilson (1973). They described the tail waveform in relation to larval movement in *Himasthla secunda* and *Cryptocotyle lingua*. The former species, swimming tail first, exhibited a relatively simple, horizontal waveform, with the tail providing the thrust on its backward stroke. In *Cryptocotyle*, waves originated near the tail base and were propagated distally with little change in amplitude. The body followed a complex helical path which was described as a "three-dimensional figure of eight", the direction of body rotation being first clockwise then anticlockwise. To improve the resolution of their analysis, Chapman and Wilson cooled their cercariae to 5°C; this had the effect of reducing the rate of oscillation considerably and

hence increasing the number of film frames per cycle. In doing this they assumed that the waveform did not vary significantly with temperature.

The purpose of this section is to describe in general terms the different types of locomotory activity seen in *Schistosoma mansoni* cercariae and to illustrate and define in more detail the normal (tail-first) mode of swimming.

2.2 MATERIALS AND METHODS

A cinematographic technique was used to record the movements of cercariae. The glass vessel into which cercariae were placed was constructed of optical glass, with a central chamber approximately 0.5mm wide. The larvae were therefore always close to being in focus. The vessel could be placed upright (as in Text Fig. 7A) during lateral filming or on its side for filming from above. Surface tension forces at the narrow chamber opening prevented loss of the contained water, regardless of the orientation of the vessel.

The relative locations of the vessel, camera, light source, etc. for overhead filming are illustrated in Text Fig. 7B. The camera (Bolex) was secured to a mobile trolley on a vertical wall mounting. The necessary magnification was obtained by attaching to the camera a series of extension tubes as well as a Pizar 25mm lens. The vessel containing the larvae was placed on a glass platform attached to a micromanipulator. Once the vessel was in position, beneath the camera, coarse focus was achieved by moving the camera trolley along its track in the wall mounting. The adjustments of the micromanipulator were used for fine focussing and also for centering the vessel in the camera field of view.

The light source consisted of a 60W tungsten filament lamp encased in a metal holder, with an aperture on one side only. It was mounted on one end of a heavy, steel optical bench and the light passed through a 25mm lens such that a parallel beam was produced. An angled mirror deflected the beam upwards through the cercarial suspension to the camera. Light intensity could be adjusted with a variable resistance transformer. A heat filter was also placed across the beam to prevent progressive heating of the cercarial suspension during filming. The

Text Fig. 7

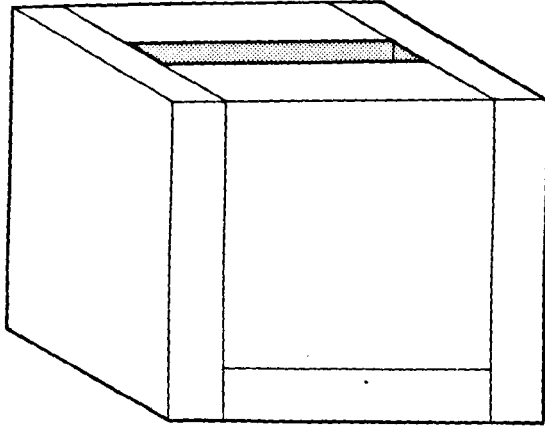
Apparatus for filming cercarial swimming movements.

- A Glass vessel into which cercarial suspension is placed. Size is approximately 1 cm. across and the walls are of optical glass.
- B Diagram to show the camera/optical bench arrangement for filming from above. The optical bench and wall mounting are shaded.

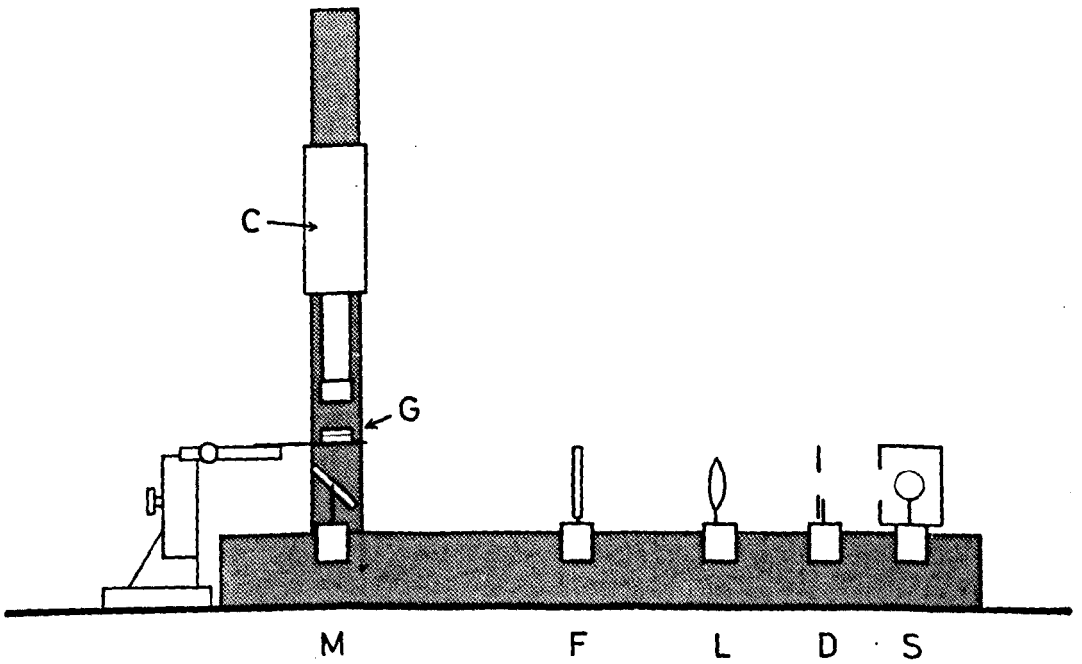
Key:

C	camera
D	diaphragm
F	heat filter
G	glass vessel
L	lens
M	mirror
S	light source

A



B



temperature inside the chamber was monitored with an electronic thermometer (Comark) fitted with a fine wire copper/constantan thermocouple. Measurements were taken before and after filming and the temperature was found to be steady at 24°C.

Other, alternative filming arrangements were found to give satisfactory results. For lateral filming the camera was mounted horizontally on the optical bench. Light ground illumination was basically as described above, but with the mirror removed, whereas dark ground illumination could be produced by placing two light sources laterally and a dark screen behind the cercarial vessel.

High magnification records of cercarial body movements (e.g. extension/withdrawal of the apical disc) were obtained by mounting the camera on a Zeiss microscope fitted with a tri-ocular head. These films were analysed by projecting individual frames onto white card and drawing in the successive images. This single frame analysis was carried out using a 'Specto' film analyzer.

The film used throughout Section 2 of the investigation was 'Kodak' Tri-X Reversal, type 7278, having an ASA rating of 400.

Because of the high rate of oscillation during swimming movements in this species, only a relatively small number of frames were included in each cycle, even at the maximum camera speed of 64 frames per second. During initial film tests, cercariae were therefore cooled; it was hoped that the rate of oscillation would be reduced, but still retain the same waveform. This technique was used with apparent success by Chapman and Wilson (1973), working with *Himasthla secunda*. Cooling of schistosomes was achieved here by placing the cercarial vessel onto a glass cooling slide. Unfortunately, at 15-18°C the oscillation of the tails appeared abnormal and further cooling led

to a complete cessation of 'fast' tail movements. It would appear, therefore, that the cooling method is not suitable for obtaining a higher resolution film record of schistosome swimming. The quantitative description of swimming movements is therefore based on higher temperature but lower resolution records, using a film speed of 64 frames/sec. It was found, however, that larvae which had been cooled to 15°C then allowed to warm up again slowly to 24°C could flex their tails fully from side to side. This was at a lower rate initially and displacement was minimal. Nevertheless, with the reservation in mind that the movements may have been altered by the treatment, an illustration of these movements is included in the following results.

Developed film was examined initially on a 'Specto' analyzer; those sections to be used in the analysis of swimming movements were marked and then printed in enlarged form. The following parameters were then recorded directly from successive prints:-

1. The angular displacement of (a) the body and (b) the distal end of the tail, from the locomotory axis.
2. The angular displacement of the furcae from a line perpendicular to the locomotory axis.

Text Fig.8 is included to clarify how these angles were measured. Appendix 8 includes some of the cine frames used.

3. The displacement of the cercaria along its locomotory axis - measured as the distance between the edge of the film frame and the body-tail junction.

The cycling values for angular deviation were examined by a lag correlation technique (computer program kindly supplied by M. Tillotson). This provided numerical confirmation of what could be seen in the original graphs in terms of the regularity of the cycling of angular

Text Fig. 8

Diagram to illustrate the way in which tail deviations etc. were measured.

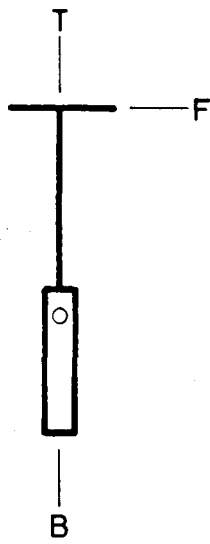
B = angular deviation of body

T = angular deviation of tail

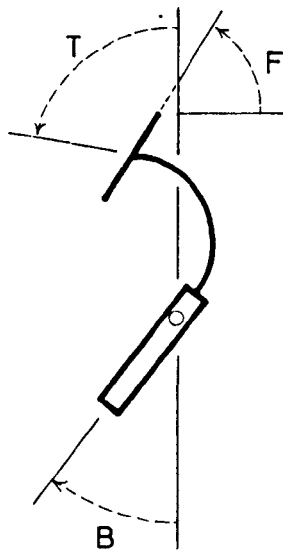
F = angular deviation of furcae

- A 'Resting' position. Tail and body vertical, furcae at right angles to main body axis.
- B Cercaria partially flexed to one side.
- C Cercaria fully flexed, so that tail stem points away from direction of locomotion.

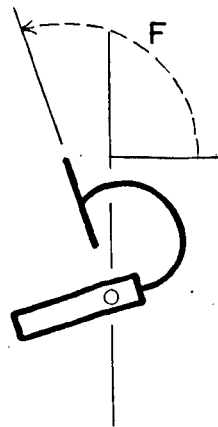
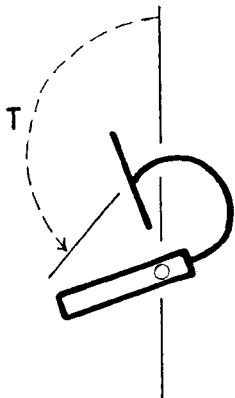
A



B

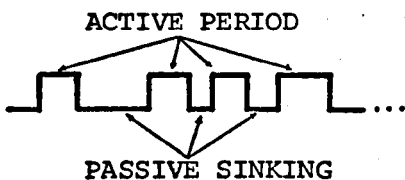


C



deviations, and the relationship between the variables. Firstly, for each test, values for one variable were correlated with those of other variables IN THE SAME FRAMES (lag zero): e.g. the tail deviations in frames 1, 2, 3 ...n were compared with the body deviations in frames 1, 2, 3 ...n respectively. Then, values for the first variable were correlated with the same or other variables in the FOLLOWING FRAMES (lag 1): e.g. the tail deviations in frames 1, 2, 3 ...n were compared with the body deviations in frames 2, 3, 4 ... (n + 1) respectively. Correlations were completed for all groups from lag zero to lag 19.

A technique which permitted rapid measurement of the duration of active periods involved the use of a morse key and a kymograph drum. An electromagnetic pointer was wired in series with the morse key and a battery (1.5v), so that depression of the key caused a deflection of the pointer. The latter was used to trace a line on a slowly rotating kymograph drum. Time intervals could be preset by adjustment of the moveable contacts on the kymograph; a buzzer sounded at the end of the set period. Individual cercariae were viewed under a stereomicroscope and the morse key depressed for the duration of each active swimming period. The following type of trace was therefore produced:



The speed of rotation of a 12" drum was set at 3.8mm/sec. Cercariae (less than 2 hours post-emergence) were observed in dechlorinated tap water at 27°C in a test chamber of the type described in Section 3. There was inevitably some delay between the observation of a change in activity and depression/release of the morse key (operator reaction

time). This feature was relatively constant : the data chosen was recorded after a period of practice during which reaction time might have been expected to change. A total of 50 cercariae were observed in this way, each for 1 minute.

Schistosoma mansoni cercariae exhibit a number of distinct locomotory mechanisms, involving the musculature of both the body and the tail. An outline of these types of activity is given here, to be followed by a more detailed description of the most common type of swimming.

a) Tail-first swimming, with passive sinking

This was the only activity of 'unstimulated' cercariae (i.e. not subject to sudden changes in temperature, mechanical disturbance etc.). Activity was not continuous, however, short bursts of swimming alternating with passive sinking periods (Table 1). Mechanical disturbance resulted in a lengthening of the active and reduction in the passive phases: this tended to cause the cercarial swarm to migrate to the water surface. A similar response occurred to a sudden reduction in light intensity - i.e. a shadow response was seen. In the absence of such stimuli, each larva was seen to oscillate in a vertical plane, first swimming upwards then sinking again. During sinking, the furcae were spread laterally, or curled towards the tail stem. The cercarial centre of gravity is presumably in the body, as larvae always sank body-first with an exactly vertical primary orientation. Most body and tail muscle was probably in a relaxed condition during sinking, although occasional contractions of the body were seen and the tail sometimes 'twitched' briefly.

Sometimes cercariae completed very extended inactive periods. These individuals consequently sank below the general population depth, if not to the bottom of the observation chamber. On contact with the latter they were usually reactivated and an extended swimming period brought them back to the surface. This behaviour may have been due to a tendency of a relatively small number of larvae to switch activity or, alternatively, all larvae might pass through one or more phases of such activity. Long, inactive periods appear more common in older larvae and are rare in freshly-shed larvae.



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TABLE 1

MEAN ACTIVE PERIOD (MAP), IN SECONDS

<u>CERCARIA</u>	<u>MAP</u>	<u>CERCARIA</u>	<u>MAP</u>
1	0.61	26	1.29
2	0.61	27	0.44
3	0.51	28	0.66
4	0.55	29	0.37
5	0.61	30	0.70
6	0.48	31	1.07
7	0.92	32	1.14
8	0.44	33	0.81
9	0.81	34	1.29
10	0.48	35	1.03
11	1.59	36	1.22
12	1.81	37	0.74
13	2.59	38	0.74
14	1.40	39	0.96
15	1.18	40	1.25
16	2.55	41	0.74
17	0.92	42	0.70
18	1.14	43	1.11
19	1.62	44	0.74
20	1.11	45	0.70
21	1.07	46	0.59
22	0.74	47	1.77
23	0.70	48	1.85
24	0.85	49	0.85
25	1.48	50	1.40

Mean : 1.02 S.D. : 0.50

Swimming was initiated with a complete lateral flexure of the tail (Text Figs. 9,10) which then continued to flex from side to side for the duration of the active period. The body swung from side to side as well, so that the larva appeared to vibrate about two nodes positioned in the region of the ventral sucker and at a point near to the distal end of the tail stem. These are the regions of minimal lateral displacement during swimming, as can be seen in Text Fig.10. The furcae were held in a laterally extended position, the junction between the tail stem and furcae (the T-joint) acting as a limited-range pivot so that the angle between the furcae and the tail stem varied during each oscillatory cycle. At the point of maximum tail flexure, a slight asymmetry of the tail action could be identified. The tail stem must effectively twist slightly so that the tips of the furcae can pass the body, either dorsally or ventrally at the end of a full stroke (e.g. see Text Fig.9, frames 35, 36).

This asymmetry is undoubtedly related to the slow rotation about the longitudinal axis exhibited by swimming cercariae. The velocity of this rotation was approximately 360° per second.

Cessation of swimming could occur apparently spontaneously but, in unstimulated larvae, contact between the furcae and the surface film of the water also inhibited activity. In very shallow water, therefore, larvae may oscillate between the surface film and the bottom. If this point is not appreciated, experimental monitoring of active periods may reflect nothing more than the depth of water!

A quantitative analysis of tail-first swimming mode is presented below.

b) Body-first swimming

This was only seen in 'stimulated' cercariae and usually occurred for short, intermittent periods; cercariae were able to switch rapidly

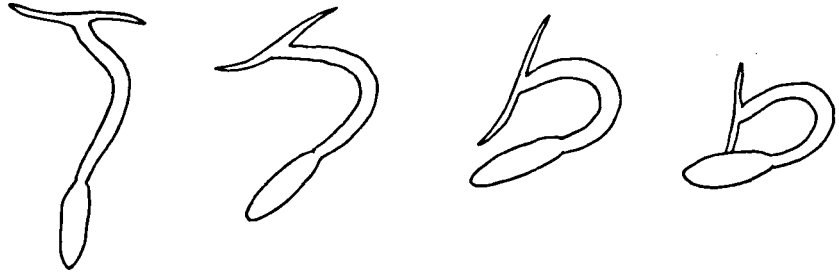
Text Fig. 9

Diagram to show positions of cercarial body, tail and furcae in 64 successive cine frames (representing about 1 second of activity).
Precooled larvae do not oscillate at the full rate so progression is limited.

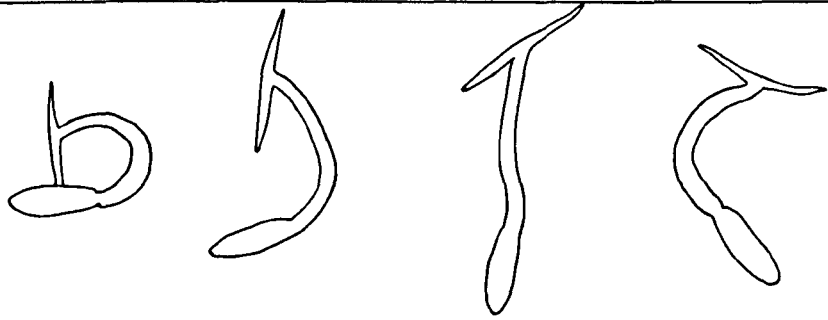
Frame numbers are indicated at the left hand side of the diagram.

64 f.p.s. 24°C, precooled to 15°C

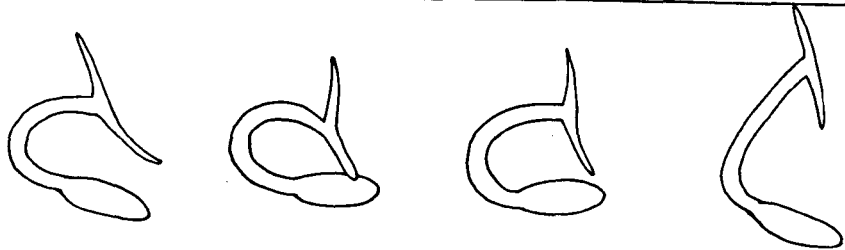
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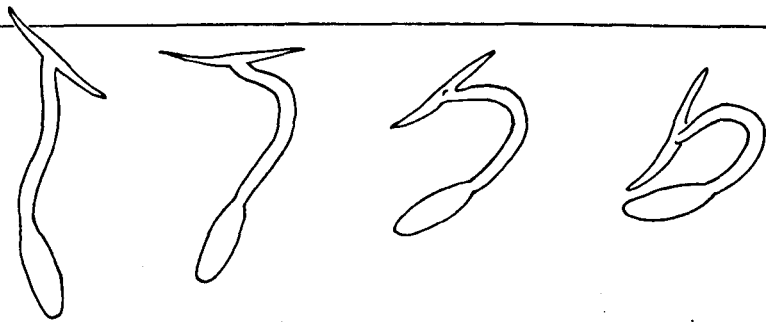
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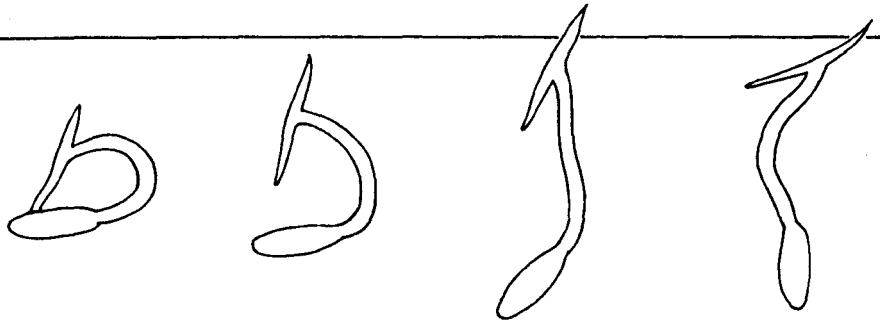
9-12



13-16



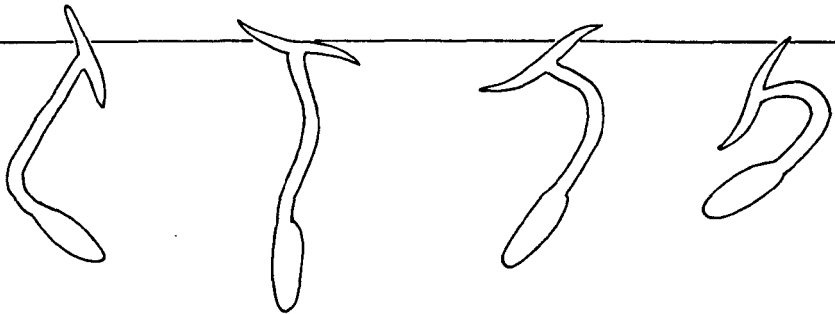
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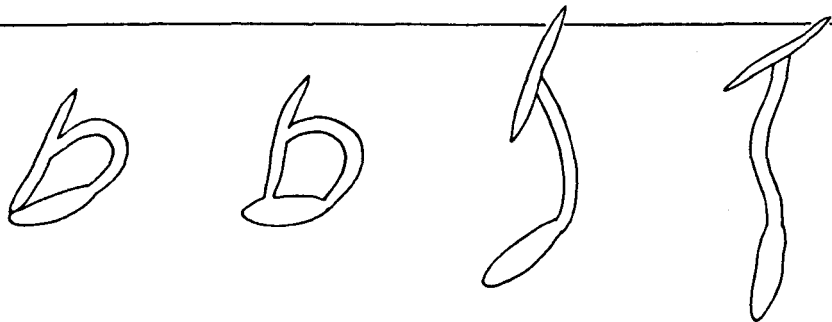
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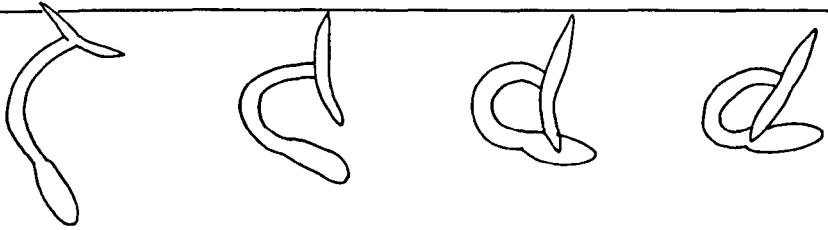
25-28



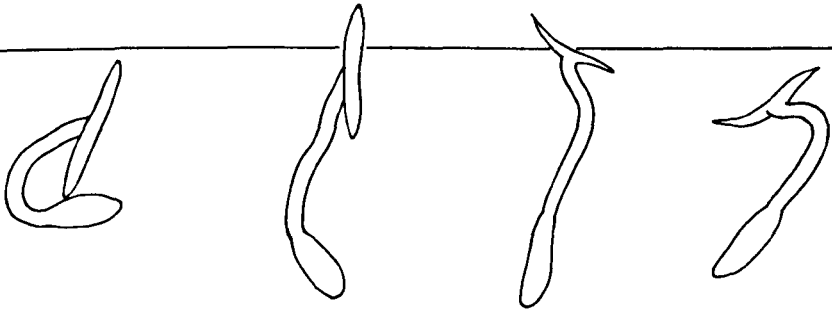
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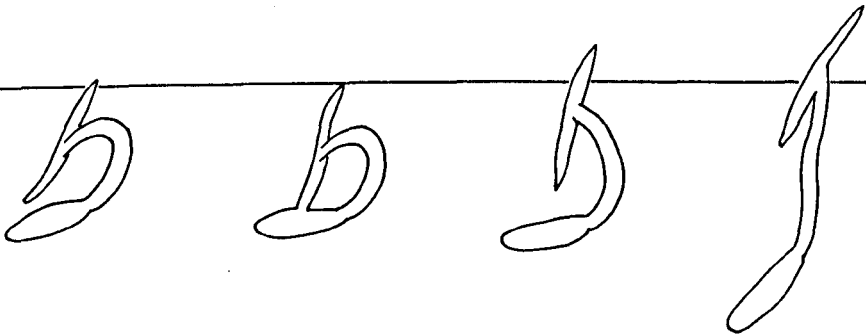
33-36



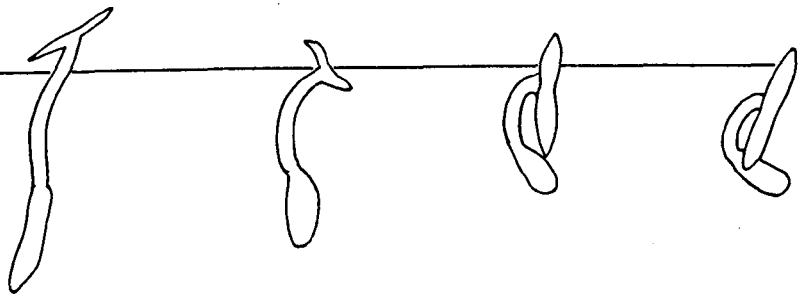
37-40



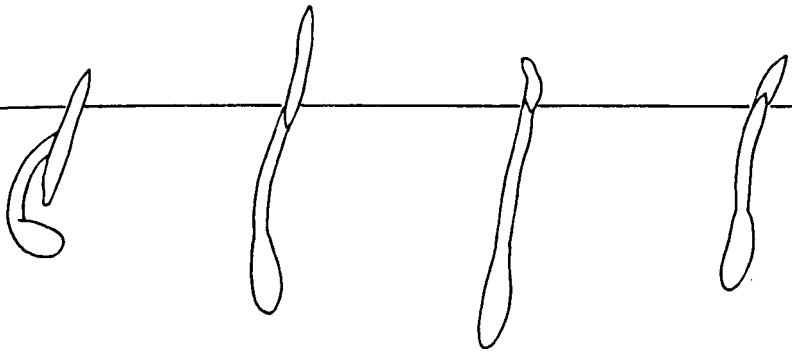
41-44



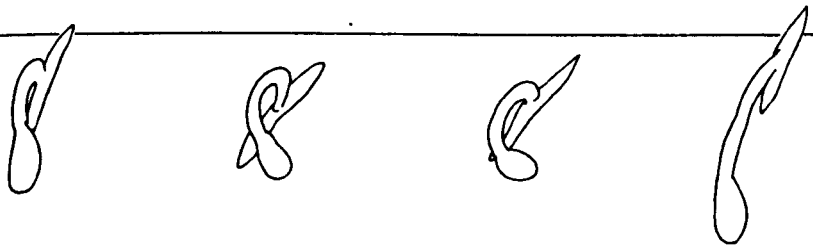
45-48



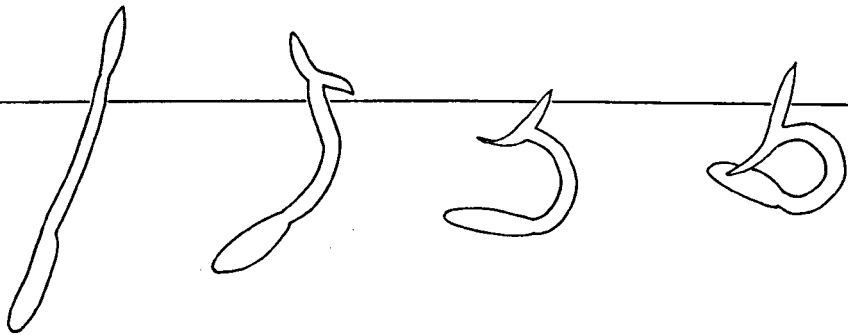
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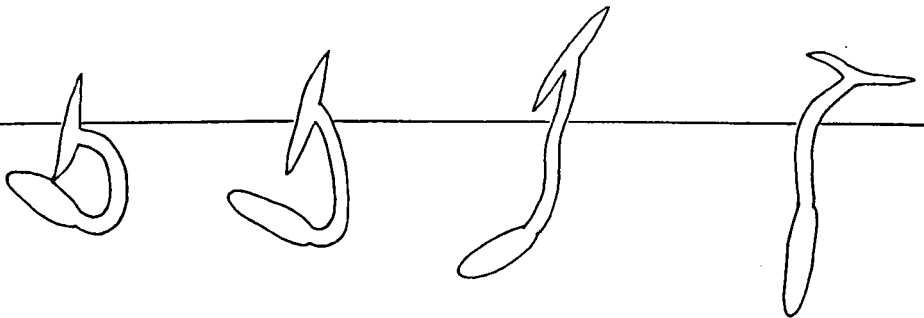
53-56



57-60



61-64



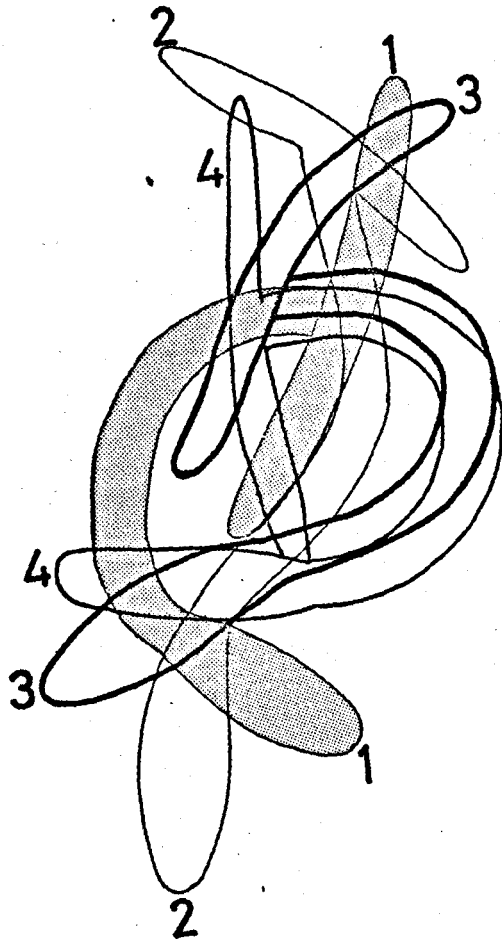
Text Fig. 10

Diagram to show relative positions of body, tail and furcae during tail-first swimming (direction indicated by the arrow).

Four successive cine frames (1 - 4) have been projected and the outline of a cercaria traced. Film speed was 64 frames per second.



64 f.p.s.



between this mode and the tail-first mode. The tail waveform differed from that described in tail-first swimming however, as the furcae were held together, in line with the tail stem. The body and tail also appeared to extend in length during body-first swimming which broadly resembled the typical undulatory swimming of a fish.

c) Crawling

Attachment to a solid surface, followed by crawling, only occurred in the presence of appropriate stimuli. There were anterior and posterior points of attachment, the latter being the protrusible ventral sucker. The anterior point of attachment could not be distinguished precisely from these films alone, but appeared to be sub-terminal. Before anterior attachment was established, the oral sucker region was considerably extended and flexed from side to side. After an anterior attachment was established the ventral sucker immediately released its hold and the whole body flexed dorso-ventrally so that the ventral sucker was brought alongside the anterior attachment. Here, the ventral sucker again gripped the substratum, the anterior attachment was released and the whole process repeated. This advance of the ventral sucker is termed *one step* of crawling locomotion. Text Fig.11 shows a sequence of such activity - these illustrations were obtained by tracing the images from consecutive projected frames. The dorso-ventral flexure and extension of the apical disc region is similarly shown in Text Fig.12. Included in Appendix 8 is a print from the cine frames used.

During crawling, the tail does not contribute to the locomotory pattern but vibrates spasmodically. The stem and furcae may also lengthen and contract in an apparently random fashion.

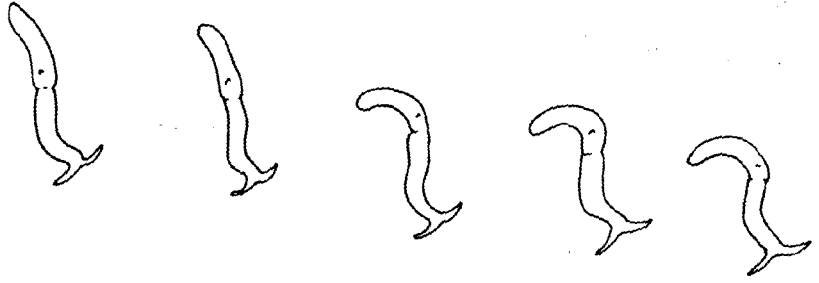
Text Fig. 11

Diagram to show successive cercarial positions during crawling (just over 1 second of activity shown). In certain frames, the precise position of the furcae could not be determined due to blurring of the film image.

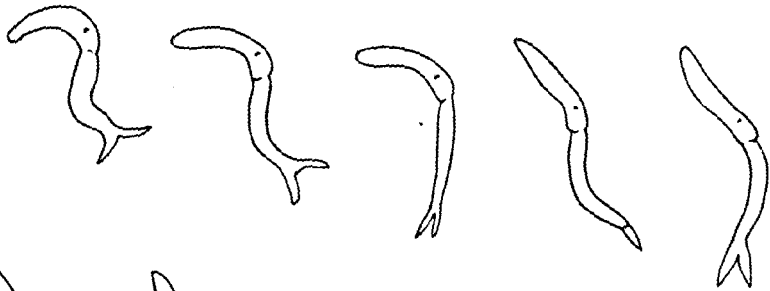
Frame numbers are indicated at the left hand side of the diagram.

64 f.p.s. 24°C

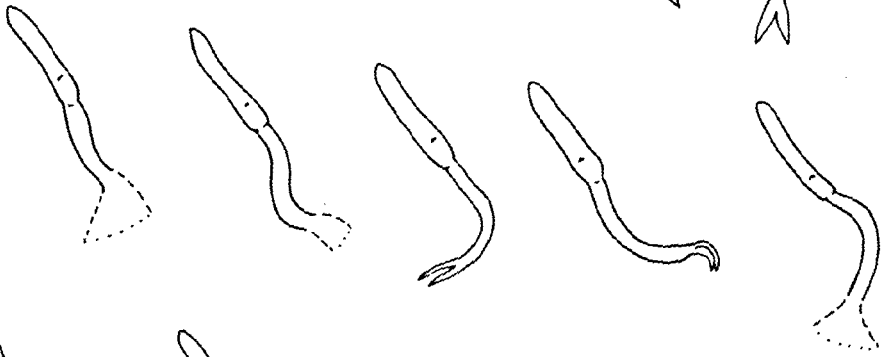
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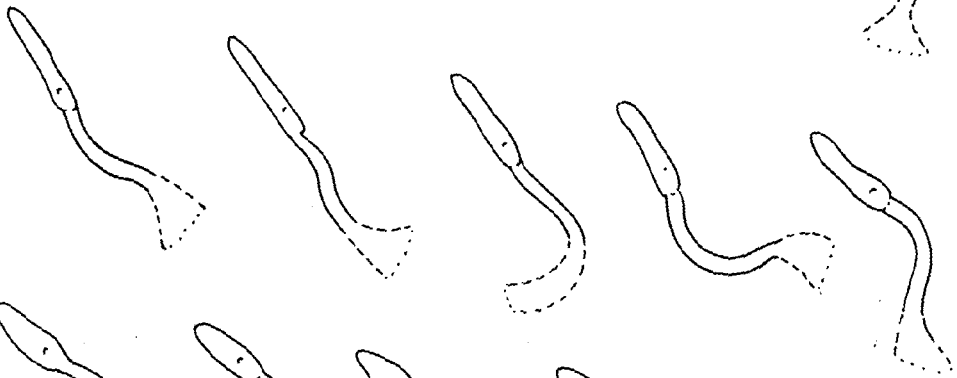
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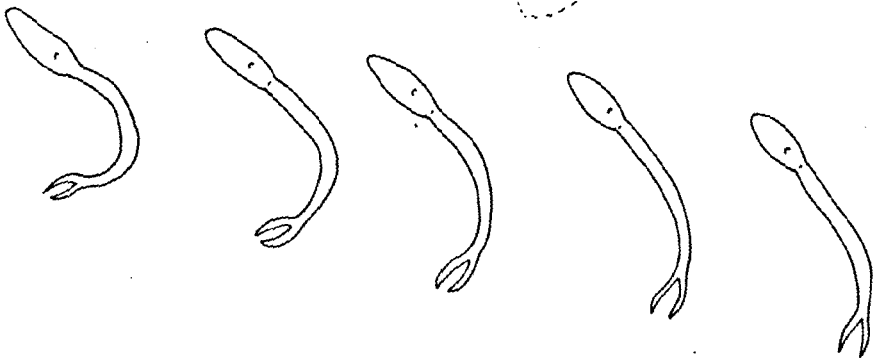
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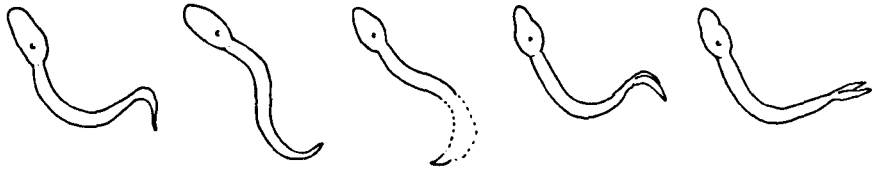
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21-25



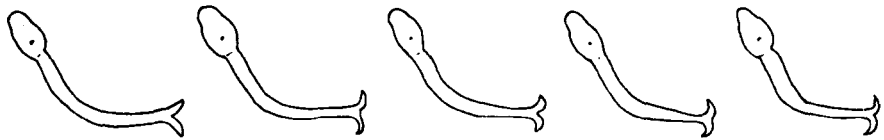
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31-35



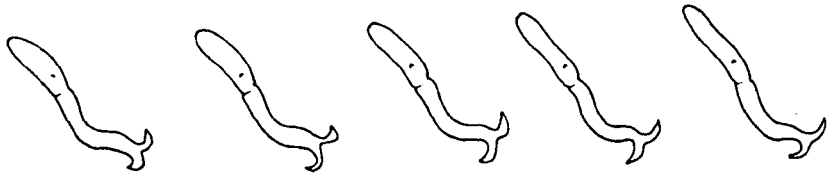
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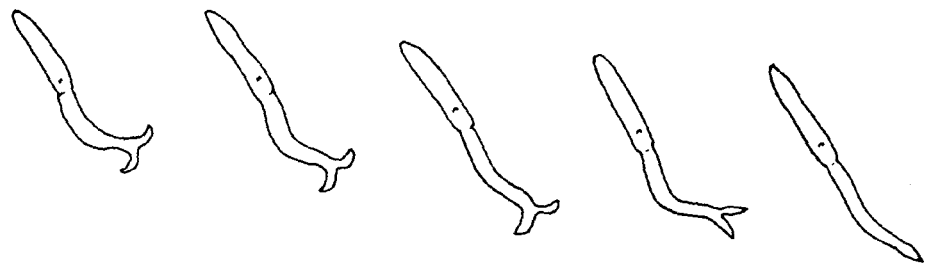
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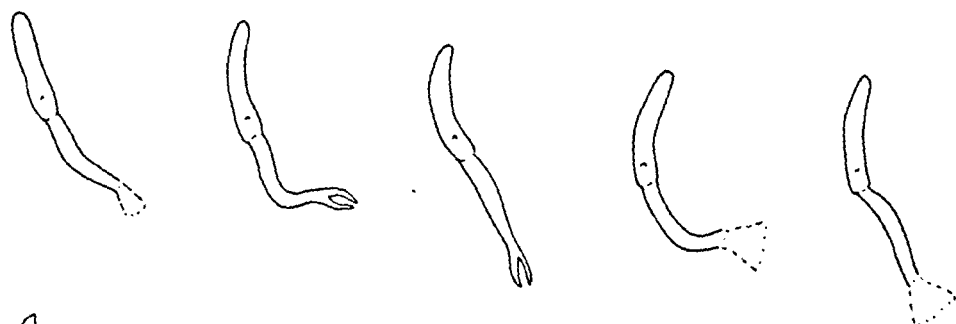
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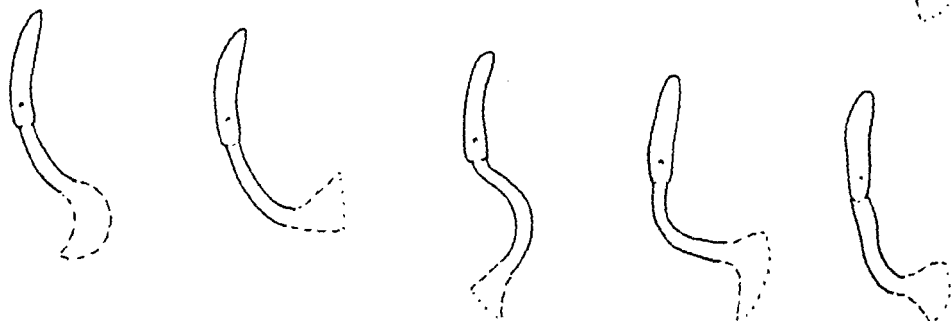
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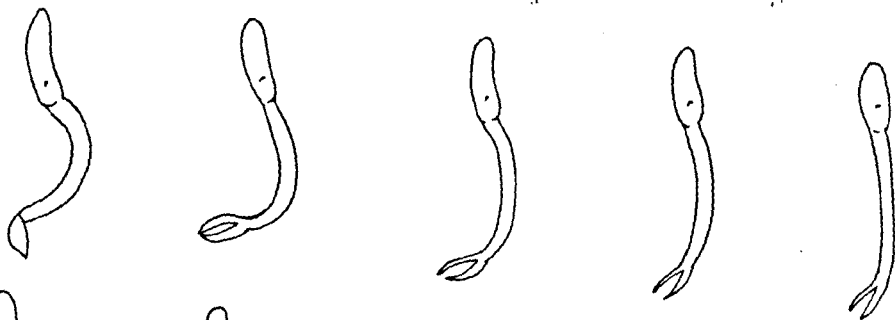
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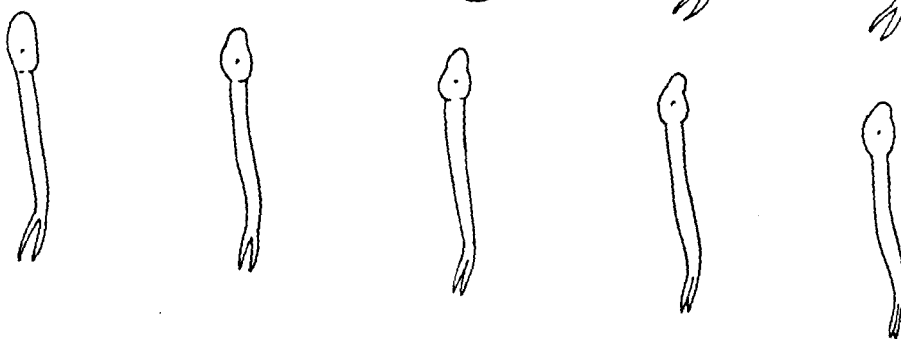
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66-70



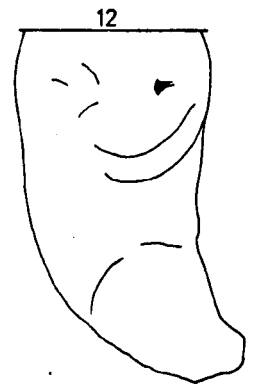
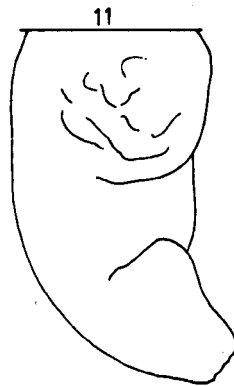
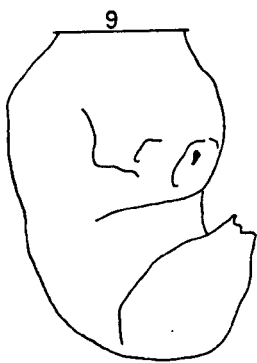
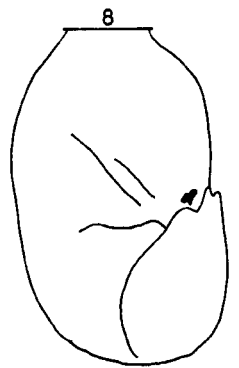
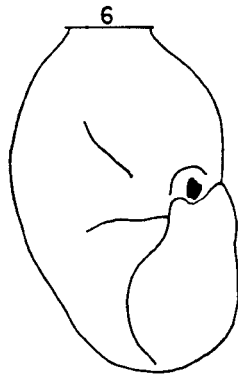
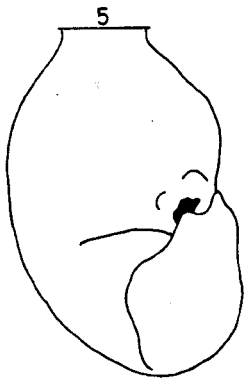
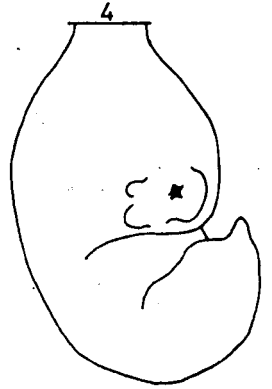
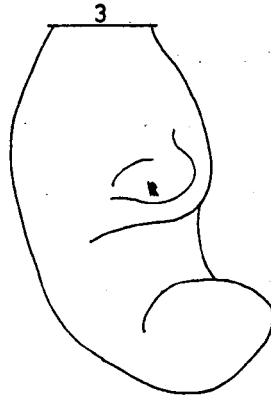
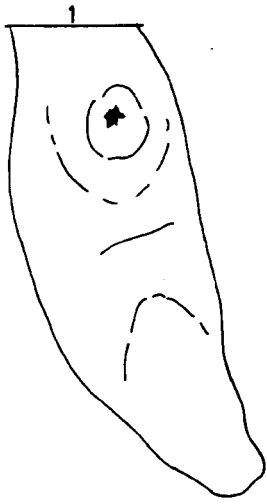
71-75

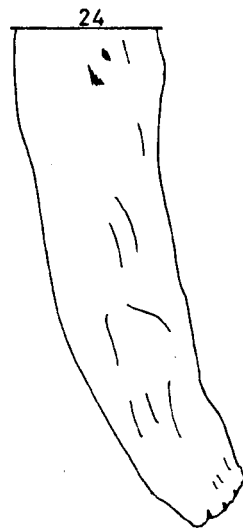
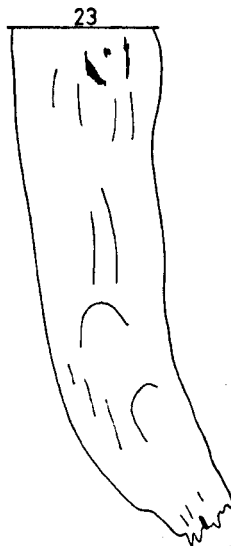
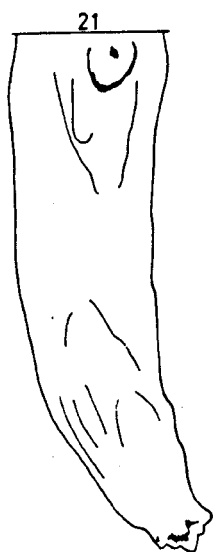
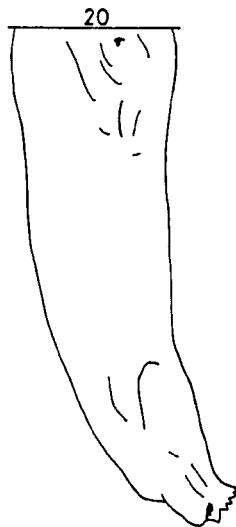
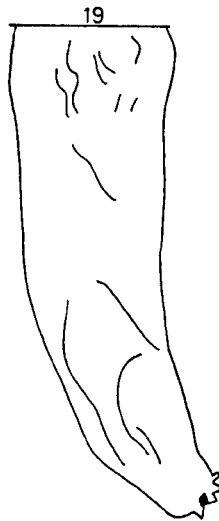
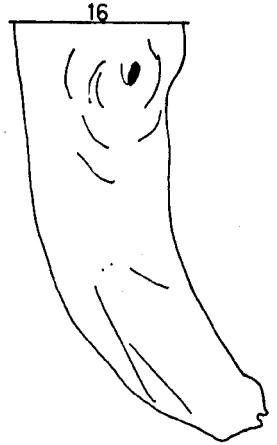
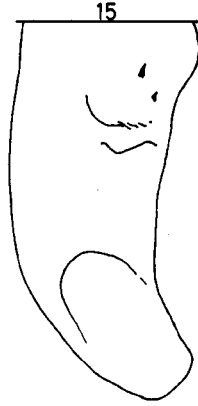
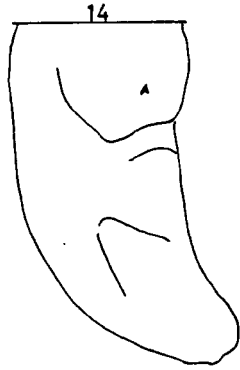
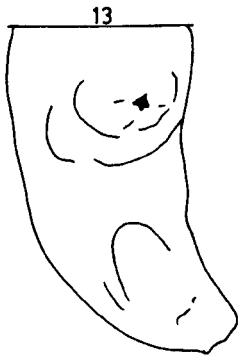


Text Fig. 12

Diagram to show successive positions of cercarial body during dorso-ventral flexure. Location of ventral sucker (shaded) is clear, and the whole sucker area is protruded as the anterior end of the body is flexed ventrally. Note the extension of the apical disc as the anterior extends forward.

Successive frames are numbered individually.





d) Burrowing

This activity was not recorded in natural circumstances, but the term is used to describe the movements of the body wall during passage through a semi-solid medium. It is assumed to occur during penetration of the host skin and is probably similar to the movements made by cercariae when slightly compressed beneath a microscope cover slip where there is insufficient room for crawling. Circular and longitudinal myofibres probably act in a broadly similar way to those of, for example, the annelid body wall.

Each part of the cercarial body and tail appears capable of considerable extension and contraction. This mobility is illustrated in Text Fig.12. The furcae are very flexible, a feature not easily demonstrated in films of swimming larvae when the furcae are usually held fairly rigidly in position, extended laterally.

Analysis of tail-first swimming

The original data showing angular deviations of body, tail and furcae in successive cine frames is illustrated graphically in Text Figs.13-16 and the original data is included in Appendix 9. Measurements were recorded for two larvae, both swimming in a straight line towards the water surface. Text Figs. 13, 14 show the movements of cercaria 1 and Text Figs. 15, 16 those of cercaria 2. It is because of this that I have sub-titled Text Figs. 13 and 14 as Cercaria 1a and Cercaria 1b respectively. Similarly Text Figs. 15 and 16 are sub-titled Cercaria 2a and Cercaria 2b, respectively. Although 'a' and 'b' represent successive time samples, several cine frames in fact intervene between those of Text Fig.13 and those of Text Fig.14; because the oscillations were viewed 'edge-on' it was not possible to estimate the angular deviation of any part of the body or tail. The same applies to Text Figs. 15 and 16. The +ve and -ve signs of the

Text Figs. 13 - 16

The angular deviation of the cercarial body, tail and furcae from the 'resting' position and the cumulative distance travelled, in successive cine frames (64 f.p.s.).

Text Figs. 13, 14 : cercaria 1

Text Figs. 15, 16 : cercaria 2

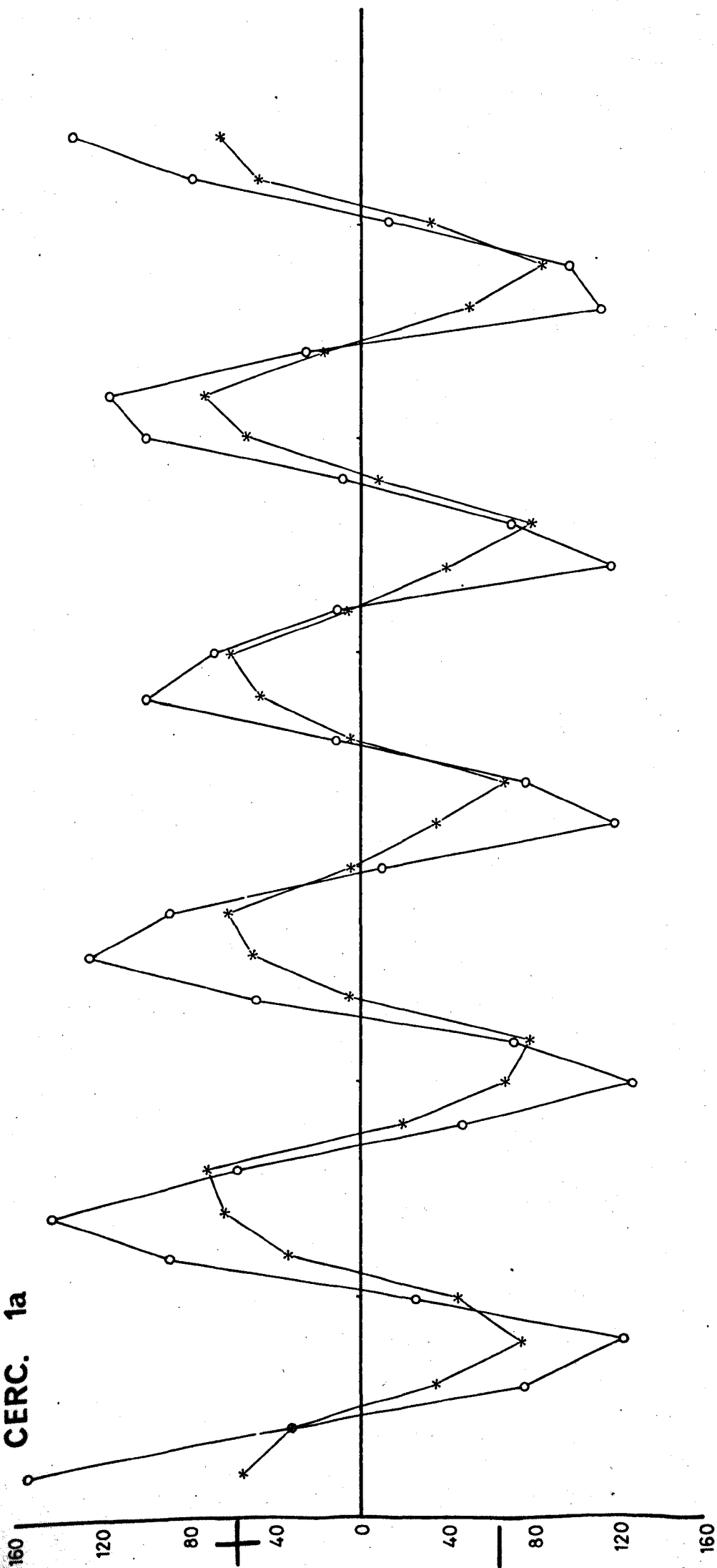
In each Figure, the Y - axis of the upper graph shows the angular deviation in a +ve or -ve direction (see text).

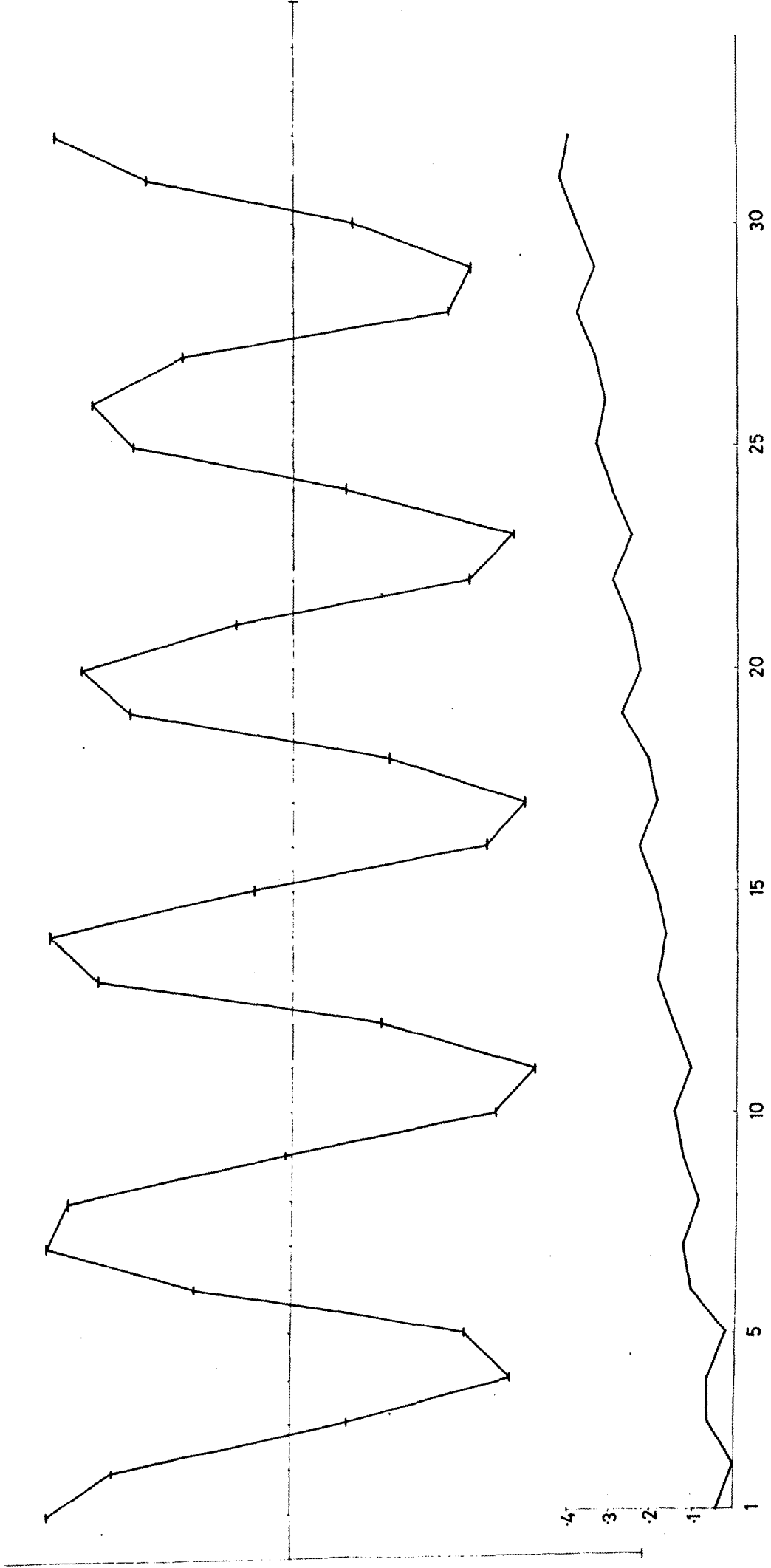
The Y - axis of the lower graphs shows the real distance travelled in mms.

The X - axis applies to both upper and lower graphs and indicates the number of the frame being analysed.

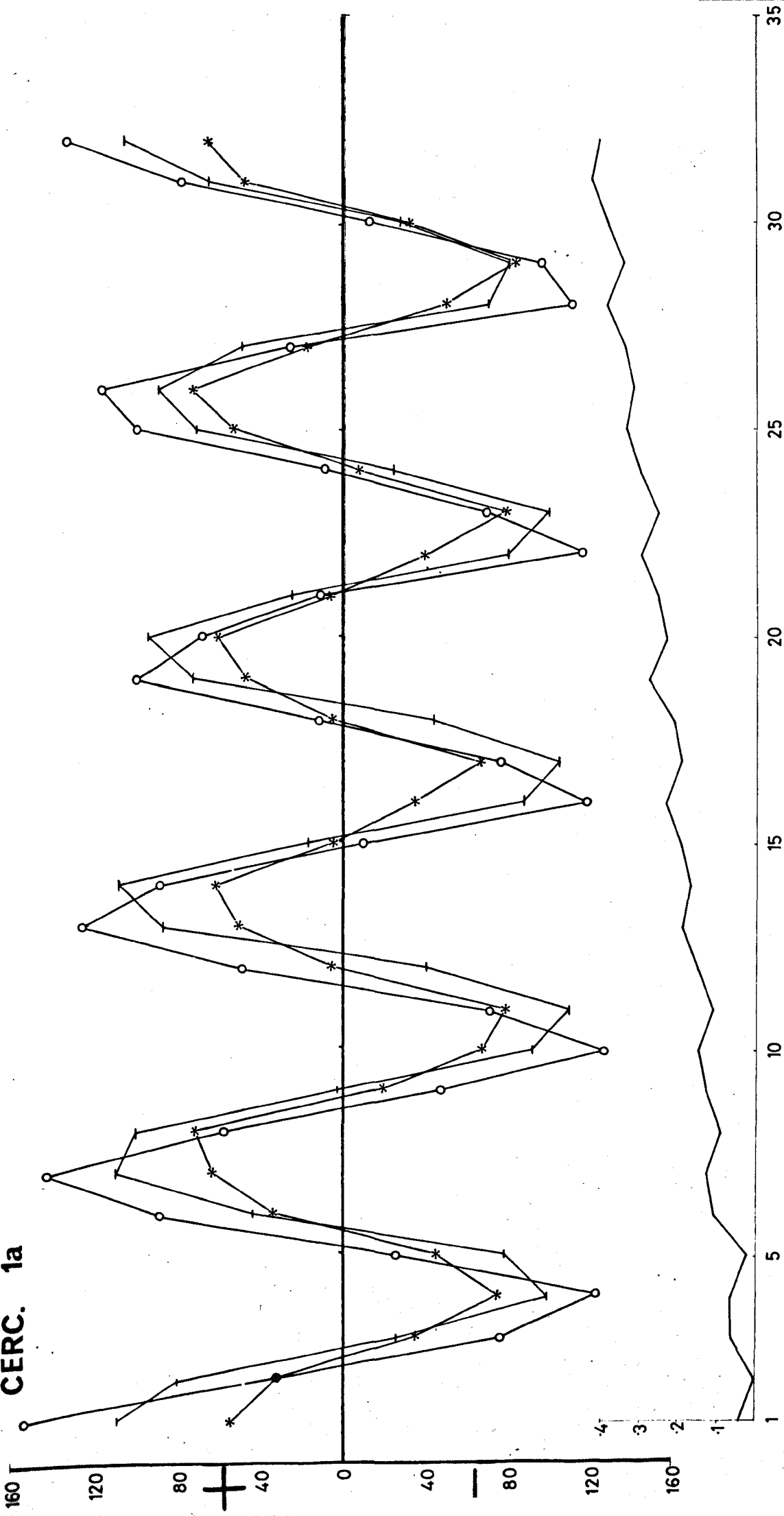
KEY: * = BODY
O = TAIL
- = FURCAE

CERC. 1a

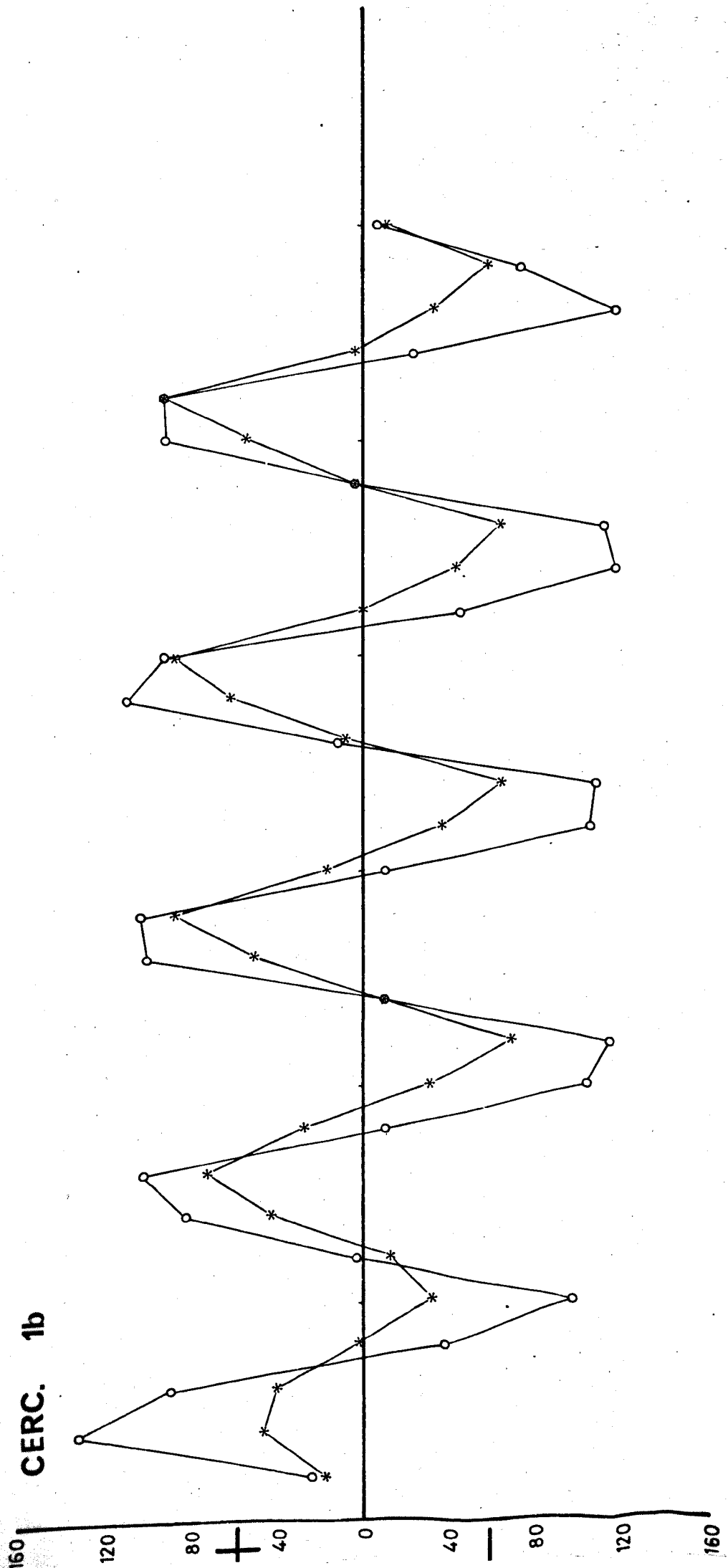


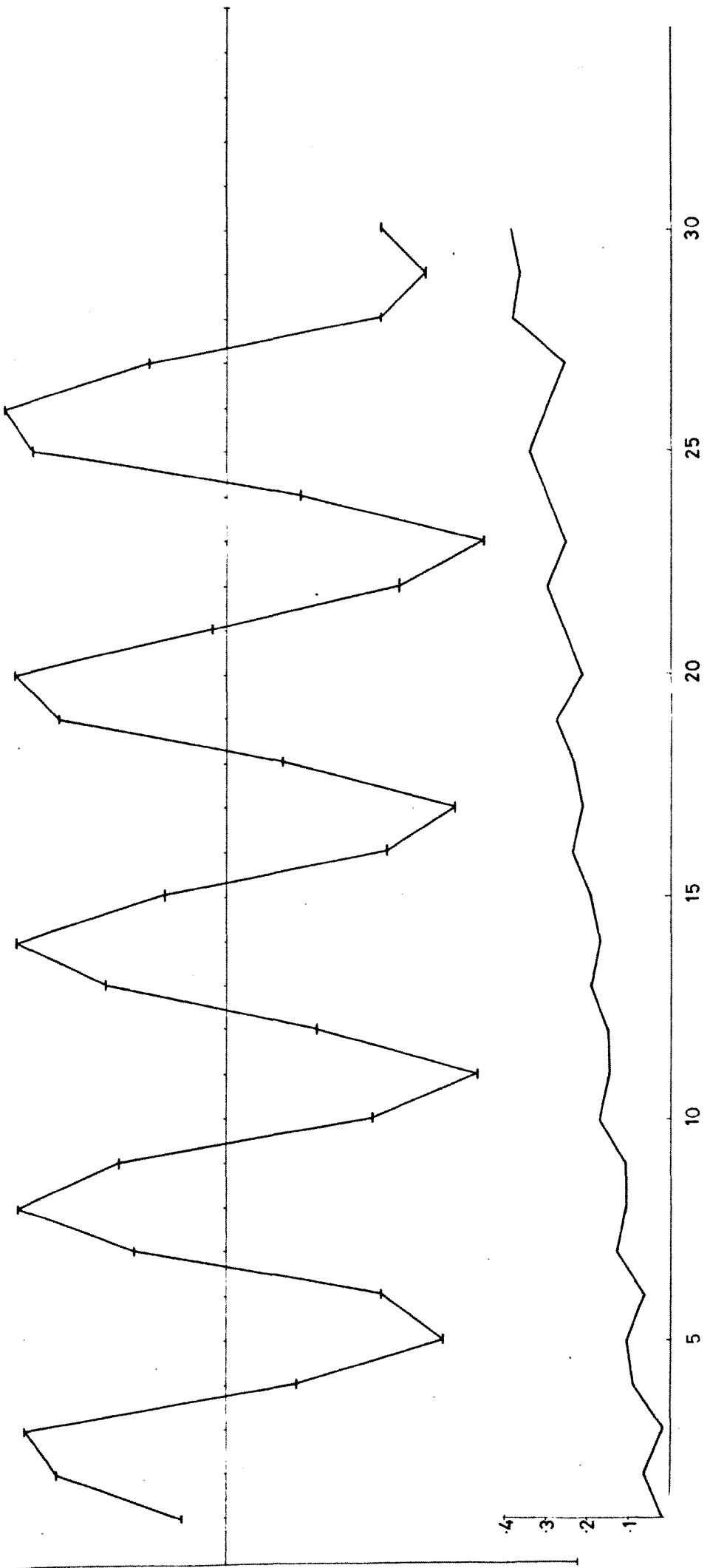


CERC. 1a

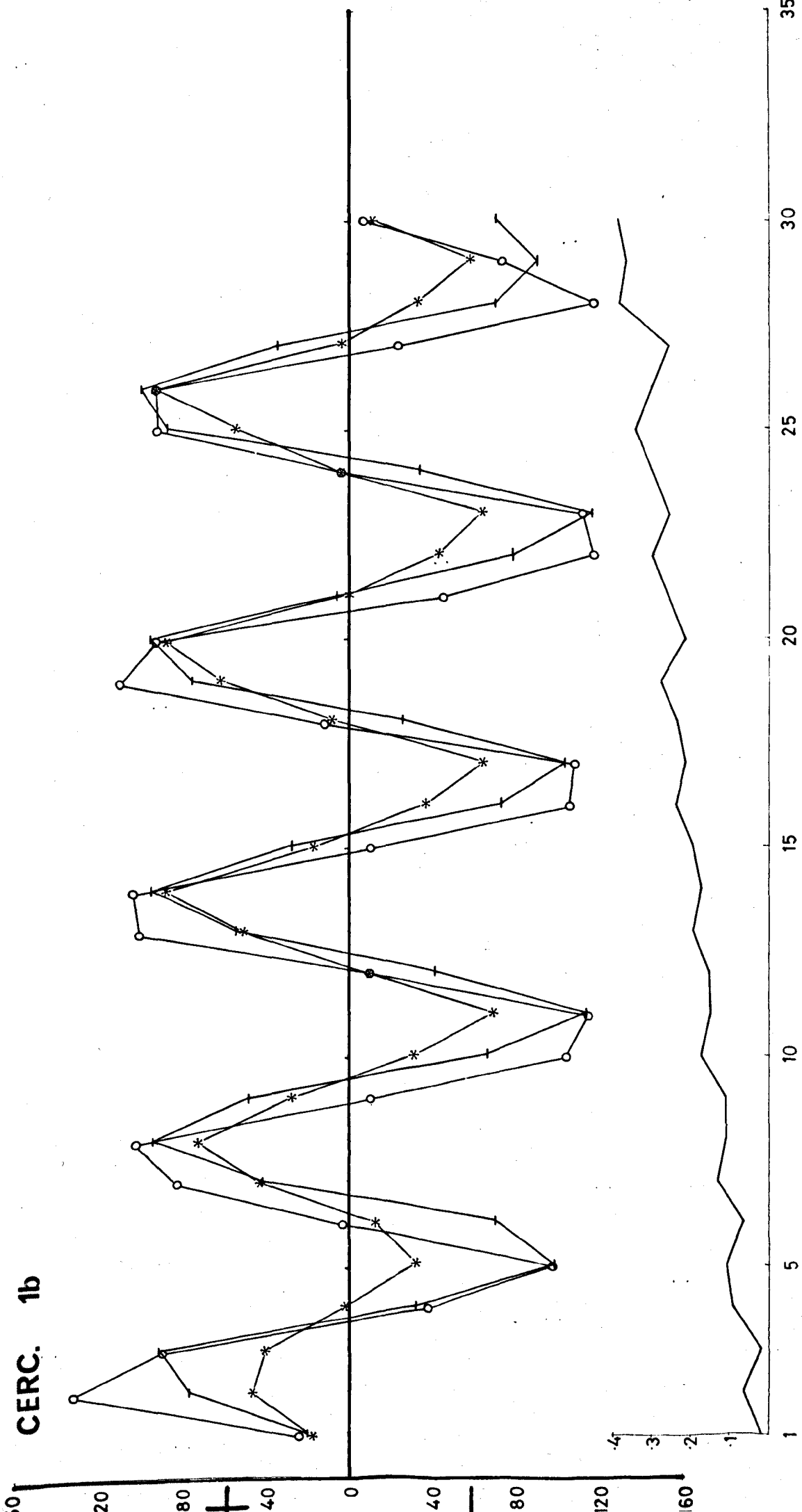


CERC. 1b

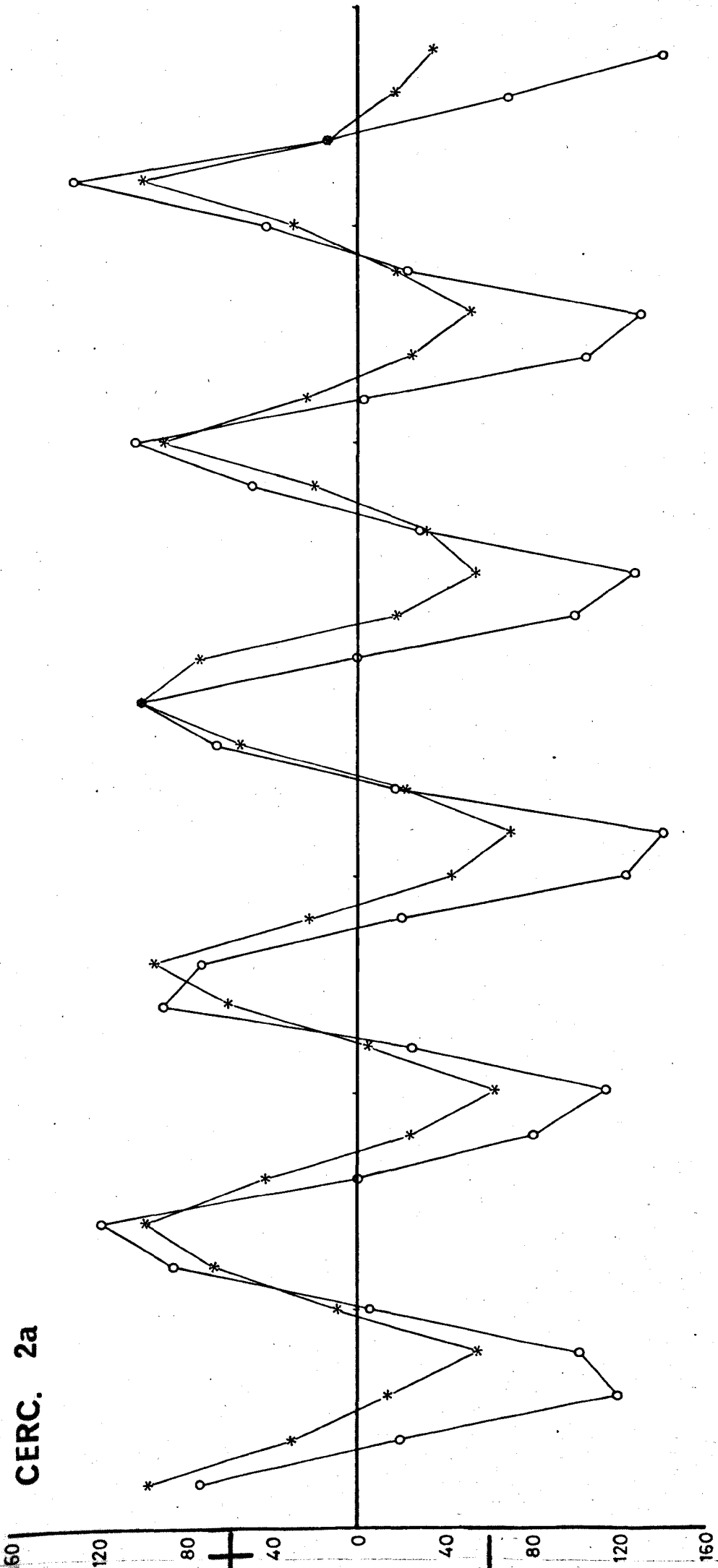


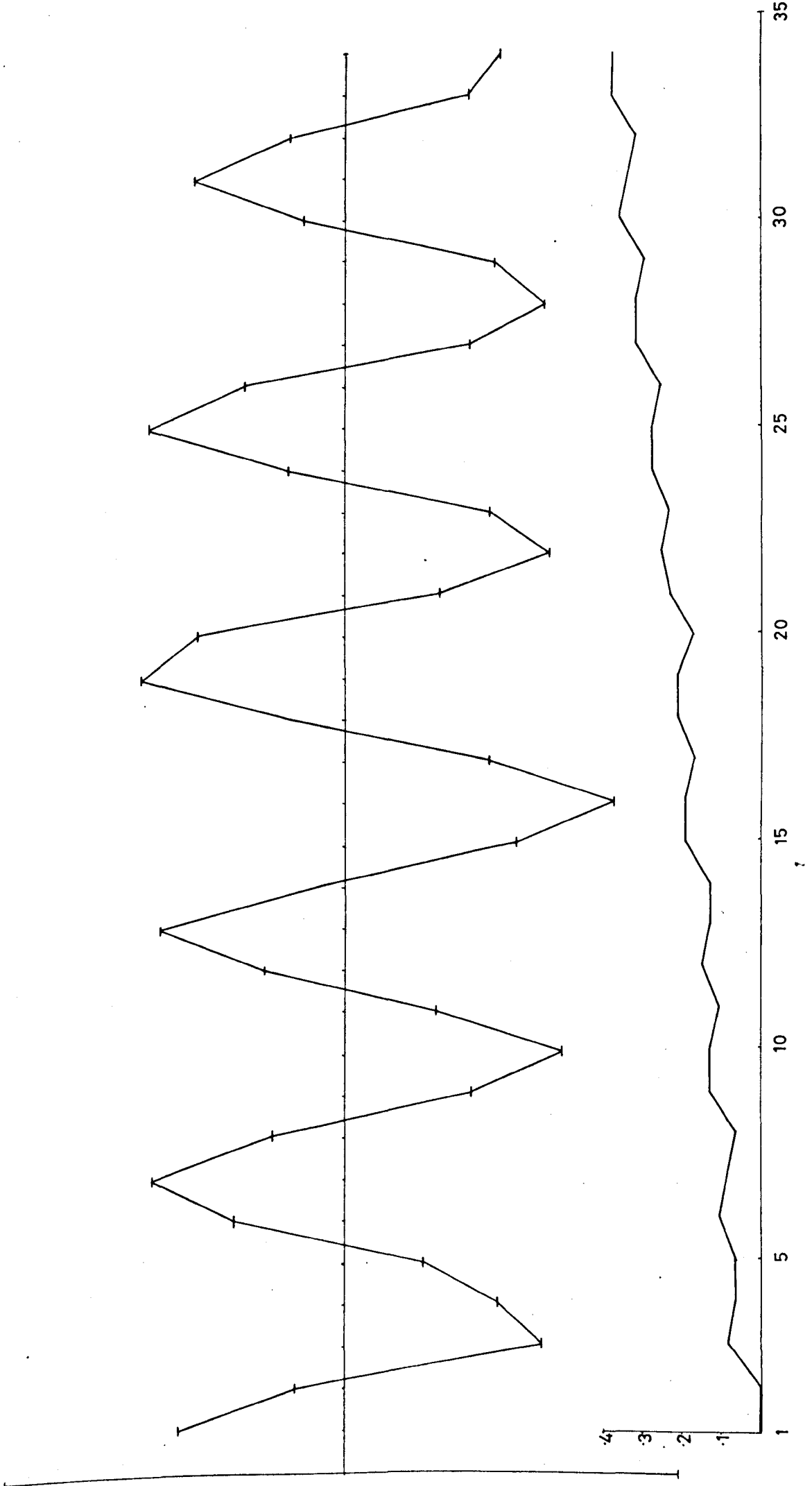


CERC. 1b

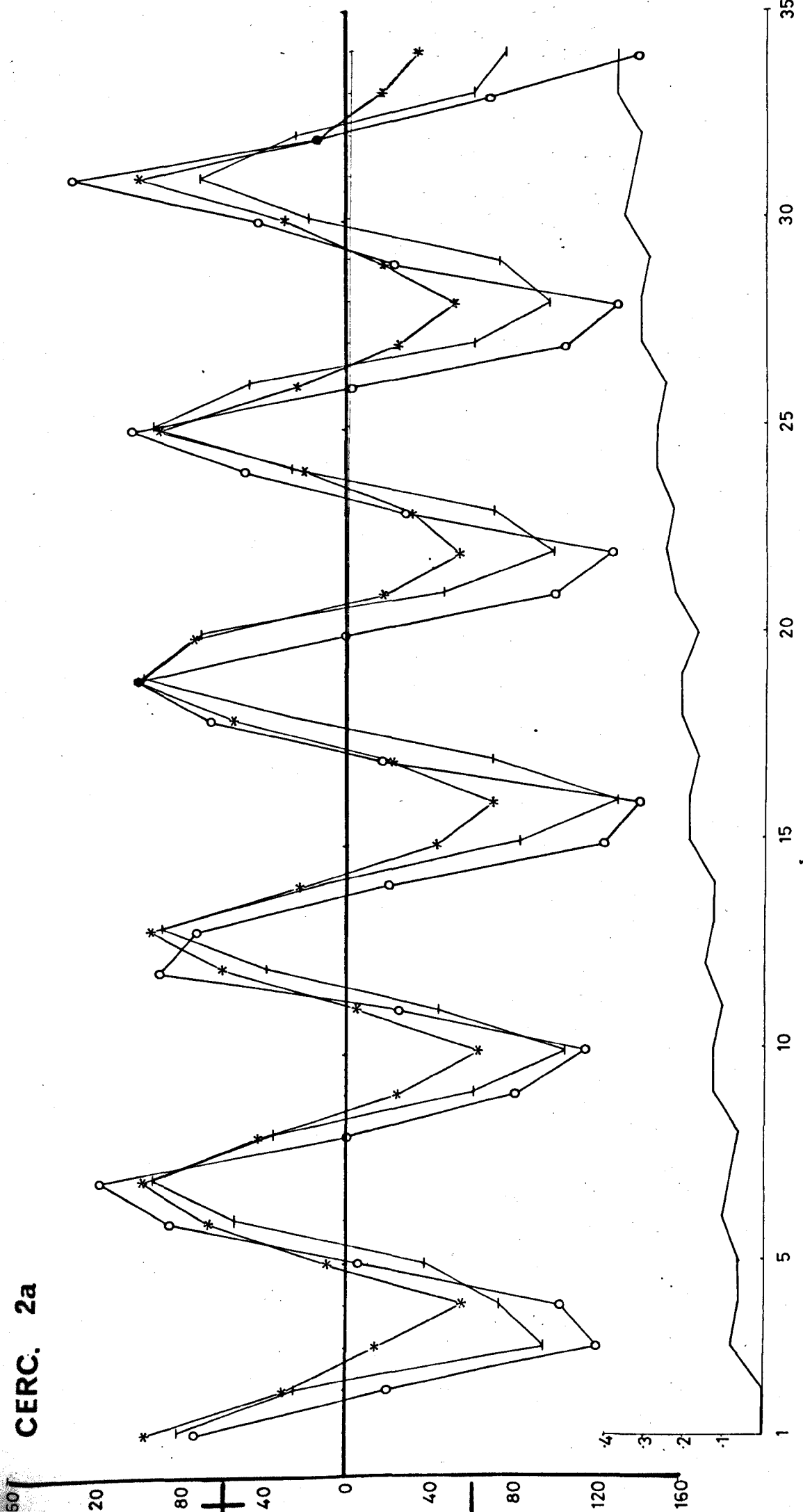


CERC. 2a

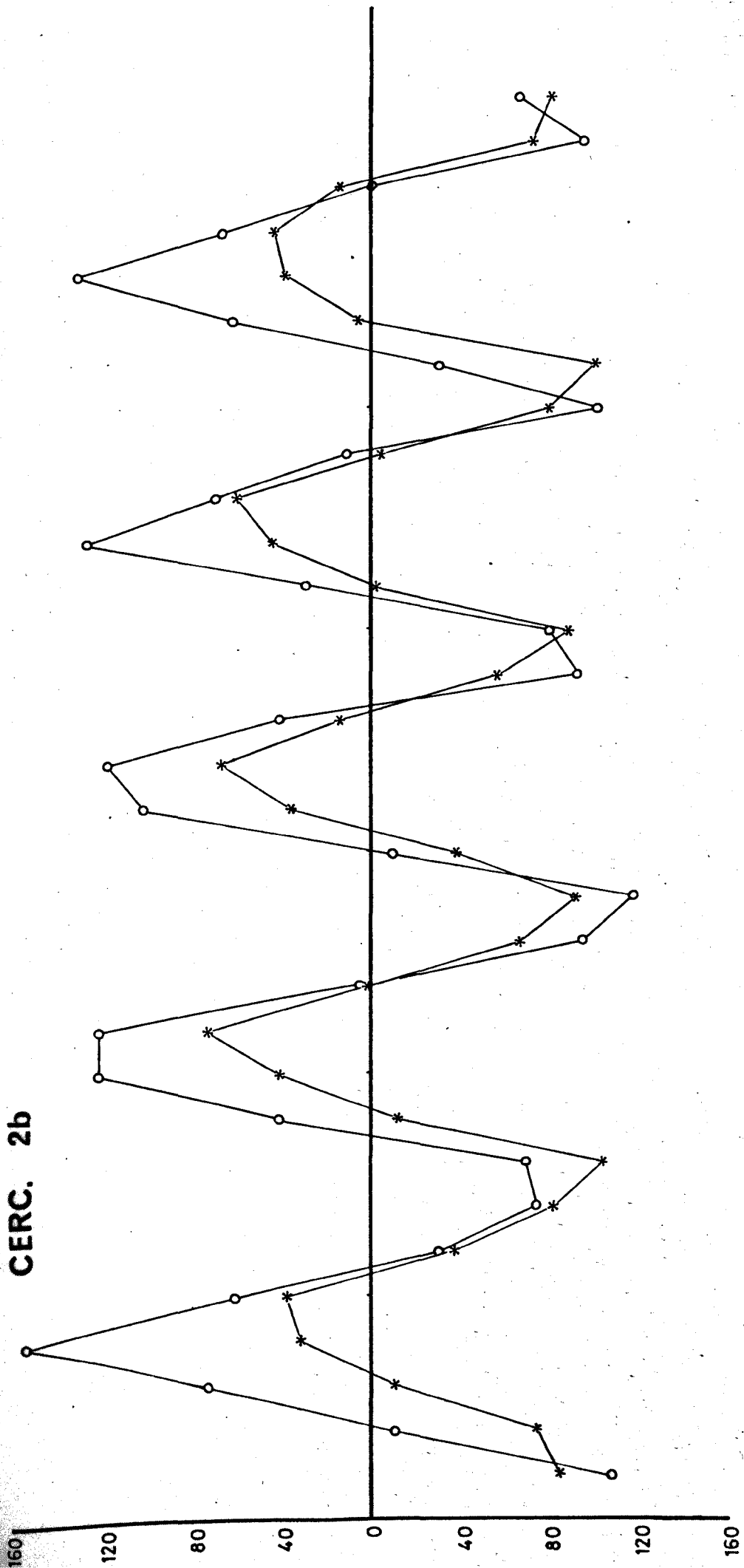


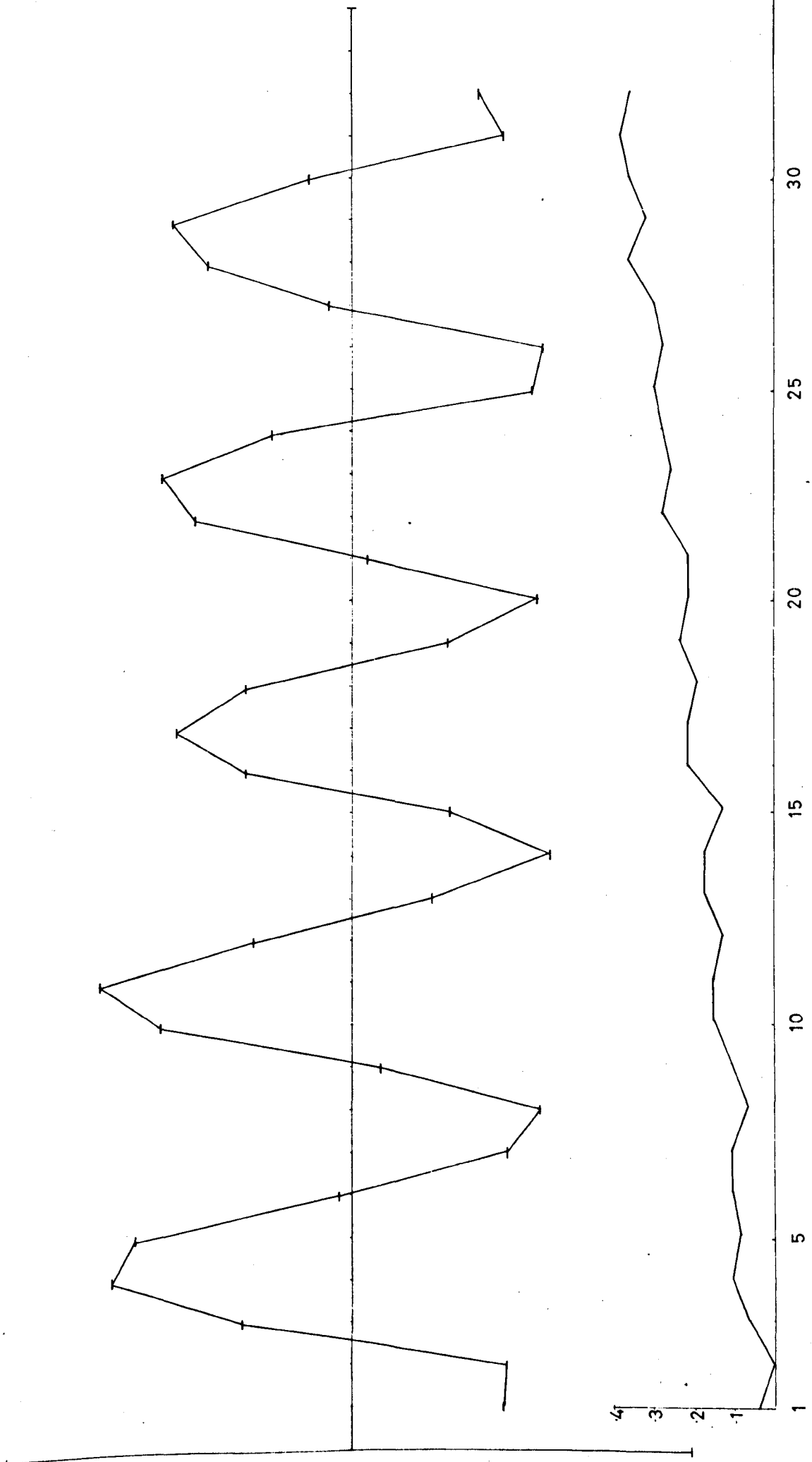


CERC. 2a

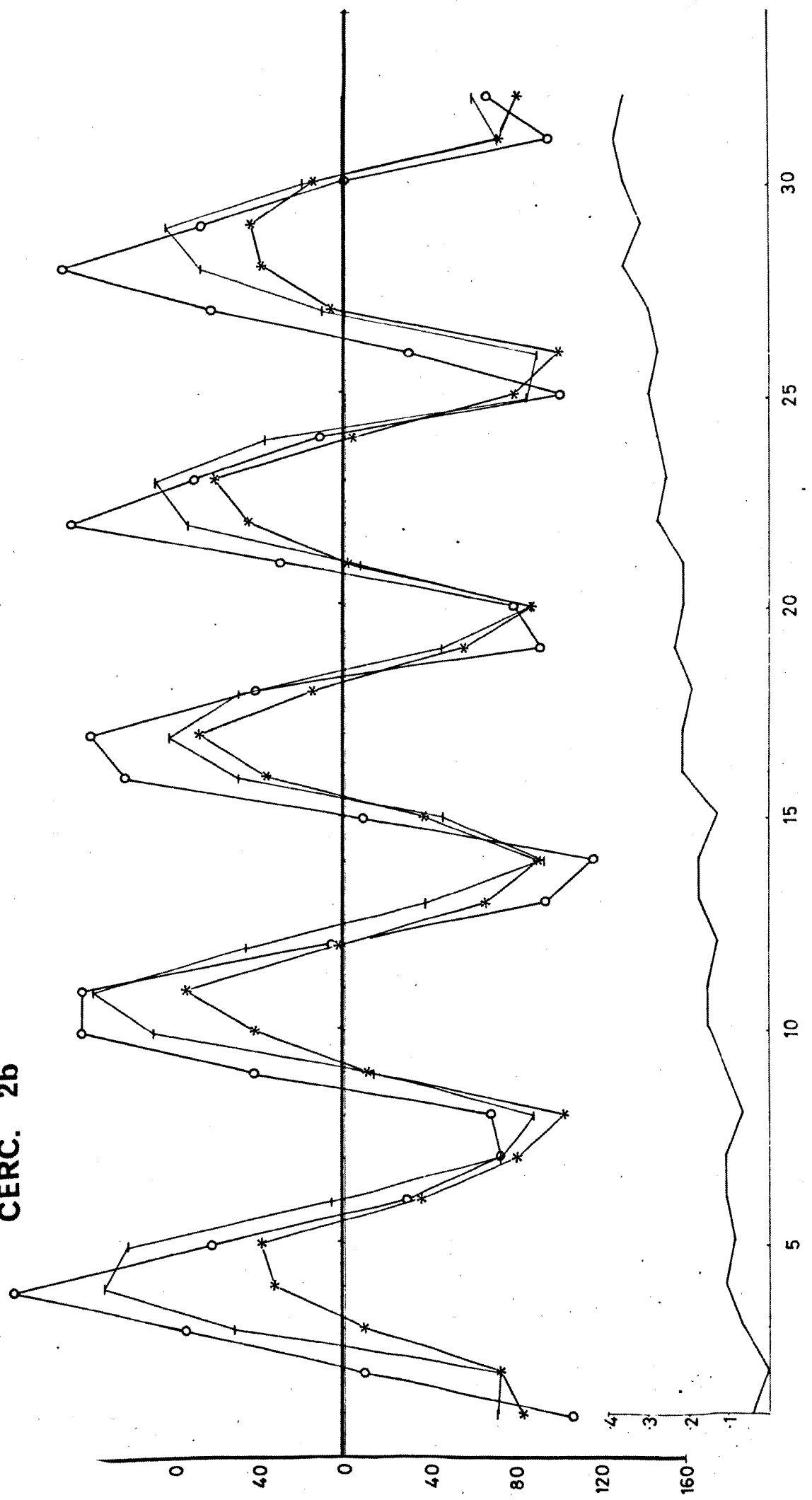


CERC. 2b





CERC. 2b



Y-axes of these graphs refer to the side of the locomotory axis towards which displacement has occurred, i.e. the left- or right-hand side of the frames being analyzed:-

left = -ve

right = +ve

Because of the rotation of a cercaria about its longitudinal axis during swimming, these signs cannot also represent the left- and right-hand sides of the animal itself; the animal's left side, for example, faces towards the left and right sides of the cine film alternately.

Consequently, in Text Figs. 13 and 15 the larvae present one side to the left of the film and in Text Figs. 14 and 16 their opposite side in the same direction. The maximum angular displacements of body, tail and furcae at each cycle are tabulated in Appendix 10 together with the maximum angular velocity achieved by the tail and the time the tail was displaced to each side. A summary of mean values (with standard deviations) is included here in Table 2.

Also included in Text Figs. 13-16 is the cumulative distance travelled. It may be seen that a net gain is made during each power stroke of the tail. The Y-axis of the lower graphs in these figures shows the real cumulative distance moved, in mms.

The speeds achieved by the two cercariae, in Text Figs. 13/14 and 15/16 respectively, were 0.85mms/sec and 0.75mms/sec.

The traces for angular deviation of body, tail stem and furcae may therefore firstly be used in a qualitative description of propulsive movements and secondly they allow measurement of a number of quantitative parameters. Text Fig. 17 relates the appearance of a cercaria to the values for T, B, F as represented in Text Figs. 13-16.

TABLE 2

Overall mean values for several parameters which define cercarial swimming movements, (standard deviations in brackets). Data has been pooled from both +ve and -ve parts of the oscillations. Fuller details are given in Appendix 10.

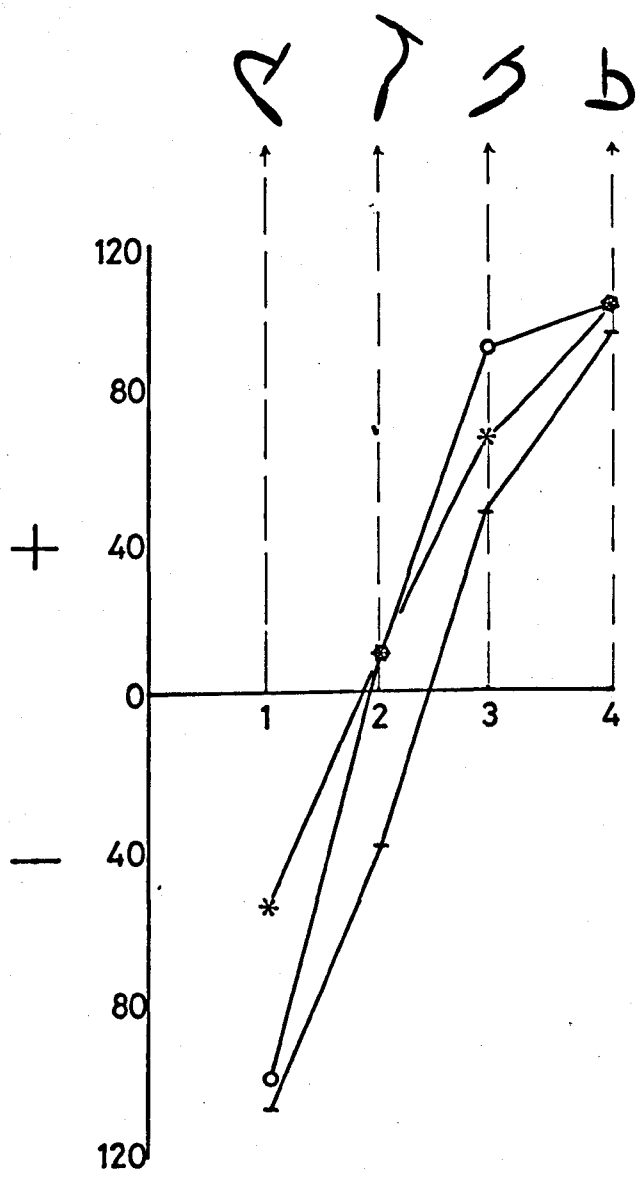
Maximum angular displacement of tail	=	T max
" " " " body	=	B max
" " " " furcae	=	F max
" " velocity of tail	=	V max
Time tail displaced to each side	=	t

	T max (degrees)	B max (degrees)	F max (degrees)	V max (degrees per 0.01 sec)	t (secs.)
CERCARIA 1	117 (16)	69 (15)	100 (9)	61 (8)	0.047 (0.005)
CERCARIA 2	118 (19)	77 (21)	95 (14)	62 (6)	0.048 (0.006)

Text Fig. 17

Diagram to relate the appearance of an oscillating cercaria to the values for angular displacement of body, tail and furcae.

Axes are as in Text Figs. 13 - 16. Frames 1 - 4 correspond to those illustrated in Text Fig. 10.



All three traces in the upper graphs of Text Fig.13-16 oscillate about the zero line, with slightly varying amplitudes. The amplitude of the tail deviation is, on average, greater than that of the other parameters and regularly exceeds 90° , i.e. the tip of the tail points away from the direction of locomotion at the end of each power stroke. If the furcae were fixed in relation to the tail stem (the T-joint inflexible) then the *difference* between the furcae and tail stem (T-F) would remain constant. This is not the case, however, the maximum difference occurring during the power stroke, the minimum nearer the point of maximum tail deviation. This is a reflection of the fact that the furcae pivot, first one way then the other, during each cycle.

Although the three traces are almost in phase, that of the tail stem is slightly but consistently in advance of the other two. The lag correlation analysis also consistently shows a peak in correlation coefficient at lag intervals of 6 (the few exceptions only differing by 1) for all pairs of variables examined. The latter included the following:-

1. (B-T) v (T-F)
2. (T-F) v (T-F)
3. B v T
4. B v B
5. T v T
6. H v F
7. (B-T) v (B-T)

.... where B = angular deviations of body
T = angular deviations of tail
F = angular deviations of furcae

The coefficient values obtained for cercaria la are presented in Appendix 11. The same patterning of coefficients was also found for 1b, 2a and 2b. This type of analysis is not, of course, as valuable in dealing with such clearly patterned data as when the periodocities are not easily identified from the graphs alone.

2.4 DISCUSSION OF SECTION 2

Trematode cercariae have generally evolved as an important dispersive stage in the parasite life cycle and even when they do not travel very far after emergence from an intermediate host, the process of emergence itself usually involves active locomotion. Most cercariae possess a muscular tail and this organ may be highly modified with fin-folds, bristles, furcae etc.; it can be very large, relative to the size of the body. Propulsive mechanisms vary too, some involving a wave of muscular contraction, initiated at the tail base, then passing distally, while others involve an 'oar-like' action. That of *Schistosoma mansoni* falls into this latter category. The development of a type of striated myofibre in cercariae with rapidly oscillating tails has been discussed in Section 1.

In some cercariae (Cercariaea) there is no tail present and these larvae move in leech-like fashion, e.g. *Leucochloridium paradoxum*. This type of locomotion is also seen in Microcercous larvae in which the tail is reduced in size. However, as *Schistosoma mansoni* has been shown, after appropriate stimulation, to be capable of crawling too, this activity may be a fairly general property of cercarial neuromuscular systems. The locomotion of larvae *after entry* into host tissues has not yet been examined in detail for any species, but the technical difficulties involved may preclude the normal experimental approach, as described here.

A description of the locomotory movements of *Schistosoma mansoni* cercariae has been presented. These larvae can swim, using their muscular tail, or move over/through a solid medium by crawling and burrowing.

In burrowing, contraction of the circular and longitudinal body musculature is roughly symmetrical and might be compared to the activity of the musculature of burrowing annelids, i.e. attachment at the anterior end is followed by contraction of the longitudinal myofibres which draws the posterior end of the body forward. A more posterior attachment is then established and the anterior is thrust forward by contraction of anterior circular myofibres. Whereas in the annelids, such as *Lumbricus*, attachment is facilitated by the setae, in *Schistosoma* the backwardly-projecting tegumentary spines may have a comparable function, supplementing the grip of the suckers.

This symmetrical pattern of muscle action has only been observed in cercariae under certain circumstances, viz in cercariae slightly compressed under glass with insufficient space for any other form of locomotion. The movement appears well-coordinated, however, and it is reasonable to assume that it could occur during and after penetration of host tissue. Examination of post-penetration larvae in a semi-solid, transparent medium would, I believe, confirm this assumption.

Prior to actual penetration, cercariae attach to and crawl over the host surface; as in burrowing, only the body myofibres are responsible. The tail, if still attached, vibrates sporadically, pulling the posterior end of the body from side to side. The actions of this relatively large, muscular appendage appear, then, to be not only non-functional in crawling but possibly something of an encumbrance. The crawling action closely resembles the 'looping' movements of a leech with, in this case, two points of attachment, one ventral and one anterior. The ventral point is easily identified as the ventral sucker and appears capable of very firm attachment to a substratum. For example, cercariae attached to glass surfaces are not easily dislodged by water flow. This strength of attachment might be anticipated from the

muscular appearance of the suckers, as described in Section 1. The anterior point of attachment is sub-terminal but has not been identified positively here. It corresponds to the expected location of the mouth and it is possible that the mouth musculature is modified for attachment. The description of the cercarial alimentary canal by Ebrahimzadeh and Kraft (1969) supports this: they concluded that the mouth area could be actively opened and closed to operate as a sucker, and, furthermore, that the other muscles of the oral sucker (or anterior organ) could also be involved in this function. It is possible, therefore, that the diagrammatic reconstruction of oral sucker musculature presented here (Section 1) may be an oversimplification: some 'retractor' muscles may, in fact, be asymmetrically placed and be functionally related to the anterior point of attachment.

The cercarial body, unlike that of the leech, is not significantly flattened dorso-ventrally. Consequently, during the body flexure of crawling, the ventral surface becomes transversely 'creased' between the two suckers. The ventral, longitudinal myofibres at this point are probably in a highly contracted state.

A notable feature associated with the extension of the oral sucker (presumably when the circular muscles contract) is the protrusion of the apical disc, with its crescents of sensory receptors. This (together with the 'creasing' during body contraction - referred to above) was illustrated in Text Fig.12. Although it may appear likely one can only speculate that these receptors function in the selection of a new point of attachment. Protrusion of the apical disc might be brought about by contraction of the radial and circular muscles of the oral sucker diaphragm and of anteriorly placed circular myofibres in the body wall. This would have the effect of reducing the diameter

of the diaphragm and also making it less convex in shape. The resulting compression of tissues anteriorly could then force the apical disc region forward. Relaxation of these muscles together with contraction of the longitudinal body wall muscles and the retractors would cause the retraction of the apical disc and an increase in cross-sectional area of this region of the body. It is these changes which are thought to underlie the movements illustrated in Text Fig.12.

Although attachment of the ventral sucker may be followed by extension and flexure of the anterior end of the body, attachment of the oral sucker is immediately followed by the release of the ventral sucker and contraction of the longitudinal body wall musculature to produce a 'step' movement. The receptors on the inner surface of the ventral sucker could be mechanoreceptors involved in this 'stepping' action and it is possible that 'squash' mechanoreceptors within the body tissues could operate as well. The only structures found, which might conceivably come into this category, are the scattered ciliated cavities and the internal laminated nerve ending (see Section 1). However, these structures may serve other sensory modes more effectively.

The myofibres extending from the ventral sucker capsule to the dorsal body wall are probably involved in the stepping reflex, lifting the sucker clear of the substratum and moving it forward as the whole body flexes.

The tail is not involved in propulsion during crawling, but continues to oscillate sporadically and to flex both stem and furcae.

In contrast, propulsive force for swimming is largely a function of the caudal musculature. It is probable, however, that tension developed in body myofibres and smooth caudal myofibres is important in efficient swimming. As may be seen in the illustrations of this Section, the

entire body and tail oscillates during tail-first swimming. This is carried out at sufficient speed to appear as a blurred image - similar to a vibrating violin string. The oscillation occurs about two nodes, one near the distal end of the tail, the other in the region of the ventral sucker. The presence of 'striated' myofibres exclusively in the tail implies that the oscillation is driven by the tail; the body, whipped from side to side by the tail (during tail-first swimming) would add to the thrust in the manner of a single, stern-mounted oar on a boat. The effective length of the tail is increased by the furcae, and the latter also become flattened, further increasing the surface area over which the propulsive thrust is applied.

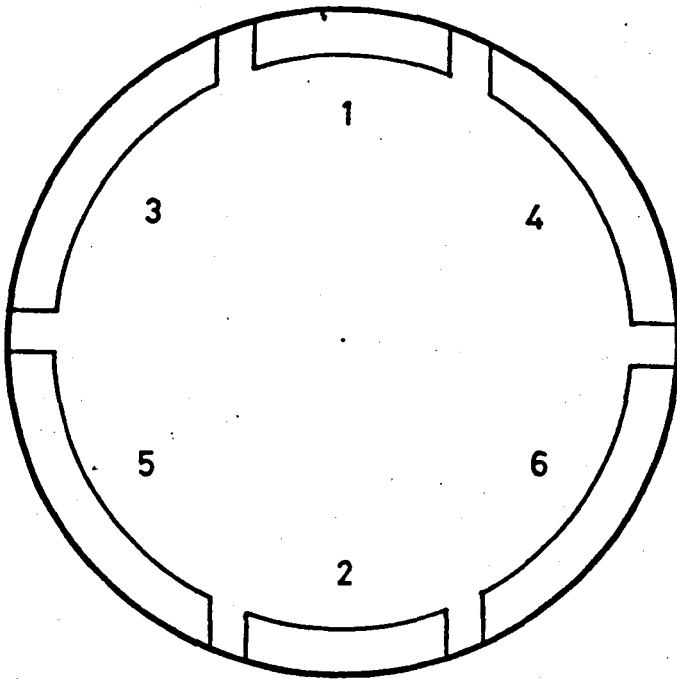
Tails which have been shed, but then quickly placed in 0.9% saline, may continue to oscillate intermittently, but without the regular switching on and off seen in complete larvae. So, although the oscillatory mechanism may reside in the tail, it is probable that the central nervous system of the body exerts a controlling influence. This could include some kind of 'pacemaker', the output of which is modified by sensory input.

The oscillation of the tail stem is achieved without a significant change in length of the stem. The musculature is therefore adapted for *bending* the stem rather than *shortening* it and the 'herringbone' pattern of longitudinal myofibres may be more efficient in this respect; it is also found in other cercarial species which bend their tails, at high frequency, from side to side. Presumably the striated myofibres of blocks 3-6 are mainly responsible (see Text Fig.18) with first 3 + 5 then 4 + 6 contracting briefly. A myofibre contraction of only 5 - 10% on one side or the other could probably account for the degree of tail-bending observed. The transmission electron micrographs (Section 1) often show very little space between the ends of thick

Text Fig. 18

Diagrammatic representation of layout of longitudinal tail muscle,
as it appears in TS.

TS TAIL STEM
PLAN OF LONGIT. MYOFIBRES



myofilaments and the ends of their sarcomeres: this must mean either that the muscle has been fixed in a contracted state or that it is only capable of shortening to a relatively small degree. The details of myofibre structure have been discussed more fully in Section 1. The non-striated myofibres of blocks 1 and 2 would not be expected to alter tension rapidly, with each oscillation, but may contribute to the overall tail movement by continuous maintenance of tension during each swimming period, as could the furcal myofibres. The contractions of the tail which occurred during crawling were almost certainly due largely to the myofibres of blocks 1 and 2 which would have been apposed by the circular myofibres.

The tail action of *Schistosoma* is therefore more 'oar-like' than wave-like in contrast to that of either *Cryptocotyle lingua* (Chapman, 1973) or *Echinoparyphium* sp. (Graefe and Burkert, 1972). In these latter species a wave appears to be initiated at the base of the tail and is then propagated distally. This propagation does not appear to take place in *Schistosoma* as here the flexure appears first in the central part of the tail stem. It may be that all the longitudinal myofibres of one side of the tail contract synchronously.

As could be seen, however, the end-result of these oscillatory movements is not simply propulsion of the larva, tail-first, through the water: a further component to the movement is added which causes a rotation of the larva about its longitudinal axis. Slight differences in the degree or timing of contraction between the muscle blocks of one side of the tail might account for the asymmetrical tail beat. Although some thrust which might otherwise be applied axially is used to drive this rotation, any loss in efficiency might be compensated for by the extra-long power stroke which is facilitated. If the oscillation was perfectly symmetrical, then vigorous tail-bending would bring the laterally-extended furcae into contact with the sides of the body. The

asymmetrical movement allows the furcae to pass the body, either dorsally or ventrally, during a full tail flexure. The rotation probably confers certain constraints on the sensory capabilities of the larvae, however, these being discussed more fully in Section 3.

The *rates* of oscillation of body, tail stem and furcae are all the same, but their *phase* differs marginally; the tail deviation is consistently just ahead of that of the other structures. Furthermore, the angular velocity of the tail is higher. These observations offer further support to the theory that the tail is 'driving' the oscillation.

Using the filming technique it was not possible to record accurately the angular deviation of any part of the larva for more than a few consecutive cycles because of the continual rotation about the longitudinal axis during swimming. When the oscillation could be viewed dorsally or ventrally, the tail stem and furcae were clearly visible and accurate measurements could be made. However, when the movement was viewed laterally, because the lashing of the tail was either directly towards or away from the camera, the degree of tail bending etc. could not be seen. Between these two extremes there was an intermediate range of positions from which measurements could be made but which included an error, the magnitude of which varied with the exact plane of oscillation.

This effect is easily demonstrated by the use of a model such as a flexible rule or piece of thick wire. The model is first bent and the angle of bending measured. It is then positioned above a piece of white card and a light used to cast a shadow of the model on the card. The card and shadow then represents what is recorded on a cine film. The model can then be rotated about its longitudinal axis, in stages, and the apparent change in the angle of bending is measured on the shadow. By this simple means it is possible to

estimate the type of error inherent in film-based data.

The effect of this error on, for example, the tail deviation measurements, was to increase high values ($>90^\circ$) and decrease low values ($<90^\circ$). Correction of errors would not, however, significantly alter the waveform shown in Text Figs. 13-16: the more prominent peaks would be trimmed back slightly; points at or near the 0° axis are subject to little or no 'rotation error' anyway; because of the high angular velocity of the tail during the power stroke, and hence very steep slope, many individual points on the trace would remain relatively close to the original line after correction.

At this point, a further comment on this type of analysis would be appropriate. Chapman and Wilson (1973) using cooled *Himasthla secunda* were able to calculate a mean value for each point of their cycle - see their Fig.6. The variation about these means also appears small. This result is only possible when the oscillation of the larva under observation is very closely *in phase* with the rate at which film frames are exposed. For example, if a full cycle occupies, say, 15 frames exactly then frame 16 will closely match frame 1. Consecutive cycles can therefore be superimposed on a graph, the X-axis of which is simply frame 1 \rightarrow frame 15 (frame 16-30 etc). If, however, a full cycle does not occupy exactly 15 frames but, say 14.5, then the two consecutive cycles cannot be superimposed in the same way. The same principle applies whatever number of frames match one complete cycle. The fortuitous match between film speed and larval oscillation was not obtained so precisely for *Schistosoma mansoni*. In this case there were approximately 6 frames per cycle, but the points within any one cycle were not always directly comparable to those in subsequent cycles. This can be demonstrated for the results in Text Fig.16 by tracing the first tail stem cycle, then superimposing

the four subsequent cycles by displacing the tracing paper along the X-axis not by 6 frames exactly, but by 6.4. The results of this are shown in Text Fig.19. It is implied, for example, that whereas the +ve peak in cycle 1 may approximate to the *true* maximum deviation, the high values in cycles 2 and 3 correspond to the tail stem deviation *just before* and *just after* the true maximum.

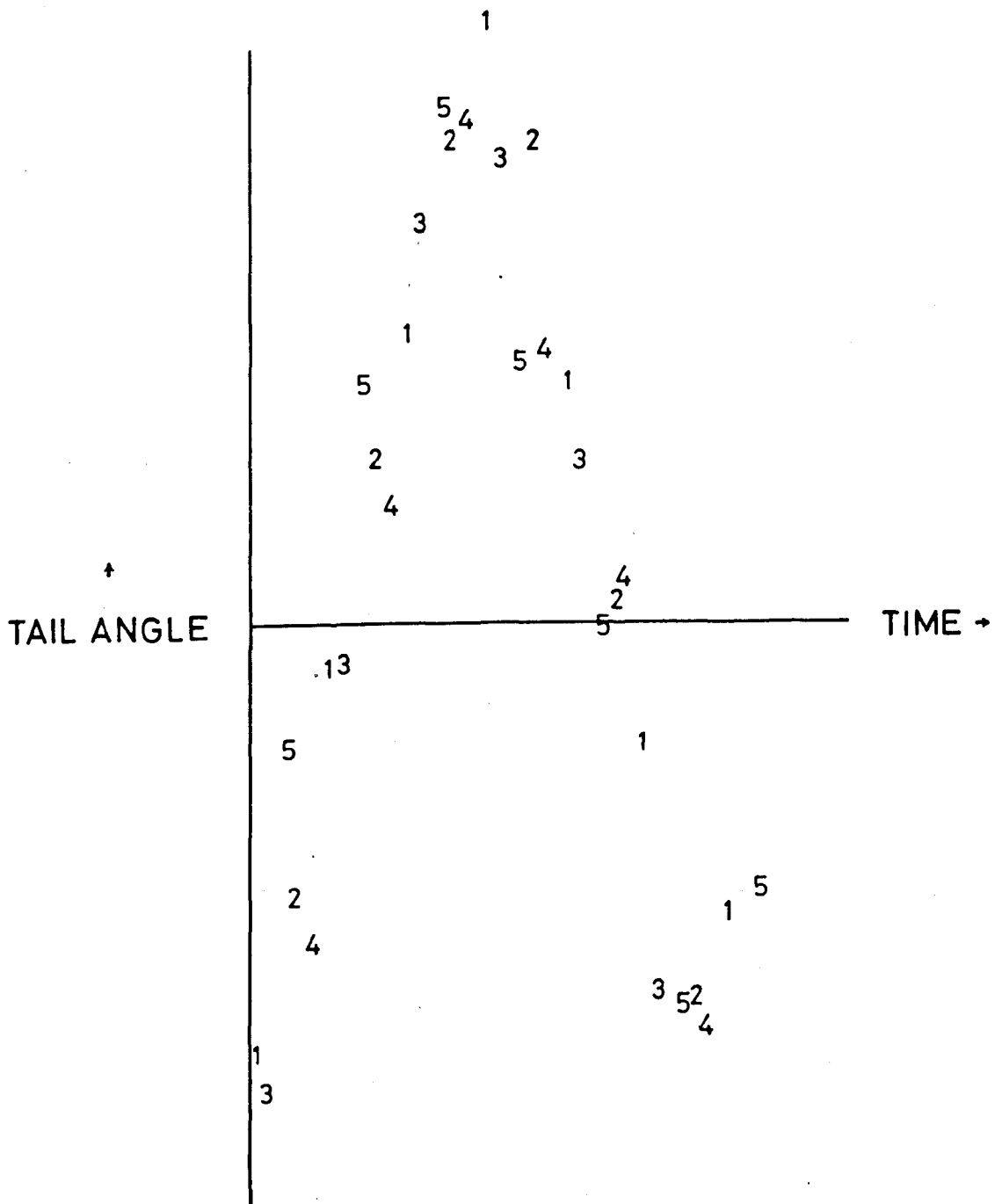
The overall larval speed which is attained by means of this oscillation depends to a significant extent on the ambient temperature. Valle et al (1974) have shown that the tail-first rate of propulsion in *Schistosoma mansoni* is less than 0.4mm/sec at 15°C but could be more than 2.0mm/sec at 40°C. (At higher temperatures, cercariae do not remain active for long). The speed/temperature relationship was found to be linear by Valle et al. Other factors may influence the speeds achieved, however, as those I recorded tended to be slightly lower than would have been predicted from Valle's data. My results are not based on a similarly large sample, however, and may not be truly representative of even the schistosome population I was using. If they *were* representative then variability between the Brazilian and British populations might still account for the differences in observed speed: size, weight, thrust achieved per oscillation will all show even individual variability. The accuracy of measurements concerning time/displacement taken from cine film is likely to be greater than that obtained by manual operation of a timer as used by Valle et al. This error has to be taken into account in the method used here for measuring active periods (the kymograph + morse key method).

Chapman and Wilson (1973) gave no information regarding the speeds attained by either *Himasthla secunda* or *Cryptocotyle lingua*. Graefe et al (1967) calculated the maximum speed for *Schistosoma mansoni* cercariae as 1.4mm/sec but they did not define their temperature

Text Fig. 19

The overall waveform produced by superimposing successive cycles from Text Fig. 16 as described in the text. The numbers represent the cycle number.

CERCARIA 2b



conditions. Graefe and Burkert (1972) subsequently also filmed the cercariae of *Diplostomum spathaceum* and *Echinoparyphium* spp. and calculated the respective speeds to be 3.7mm/sec and 2.5mm/sec.

Diplostomum spathaceum therefore swims not only faster in absolute terms but at a speed equivalent to about 10 times its own length per sec. These authors again did not define the temperature at which their experiments were conducted.

These differences in speed between species cannot invariably be correlated with the different types of tail. The large echinostome cercaria (above) has an unbranched tail which develops a waveform quite different from that of either *Schistosoma* or *Diplostomum*. The furcae of *Schistosoma* remain relatively rigid and straight during oscillation of the tail whereas those of *Diplostomum* are much longer, and more flexible; during the power stroke, the 'trailing' furca of *Diplostomum* bends right back, offering minimal resistance to the water while even the 'leading' furca applying the thrust bends to some extent. A whip-like effect is therefore produced at each cycle in these long furcae.

In the furcocercous cercariae, the myofibres of the leading, perhaps both, furcae are likely to be held under tension during locomotion, so that flexing is minimized and the maximum surface area used for the thrust. As there is no striated muscle in the furcae of schistosome cercariae one would not expect this tension to be varied during the course of a single cycle, but rather that an overall tension would be maintained for the duration of a swimming period. The bending observed (possibly in *Diplostomum* too) could be solely a hydrodynamic effect on tails which are effectively "sprung" in the 90° or T-position by both muscle action and connective tissue layout etc. In the 'resting' position, *Schistosoma* furcae are frequently curled round towards the tail stem: this could indicate an underlying tendency to resist bending back during the power strokes of the tail, in addition to the tension

which may be developed in the furcal longitudinal myofibres.

The observed layout of furcal longitudinal myofibres is problematical.

The above arguments would imply that any muscular effort needed in the furcae during swimming would be directed towards bending the furcae towards the tail stem, i.e. against the resistance of the water.

However the myofibres on the posterior side of the furcae in *Schistosoma mansoni* are greater in cross-section than those on the anterior side (see Section 1). Contraction of the posterior fibres would cause bending *away* from the tail stem. They might be involved in moving the furcae into line with the tail stem when the cercaria swims body-first.

Not only is tail-first swimming switched on and off very rapidly but immediate switching from tail-first to body-first swimming sometimes occurs. This is seen in stimulated larvae, but its adaptive purpose is not clear. Croll (1972) described the movement patterns of nematodes in which forward progression was periodically interrupted by reversals. Sudden change in environmental conditions and larval age were two of the variables which could influence the frequency of these short reversals. Clearly, however, the action formed a normal part of the locomotory behaviour of the nematodes studied (*Trichonema* and *Ancylostoma* spp.) in contrast to the reversals (*to body-first swimming!*) in schistosomes which are only displayed by stimulated larvae.

The 'normal' tail-first mode does seem to be inconsistent with the general rule that organisms tend to develop their highest concentration of sense organs on that part of their body which is foremost during locomotion. The advantages of sampling environmental stimuli just in front by the actively moving animal are often quoted as an important selective force in the development of this concentration of sense organs.

The speed of locomotion in schistosomes is similar by tail-first and body-first modes and so an explanation other than speed must be sought for the presence of the body-first mode. One possibility is that although speed is similar, efficiency may be higher in the tail-first mode, i.e. less energy is expended. In relation to this, the normal, unstimulated swimming pattern should be considered. As described earlier, unstimulated larvae do not swim continuously but in short bursts lasting only seconds. As progression is tail-first and as the cercarial body is heavier than its tail, each larva alternately swims towards the surface then sinks back again. The general 'stream-lined' shape and the laterally-projecting furcae are probably important elements in the maintenance of a vertical primary orientation during sinking. The furcal receptors which, as previously suggested may trigger inhibitory reflexes, are therefore always first to strike the surface film when this is reached. If the purpose of the on-off activity is indeed simply to maintain position, clear of the substratum (see Nuttman, 1974; also Section 3 this report) then the observed swimming behaviour involves the absolute minimum of muscular activity, orientation reflexes etc., and would therefore appear very efficient in this respect.

A predominance of tail-first activity, at least during 'unstimulated' activity and possibly during 'stimulated' activity too might consequently be expected. The original question remains however, 'Why does the cercaria periodically resort to body-first swimming, utilizing different patterns of apparently well-coordinated muscular activity?'

The precise waveform taken by the schistosome tail during body-first swimming has not been examined yet in detail. It appears that a travelling wave, initiated at the base, may be involved. The furcae are closely apposed and effectively lengthen the tail. Changes in the

overall length of the body occur and both body/tail and tail/furcae junctions appear broader and less flexible. This would presumably be due to contraction of the longitudinal myofibres at these locations. These changes were also noted by Graefe et al (1967).

Schistosome cercariae are therefore able to propel themselves through water or over solid surfaces by several distinct mechanisms: swimming tail first (the normal mode), swimming body-first (only sporadically, when stimulated), crawling by means of attachment with suckers, and finally a (presumed) technique for burrowing. Whereas the techniques for moving over or through the substratum may be similar in different species, the cercarial tail appears to be capable of a number of distinct, well-coordinated movements, some of which are associated with an unusual type of striated myofibre.

SECTION 3 : BEHAVIOUR

2.1 INTRODUCTION TO SECTION 3

"Dogs ... exhibit a manifest superiority to whole races or classes of man, both civilized and savage, in the following respects, which include the noblest of human virtues:

1. Heroism, patriotism, self-sacrifice
2. Compassion or sympathy, charity, benevolence, forgiveness
3. Love and adoration of a master
4. Fidelity to trust, duty or friendship etc."

This quotation, from Lindsay (1880) may appear somewhat remote from the behaviour of parasites as described later in this section, but it serves to illustrate a particular stage in the conceptual development of studies in animal behaviour. Whereas the Darwinian theory of evolution had given Biology a new unity, the comparative psychologists of the time were still searching for a similarly all-embracing approach. Evolutionary theory eroded the previously rigid and basic distinctions between man and the other animals and this led to a tendency to attribute other vertebrates and even invertebrates with high-order mental processes - hence the above quotation.

The 'backlash' was inevitable. It gained momentum towards the end of the last century, the most prominent worker in the field being Jacques Loeb. Using a terminology previously used by the botanists, Loeb applied the principle of the TROPISM to animal species, *viz* 'forced' movement in direct response to environmental stimuli. Bilateral muscle 'tonus' as a reflection of bilateral stimulation of receptors was an integral part of the theory. Some of Loeb's ideas could, in fact, be developed to account reasonably for the reflexive behaviour of certain invertebrates, but over the years he insisted on the theory being all-embracing. His refusal to admit to its limitations has consequently made him appear as much an extremist as Lindsay. The term 'tropism' soon became restricted, first to the movements of

sessile animals and then to the growth movements of plants (from whence it came!). It is, however, interesting to note its appearance more recently in Kloetzel (1958, 1960).

Reflexive orientation movements in animals are now classified in terms of the nature of the stimulus, the capacity of the receptors and the manoeuvres made by the animals. The first such classification was that of Kühn (1919) and this was later modified by Fraenkel and Gunn (1961) to include three basic categories. These were KINESIS, TAXIS and TRANSVERSE ORIENTATIONS. The last of these included the light compass and dorsal light reactions and the orientation with respect to gravity. Kineses are divided into two groups:

ORTHOKINESIS is an undirected locomotor response in which the speed or frequency of movement is a function of the intensity of stimulation.

KLINOKINESIS is a response in which the frequency or amount of turning per unit time is a function of the intensity of stimulation.

i.e. kineses are undirected responses, there being no continuous orientation of the body axis with respect to the stimulus source. The direction of turning is random. Taxes, on the other hand, are directed responses in which such an orientation is maintained and locomotion is either directly towards or away from the source.

In KLINOTAXIS, attainment of orientation is by means of regular lateral deviations of part or all of the body and comparison of the intensities of stimulation at successive time intervals. As for the kinetic responses, klinotaxis could, therefore, be attained with the use of a single intensity receptor (in theory).

In TROPOTAXIS there is a simultaneous comparison of the intensities of stimulation on the two sides and the animal turns towards the more stimulated side. Paired intensity receptors are therefore required.

TELOTAXIS involves orientation directly towards a definite source, without bilateral comparison of intensities; a number of receptors, pointing in different directions are required.

These types of responses have frequently been assumed (sometimes with experimental backing, sometimes without) to underlie the observed behaviour of a variety of invertebrate species. The means by which an animal might locate and move towards (or away from) a stimulus by a tactic response can easily be appreciated, but there is relatively little direct evidence to show that klinokinesis can effect displacement along a stimulus gradient. Ulliyott (1936a) reasoned that sensory adaptation would have to be an integral part of the response and when Rohlf and Davenport (1969) simulated this type of behaviour using a digital computer, they corroborated Ulliyott's conclusion. They found that displacement of organisms along a gradient could occur by kinetic responses but only if sensory adaptation was incorporated into the model: inverse klinokinesis (less turning at higher stimulus intensity) resulted in displacement towards the 'higher' end of the gradient, whereas direct klinokinesis (more turning at higher stimulus intensity) resulted in displacement to the 'lower' end.

The terminology of Fraenkel and Gunn has often been used rather loosely - this despite the clear intention of these authors to bring together information from many behavioural investigations and to organize a previously confused terminology. They stressed, however, that theirs should not be regarded as a final or even fully adequate classification. Any divergence from the scheme should be carefully defined, though. This is necessary because of the implications of the behavioural terms, for example in relation to the distribution and nature of the receptors involved. Demonstration of the accumulation of organisms at one end of a gradient, over a period of time, is not sufficient evidence for a tactic response. The supposed "positive phototaxis" of *Posthodiplostomum*

cuticola (Dönges, 1963) may well occur, but the evidence provided does not, in itself, justify classification of the response as a taxis rather than a kinesis.

Many of the earlier investigations of taxes and kineses in invertebrates were carried out using turbellarians, e.g. Walter (1907), Taliaferro (1920), Koehler (1932) and Ulliyott (1936a,b). For a detailed review see Fraenkel and Gunn (1961). Koehler (1932) considered that *Planaria lugubris* showed a wide range of behavioural responses to a food source, including ortho- and klinokinesis, klino- and tropotaxis. Chemoreceptors were located anteriorly in two symmetrically placed areas. At some distance from the source, the first overt sign of stimulus reception was an increase in activity. The anterior end of the planarian was then swung from side to side, possibly so that successive comparisons of stimulus intensity could be made, allowing orientation by chemo-klinotaxis. Close to the source, at high stimulus intensity orientation was thought to be tropotactic.

The 'mechanistic' approach can still provide useful information in behavioural investigations of relatively simple organisms, but as Fraenkel and Gunn (1961) have said "... the more complex the receptor - nervous - effector system, the more difficult it is to describe an animal's behaviour in terms of one of these simple types". The behavioural repertoire of some helminths is probably amenable to this type of analysis, but relatively few quantitative studies have been reported.

The process of infection of a host by a motile parasitic larva may be considered in several chronological stages, (based, in part, on Wright, 1959) :

- (a) The parasite must be attracted to, or otherwise reach, the host's environment,

- (b) remain viable until approached by the host,
- (c) either actively orientate towards the host or make direct contact by chance movements alone,
- (d) enter the host's body, e.g. by direct penetration or by being ingested,
- (e) migrate to its specific site within the host.

Most investigations have concentrated primarily on one or two of these stages at a time, but there are several recent reviews of helminth behaviour e.g. Cable (1972), Croll (1972), Llewellyn (1972) and Ulmer (1971). Ulmer emphasized site selection within the host, a field in which relatively little information appears to be available. Many examples of migration routes and final site specificity were given but, as Ulmer said "... factors responsible for this localization remain speculative or wholly unknown".

The behaviour of free-living parasite larvae is more amenable to analysis and a great deal more information is consequently available. However, this has not precluded a measure of disagreement as to whether host location is the result of active orientation by the larvae or of purely random movements; this has applied in particular to studies of miracidia. One of the first accounts of an apparent attraction of miracidia to a host snail was that of Leiper and Atkinson (1915), the species used being *Schistosoma japonicum* and *Oncomelania nosophora*. Subsequent authors who have provided evidence for, or who agree in principle with, the chemo-attraction hypothesis are listed below, with an indication of the parasite species studied:

SCHISTOSOME SPP. - Lutz (1919), Faust (1924), Faust and Meleney (1924), Faust and Hoffman (1934), Brumpt (1940), Neuhaus (1952), Davenport et al (1962), MacInnis (1963, 1965), Etges and Decker (1963), Plempel (1964), Plempel et al (1966), Kloetzel (1958, 1960), Shiff (1968, 1969),

Chernin and Perlstein (1969), Chernin (1970, 1972), Shiff and Kriel (1970), Wright and Ronald (1972).

FASCIOLA - Neuhaus (1941, 1953b), Kendall (1965), Wilson and Denison (1970 a, b).

OTHER SPECIES - Barlow (1925), Mathias (1925), Tubangui and Pasco (1933), Campbell (1961), Kawashima et al (1961), Ulmer (1971).

No significant 'attraction' by host chemicals was found by the following authors:

SCHISTOSOME SPP. - Cowper (1947), Stirewalt (1951), Chu and Cutress (1954), Najim (1956), Barbosa (1960), Sudds (1960), Chernin and Dunavan (1962), Wajdi (1964).

FASCIOLA - Mattes (1926, 1936, 1949a, 1949b), Griffiths (1939).

OTHER SPECIES - Stunkard (1943), Ulmer and Sommer (1957), Crandall (1960).

A variety of techniques was used in these studies, but the majority involved, in principle, measurement of the same parameter, i.e. the change in distribution of larvae after an applied stimulus. Despite the frequent use of the terms 'taxis' and 'kinesis', very few authors indeed have attempted to actually monitor the movements of larvae accurately and to quantify them; many give no details of locomotory behaviour at all, but merely record distribution 'before and after'.

MacInnis (1965) has attempted a classification of miracidial responses in the presence of chemical "attractants". The system was not suitable for quantitative comparative studies, however. A vital return to the use of quantitative parameters was made by Wilson and Denison (1970 a, b). They measured the distance moved per second and the total angle turned per mm by the miracidium of *Fasciola hepatica*. Environmental factors such as temperature flux and pO_2 influenced the distance moved but this

was assumed to be a direct metabolic effect, not mediated by sensory receptors. It was thought that the increase in the angle turned, in the presence of snail mucus, was a receptor - mediated effect.

The most sophisticated 'hardware' to be used so far in recording larval locomotion was that developed by Davenport and his team (Davenport et al 1962; Davenport, 1969; Davenport et al 1970). The movements of organisms were recorded on videotape and the video signal was processed for quantification of the data and analysis by digital computer.

Little clear progress has been made in the identification of chemicals responsible for modifying miracidial behaviour, in natural circumstances. Chernin (1970) has found that a thermostable, water-soluble molecule (M.W. <500) is emitted from *Biomphalaria glabrata* and which significantly modifies miracidial behaviour. Wilson and Denison (1970) found that short-chain fatty acids (C6-C10) caused a progressive increase in the rate of turning of *Fasciola hepatica* miracidia. Short chain fatty acids and some amino acids had previously been found to stimulate *Schistosoma mansoni* miracidia (MacInnis, 1965).

Kearn (1967, 1971) has described the behaviour of the oncomiracidium of *Entobdella soleae*. It appeared that chemicals present in the epidermis of the sole allowed a fairly accurate degree of selection by larvae, even in the presence of skin from related fish. The stimulant was thought to occur as a component of the mucus secreted by the epidermal cells.

The literature pertaining to cercarial behaviour is not as extensive as that for miracidia, but a number of recent publications have been concerned with the identification of stimulatory chemicals from the host surface. It would appear however, that different parasite species respond to different types of chemical stimulus (e.g. compare Clegg, 1969; Shiff et al, 1972; this report). These papers, and the others

relating to the behaviour of cercariae are reviewed in more detail in the following chapters.

The technique of tracking has been applied to nematode behaviour (e.g. Sandstedt et al, 1961; Croll, 1965). This has usually been accomplished by allowing the worm to move across the surface of an agar plate. The resulting undulating track can then be photographed. Croll (1972) said that "... there seems sufficient data to assume that taxes as well as kineses ... occur in larval nematodes"; but, in fact, in few instances has there been any attempt to quantify data in a way that would justify this statement. Computer simulation of tracks (Croll and Blair, 1973) allows some inferences to be drawn with regard to the adaptive significance of observed track patterns.

In Section 2 the mechanisms of cercarial locomotion were described, including both swimming and crawling phases. Another perspective into the behaviour of cercariae may be developed by monitoring the overall displacement of larvae as a function of time, i.e. track analysis. This approach should be distinguished from that in which the initial and final distributions of a population of larvae are recorded. The latter technique gives no information on the way in which the new distribution is achieved, *viz.* by kinesis or taxis. In view of the long-established principles for quantitatively discriminating between the various reflexive orientation movements, (Fraenkel and Gunn, 1961), it is surprising that no information of this kind has ever been published which relates to cercariae.

Contact between cercaria and host has been regarded by many as a chance event only, host recognition being achieved only after direct contact had been made. Other authors have observed that cercarial behaviour is modified in the proximity of the host. There are a number of ways in which a host might signal its presence to an infective larva and any

of the main sensory modes could be involved. Chemicals diffusing from the host surface could supply general or specific information; heat energy from a warm-blooded host might diffuse some distance into the surrounding water; the host's shadow might be monitored by larval photoreceptors.

A positive shadow response (i.e. locomotion initiated) has been observed in a number of schistosome species (Cort and Talbot, 1936; Bracket, 1940; Neuhaus, 1952; own observations) and in several other groups by Miller and McCoy (1930), Miller and Mahaffy (1930), Dönges (1963, 1964), Haas (1969, 1971). Not all species studied respond positively, however, (Cort and Talbot, 1936; Bracket, 1940). Where found, the swimming response only lasts a short time, e.g. Haas (1969).

Responses to chemical stimuli were not thought to occur by Miller and McCoy (1930) and McCoy (1935). In a review of cercarial behaviour Smyth (1966) concluded that "There is no evidence that chemotaxis is involved in cercarial location of a host". Komiya (1966) considered that there was no evidence for active host location by *Clonorchis sinensis* cercariae in response to chemical stimuli, although this does not seem consistent with the observations of Faust and Khan (1927) who noted behavioural modification some distance from the host. Although track analyses have not been made, several authors report that cercarial behaviour changes, presumably in response to chemical stimuli, when the host surface is approached, e.g. Bolwig (1955) and Stirewalt (1971) in *Schistosoma mansoni*, Goodchild (1960) in *Spirorchis elegans*, Campbell (1971) in *Cotylurus flabelliformis*.

Where a positive response has been recorded, the assumption has usually been that the probability of contact between the larva and its host would be increased. It is not always so obvious, however, that this would necessarily be the case.

The main aims of Section 3 were firstly to record the movements of cercariae and carry out a quantitative track analysis and secondly to examine the relationship between chemical/thermal stimuli and the attachment response of *Schistosoma mansoni* cercariae.

3.2 HOST RECOGNITION (1)

THE HEAT STIMULUS

INTRODUCTION

An early attempt to quantify the temperature response of schistosome cercariae was that of Bolwig (1955). He added a suspension of *Schistosoma* cercariae to each of a pair of small glass tubes. The temperature of one tube was then raised 4 - 16°C above that of the other and after 10 minutes the number of "settlers" was counted in each. The results indicated that the number of cercariae attaching to a solid surface was directly proportional to the increase in temperature. It is not clear, however, if a temperature gradient existed in the heated tube during the 10 minutes or, alternatively, if a higher overall temperature was quickly attained and then maintained.

Bolwig also related the observed response to the temperature at which larvae were shed from infected snails. If the shedding temperature was 28.5°C then none of the larvae attached as long as the temperature was held constant. If it was reduced to 21°C, however, and then raised slightly, attachments could be observed even if the final temperature was less than 28.5°C.

More recently, Stirewalt and Uy (1969) and Stirewalt (1970, 1971) have shown the importance of a temperature differential in stimulating cercarial penetration responses. The latter were measured in terms of the schistosomule harvest in an *in vitro* system during a 3 hour collecting period and the temperature differential used was up to 13 - 14°C (collection medium warmer). The absence of any differential, even in the presence of chemical stimulants, was associated with low schistosomule harvests. Stirewalt (1970) also counted the numbers of cercariae observed attaching to the test membrane within 8 minutes of exposure to the differential: no responses were recorded within this

time if a uniform temperature existed throughout the apparatus; a 100% response was obtained in the presence of a differential. The minimum gradient necessary was not determined.

Stirewalt and Fregeau (1965) studied the percentage of penetrants (of mouse tails) for a range of constant temperatures. They found that penetration by at least some larvae occurs throughout the range 7 - 45°C during a one hour exposure; usually, between 13 - 35°C over 40% penetrated, there being no clear optimum within these limits. De Witt (1965) harvested adult worms from mice exposed to infection for 30 minutes at a range of temperatures from 0 - 45°C and demonstrated a clearer optimum at approximately 30 - 32°C, with a relatively sharp fall-off above 35°C. Purnell (1966) obtained the slightly lower optimum of 24 - 27°C. He also harvested adults from infected mice but allowed one hour for cercarial penetration.

The 'temperature effect' has also been found in other species: Clegg (1969) found that four times as many *Austrobilharzia* cercariae penetrated a gelatine membrane (with added cholesterol) at 40°C than at 25°C.

It should be noted that all of these experiments involved either *in vivo* infections or penetration of test membranes to which stimulant chemicals had been added. Under certain conditions, larvae may attach temporarily to a warm but clean glass surface and crawl for a short time before swimming again, without any attempt to penetrate. A comparable response was noted by Austin et al (1972). This serves to illustrate the important point that the processes of (a) host location (b) attachment and (c) penetration may not necessarily be brought about by the same stimulus.

It seems to be accepted at present (e.g. by Stirewalt 1971, Austin et al 1972) that heat is the remotely sensed stimulus in host location by schistosome cercariae and that chemicals on the skin surface trigger both attachment and penetration responses. The latter have not usually been distinguished in investigations of infection by trematode larvae. An exception was Haas (1971) who did consider that different stimuli were responsible for attachment and penetration in *Diplostomum spathaceum*.

The infection of a mammalian host by *Schistosoma mansoni* cercariae should not be approached as an 'all-or-nothing' process but more a graded response which changes as a function of time and environmental conditions. Swimming behaviour may be modified by host-related factors without attachment and crawling taking place. Similarly, crawling may be triggered without the glandular activity associated with actual penetration.

The aim of this section of the investigation was to define in quantitative terms how *Schistosoma mansoni* cercariae respond to both constant and varying temperature.

Materials and Methods

A glass observation chamber (Fig. 153 and Text Fig.20) was mounted on the stage of a binocular dissecting microscope and cercariae pipetted into the central chamber for each of the tests described below. A hand tally was used in counting cercariae, and a 'Comark' electronic thermometer with a copper/constantan thermocouple used to measure the temperature inside the chamber.

Only cercariae which had been shed less than two hours previously were used.

A control experiment was first carried out to determine the proportion of cercariae swimming, crawling and inactive in water kept at the same temperature from the time of shedding. Modification of the water flow through the glass cell allowed similar counts to be made either in the presence of stable temperature gradients or after a sudden increase in temperature.

a. Constant temperature controls

For all tests cercariae were obtained from infected snails by placing 6 - 8 of the latter into 50mls of filtered, dechlorinated tap water at 20°C for 1 hour. Approximately 20 - 25 cercariae were then pipetted into the central (observation) chamber of the glass cell, (see Text Fig. 21 A for water flow pattern). The temperature of the whole cell was maintained at 20°C. Because it was found that pipetting could cause at least some larvae to attach and crawl temporarily, time was allowed after pipetting for this enhanced level of activity to subside. Counts were then made of the number of larvae:

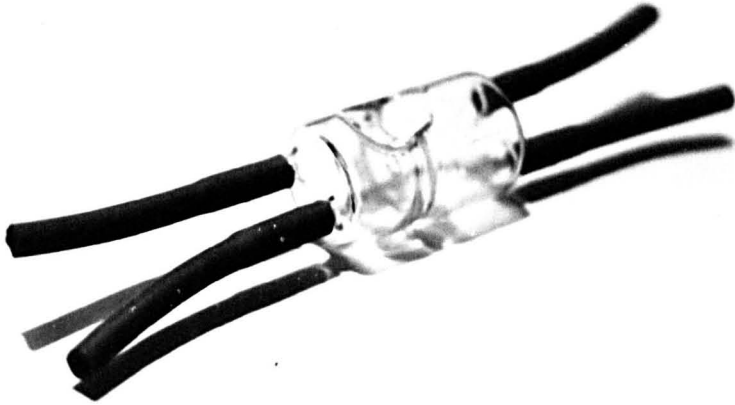
- (a) swimming (both active and passive phases included in this category);
- (b) inactive (those lying motionless on the floor of the chamber);
- (c) crawling on the glass surface.

Fig. 153

Photograph of flow cell used in behaviour experiments. Short lengths of rubber tubing are attached to illustrate their position. Normally this tubing leads to the reservoir/circulator.

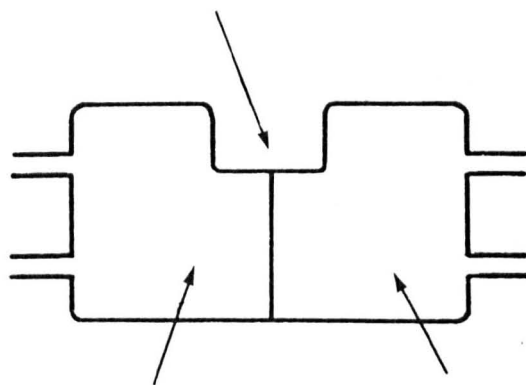
Text Fig. 20

Diagrammatic representation of section through flow cell.



DIAG. of SECTION through CELL

OBSERVATION CHAMBER



WATER at $T_1^\circ\text{C}$

WATER at $T_2^\circ\text{C}$

10 samples were counted in this way, and a total of 232 cercariae were examined at this temperature.

The entire procedure, including the shedding of cercariae from infected snails, was then repeated at each of a series of higher temperatures, up to 34°C. Above 34°C the shedding snails tended to react by withdrawing their antennae and by cessation of locomotion and a high proportion of the cercariae were found to be inactive. These high-temperature readings are not included in the accompanying figures.

b. Rapid rise in temperature

Cercariae were shed at 29°C and an individual was then transferred into the observation chamber (water flow as in Text Fig. 21 C) equilibrated at the same temperature. After 1 minute the water supply to the cell was switched to 39°C. After 1 further minute the activity of the cercariae was noted. A total of 100 cercariae were each examined in this way. The inside of the chamber was found to rise to the new temperature during the course of just over a minute - see Text Fig. 22.

c. Temperature gradient

Water flow was as shown in Text Fig. 21 B. where $T_1 = 29^\circ\text{C}$ and $T_2 = 39^\circ\text{C}$. 1ml of water was added to the central chamber and time allowed for equilibration. A sample of approximately 20 cercariae, shed at 29°C was then added to this in a minimal volume of water. After a further minute had elapsed, the location of cercariae in the chamber was noted.

Further samples were tested similarly until the responses of 128 cercariae in all were monitored.

Replication of this experiment on four successive days, using similar numbers of freshly-shed cercariae, gave very consistent results.

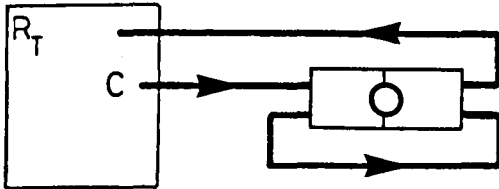
Text Fig. 21

WATER FLOW CIRCUITS

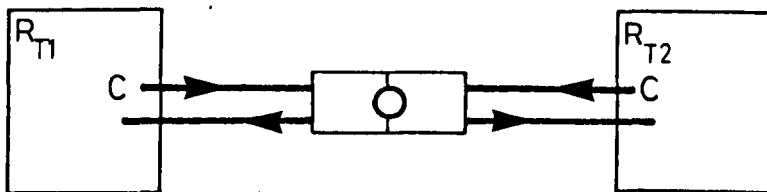
(Not to scale)

R=Reservoir C=Circotherm unit * =Switch

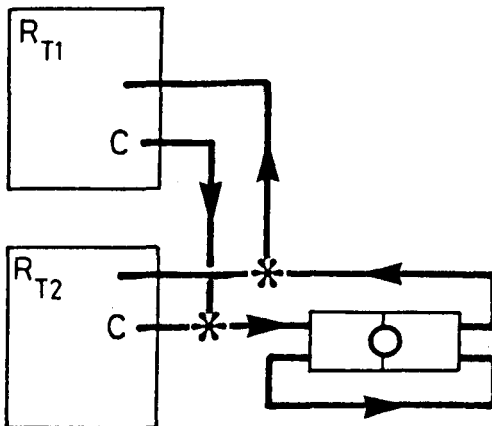
A. SAME TEMP. BOTH SIDES $T_1=T_2^\circ\text{C}$



B. TEMP. GRADIENT $T_1-T_2^\circ\text{C}$

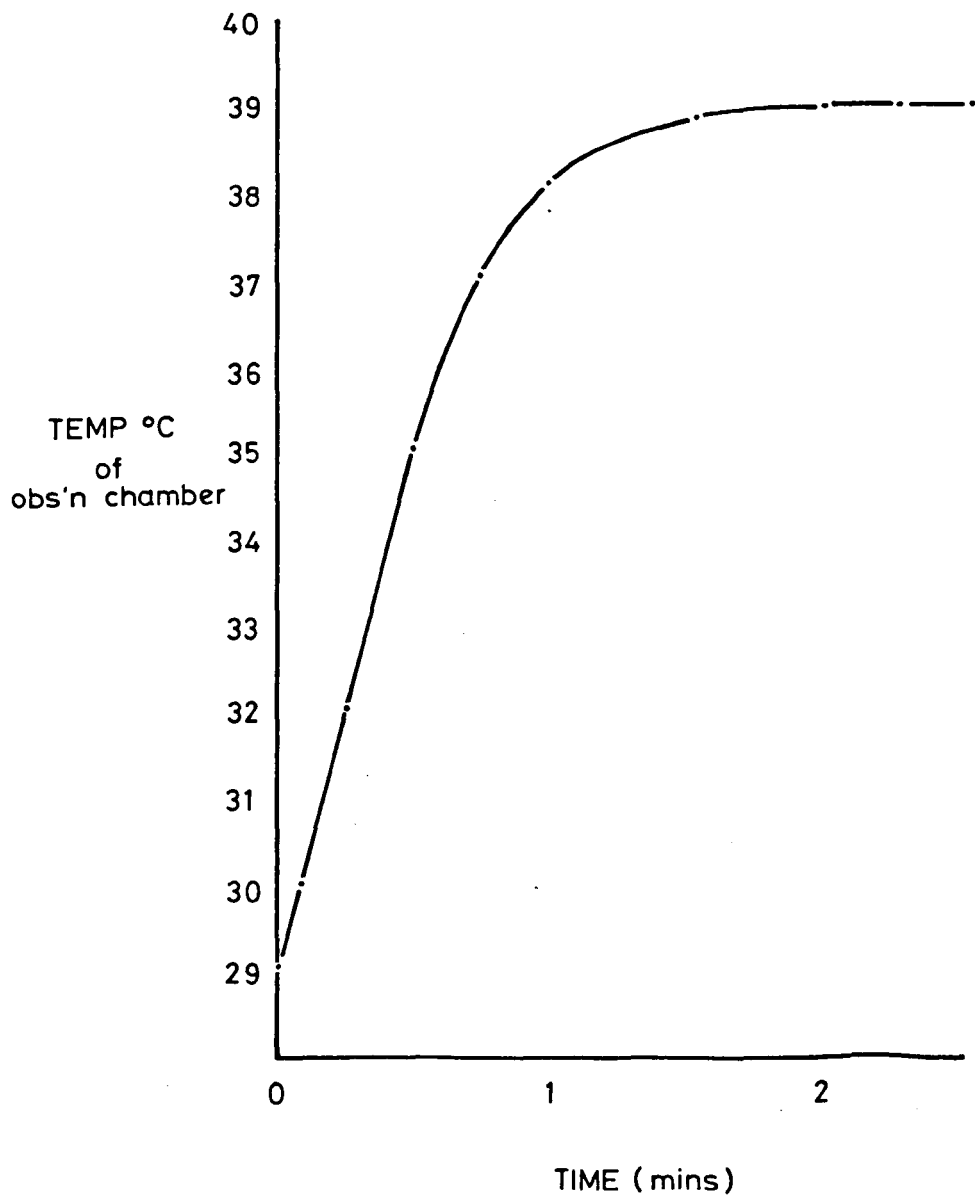


C. RAPID SWITCHING $T_1 \rightarrow T_2^\circ\text{C}$



Text Fig. 22

Temp / Time curve for glass cell after water temp switched from 29 - 39 °C



In order to test for the possibility that some factor other than temperature, e.g. the cell itself or lighting conditions, might have affected the distribution, the following experiment was carried out:

Firstly, the cell was equilibrated at 29°C (both sides) and the distribution of added larvae noted as described above. The glass cell alone was then disconnected and reversed in position and the observations repeated with fresh larvae. Totals of 70 and 71 cercariae respectively were used.

Further Tests using smaller temperature differentials and in the presence of human skin lipid (crude extract) were also carried out.

d. Skin surface temperature

Human forearm surface temperature was measured with a Comark electronic thermometer and Copper/Constantan thermocouple, the same point location on each subject's arm being used. After the measurement in air, each subject immersed his/her arm in a tank of circulating water maintained at 28°C. The surface temperature was then measured again after 1, 2 and 5 minutes of immersion.

This procedure was then repeated using each of a range of water bath temperatures, from 21 - 35°C.

RESULTS

a. Constant temperature controls

If cercariae emerged from infected snails into water maintained at constant temperature, then, within the range 20 - 32°C, most were found to be swimming while usually less than 10% were inactive (see Text Fig. 23)

At 34°C, however, a different pattern was seen, as 60% were inactive and the variance of results increased. It should be noted at this point that this inactivation did not take place as a result of only 1 - 2 minutes exposure to the higher temperature. As the larvae were shed at the same temperature they were exposed for up to an hour to each test temperature. No larvae were seen to attach to the glass surface.

An analysis of variance based on the results from 20 - 32°C indicated that neither temperature nor sample number had a significant influence on the result (at 5% level). The proportion of larvae active at 34°C, however, was found to be significantly reduced (at 0.01% level) compared with the proportion active below this temperature, using the χ^2 test. The original data is included in Appendix 12.

b. Rapid rise in temperature

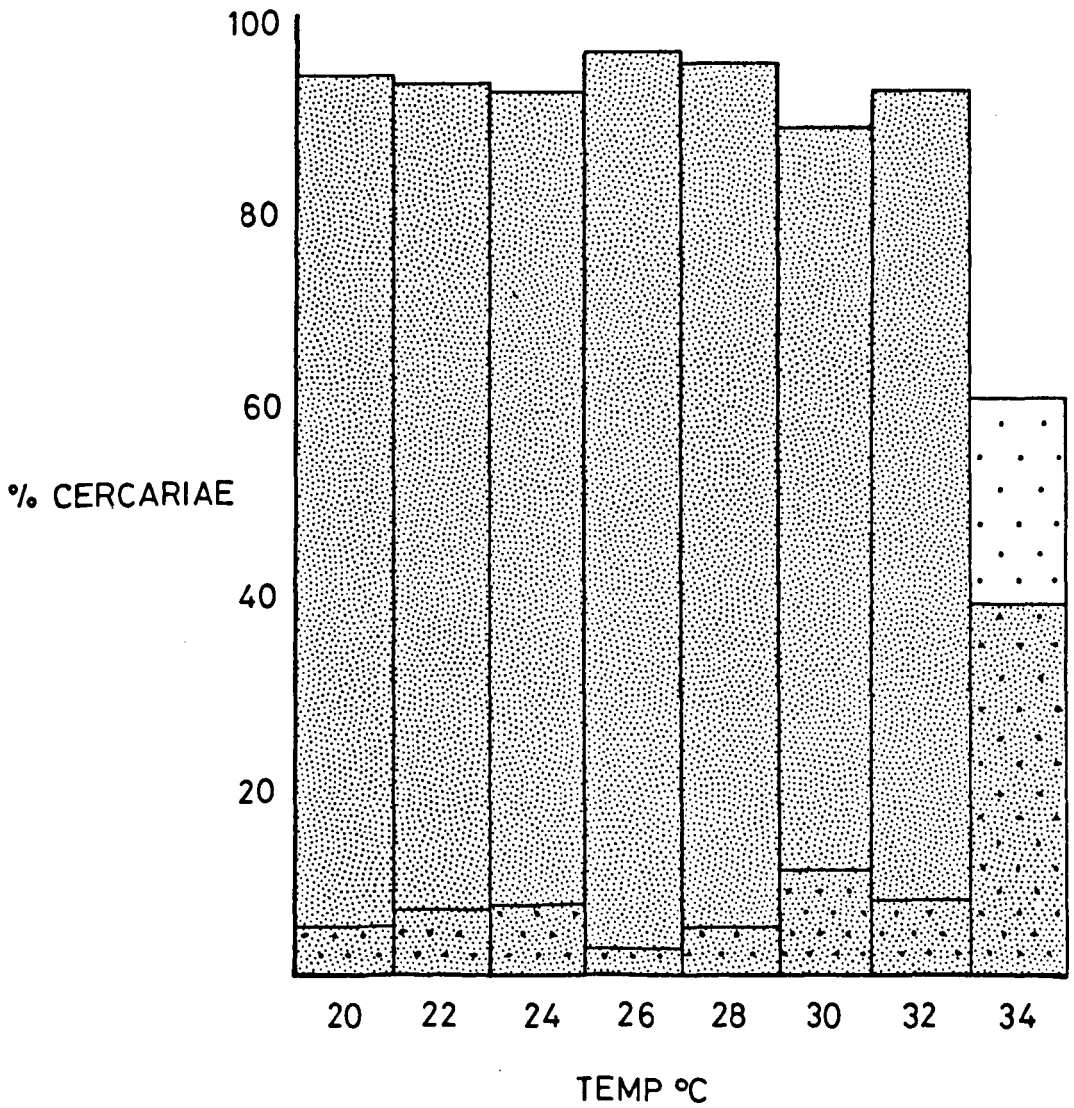
One minute after the water supply to the glass cell was quickly switched from 29 - 39°C, 21 out of 100 cercariae tested individually were found to have attached to the glass surface and started to crawl. Although the temperature within the chamber took up to a minute to rise to the higher level, the response of some cercariae was almost immediate.

If cercariae were kept in the chamber at 39°C for more than 5 minutes the inactivation demonstrated in (a) tended to occur. The effects of a temperature change of less than 10°C were investigated but a crawling response was not seen if this was less than 6°C.

Text Fig. 23

ACTIVITY of CERCARIAE at CONSTANT TEMPERATURE

(10 samples summated at each temp)



 inactive

 swimming

TABLE 3

The distribution of cercariae in a temperature gradient;

	Samples	T1	T2	Sample Totals	Day Totals
Day 1	1	8	16	24	
	2	10	18	28	
	3	10	20	30	128
	4	5	14	19	
	5	11	16	27	
Day 2	1	7	16	23	
	2	9	20	29	
	3	7	17	24	127
	4	10	22	32	
	5	6	13	19	
Day 3	1	8	13	21	
	2	10	17	27	
	3	11	24	35	135
	4	8	11	19	
	5	13	20	33	
Day 4	1	6	16	22	
	2	9	16	25	
	3	11	17	28	113
	4	7	13	20	
	5	7	11	18	

c. Temperature gradient

More cercariae were consistently found at the warmer end of the gradient, even after the short time interval allowed. Table 3 shows the numbers of cercariae of each side of the chamber after 1 minute, T2 being the higher temperature. An analysis of variance showed the temperature effect to be significant at the 0.1% level. Neither the day nor sample number (the latter being an indication of the TIME of day) significantly affected the results.

The results of the preliminary controls indicated that:-

- a. there was no cell-related factor causing significant asymmetry in cercarial distribution: see Table 4 (i) and (ii)
- b. the cercarial distribution could be skewed to the left, Table 4 (iii), or right, Table 4 (iv), depending on the polarity of the temperature gradient alone. This shows that external factors, such as lighting for example, were not responsible for the asymmetry.

TABLE 4. For explanation see above.

(i)		(ii)	
<u>Original Cell Position</u>		<u>Reversed Cell Position</u>	
<u>Side 1</u>	<u>Side 2</u>	<u>Side 2</u>	<u>Side 1</u>
38	32	39	32
(iii)		(iv)	
<u>Original Gradient Polarity</u>		<u>Reversed Gradient</u>	
<u>Side 1</u>	<u>Side 2</u>	<u>Side 1</u>	<u>Side 2</u>
12	50	56	9

The results do not differentiate between larvae which are swimming and crawling in the chamber. It was in fact rare for cercariae to attach to the glass in the established gradient experiments (0 - 4%). In the presence of crude lipid extract from human skin, however, a differential of only 5°C stimulated many (85%) cercariae to attach and crawl.

Skin surface temperature

The recorded temperature in air varied from 32.5°C to 33.5°C. Within one minute of immersion, however, in three out of four cases, a temperature differential of only 0.5°C existed between the skin and surrounding water. In the fourth case, surface cooling took slightly longer, but had stabilized in about three minutes to 29°C. These results are tabulated below:-

TABLE 5

Skin temperature before and after immersion in water

<u>Subject</u>	<u>Skin temp in air</u>	<u>Temp after immersion in water @ 28.5°C</u>		
		<u>1 min</u>	<u>2 mins</u>	<u>5 mins</u>
1	33.5	30.5	30.0	29.0
2	32.5	29.0	29.0	29.0
3	33.0	29.0	29.0	29.0
4	33.5	29.0	29.0	29.0

With the circulating water maintained at 21°C, surface temperatures measured approximately 22.5°C after 1 minute. At 25 - 35°C water temperature, the skin surface temperature did not normally deviate by more than 0.5°C from that of the water, after equilibration.

DISCUSSION

It is apparent that a proportion of schistosome cercariae remain infective over a wide range of temperatures and the degree to which this is so has been demonstrated in a number of ways, *in vivo* and *in vitro*. For example, adults may be recovered from infected laboratory animals by perfusion and their relative numbers expressed as a percentage of the number of cercariae used in the infections.

Although displaying a great deal of replicate variability, the results of Stirewalt and Fregeau (1965), which consisted of counts of the number of cercarial penetrants, demonstrated that some cercariae are infective in water maintained at only 7 - 9°C. Similarly, a small number also penetrated mice from water maintained at 45°C. However, the range within which an average of over 40% penetrated was approximately 13 - 35°C with a rapid reduction in penetrations outside these limits.

Not discussed by the above authors, however, was the possible effect of the temperature regulatory mechanism of the experimental mice which are, of course, homeotherms. At the colder end of the temperature range, the tail could be maintained, initially, at a higher level than that of the surrounding water, resulting in a gradient. At the central and higher temperature ranges this gradient would be smaller or non-existent. Even in cold water the gradient would reduce with time, especially near the distal end of the tail which would quickly approximate to water temperature.

It should, therefore, be noted that, when comparison is made between points on the graphs presented by Stirewalt & Fregeau (1965), Dewitt (1965) etc., some of these represent a response in the presence of a temperature differential, however transient, and others in the absence of such a differential. This error, however, would tend to

increase the recorded numbers of penetrants in cooler water. If it could be corrected, the temperature optima observed would not be altered but, in fact, highlighted. That a gradient did exist in the above experiment (Stirewalt & Fregeau, 1965) is suggested by the results of Stirewalt and Uy (1969) who counted the number of *Schistosoma mansoni* larvae which penetrated a rat skin membrane. If both the cercarial suspension and the collecting medium were maintained at a uniform temperature of 23 - 24°C only 4% larvae penetrated. A differential of 23 - 24°C (in the suspension) to 37°C (in the collection medium) was optimal, inducing 45% to penetrate.

It can be seen from the results of Stirewalt and Fregeau (1965) that in their 'uniform' temperatures within the range 21 - 26°C over 40% penetrated. Unless the rat and mouse skin preparation have very different stimulatory properties, and this seems unlikely, then it must be assumed that a temperature gradient existed in the mouse infections for at least some of the exposure time.

Despite the degree of variability of optimum constant temperatures found by previous authors, using the different assay techniques, (e.g. Purnell 22 - 27°C, De Witt 30 - 32°C, Stirewalt and Uy 37°C), two general conclusions may be drawn: Firstly, within relatively wide temperature limits, at least some cercariae are able to penetrate the skin of a primary host. Secondly, if the skin surface is warmer than the surrounding water a higher proportion of the available cercarial population will attach to and penetrate through the skin.

Stirewalt (1971) stated that, up to that time, cercarial sensitivity to small temperature differentials was not known and that cercarial behaviour in constant temperatures other than 24°C ("room temperature"?) had not been observed. In the control experiment described here (see Text Fig. 23) the proportions of swimming and inactive cercariae were

determined for a range of constant temperatures. Although over most of the range covered a high proportion of larvae were active, after a period in water at 34°C (or above) many were inactive, having sunk to the bottom of the observation chamber.

This result can be compared with the vertical distribution of cercariae observed by Stirewalt and Fregau (1965). At 37°C they found the distribution limited to the lower half of their exposure tubes after 15 mins. but that "locomotion in this area appeared normal." I would concur that those cercariae which do swim at the higher temperatures all appear to swim normally. The type of quantitative study presented here, showing the proportions of active/inactive larvae for a range of temperatures, has not previously been carried out, but Valle et al (1974) have clearly illustrated the general effect of temperature level on larval physiology by plotting backward propulsion speed in relation to temperature. They found a linear relationship, between 15 - 30°C, with a doubling of speed per 10°C increase.

Inactivity in cercariae may be due to a number of factors. Becker (1971) argued that the decrease in cercarial activity with time in *Schistosoma mansoni* was due to a constant loss of ions via the excretory system. On the other hand, having established that the infective capacity of *Schistosoma mansoni* cercariae decreased rapidly with time, Olivier (1966) attributed this to an eventual loss of the glycogen energy reserves. Ginetsinskaja and Dobrovolskii (1963) and Palm (1962) came to the same conclusion for *Cotylurus brevis* and *Cercaria limnaea ovata* respectively. Lewert et al (1966) have discussed the probable importance of the metal ion concentration of natural water in explaining the variability of infection success in different laboratories and of infectivity in different endemic areas. It may be that this loss of ions causes, initially, a reduction in the mean length of swimming

periods and, eventually, inactivity which is correlated with greatly lowered penetrative capabilities.

Whatever the cause of inactivation in *Schistosoma mansoni*, it would appear that the process (or processes) is markedly accelerated at temperatures over 32°C.

Although a temperature gradient may be of importance in infection, no quantitative study has been carried out to characterize the behavioural response to temperature flux alone. Furthermore, when applied in conjunction with a chemical stimulus the temperature differential has been equilibrated as high as 13 - 14°C (Austin et al, 1972). A realistic assessment of the significance of any kind of cercarial reaction to temperature flux ought, however, to follow a consideration of the temperature gradients likely to be encountered by the larvae under natural conditions. The presence of a gradient steep enough to cause modification of cercarial behaviour is assumed by authors who postulate temperature as the "remotely" sensed stimulus in host location, e.g. Stirewalt (1971), Austin et al (1972). Direct evidence, however, has not been quoted to support this supposition.

The results presented in Table 5 were based on measurements on human subjects whose forearm skin surface temperature in air was 32.5 - 33.5°C. This temperature was not maintained on immersion into cooler water, however. Usually, immersion for one minute in water maintained at 28.5°C led to equilibration, with skin surface temperature 0.5°C higher. Within the whole water temperature range 25 - 35°C, no more than a 0.5°C differential existed. As there is a rapid reduction in temperature with distance from the skin surface, it would appear unlikely that heat could play a significant role in host *location* under natural conditions. Indeed, such a small differential on its own does not even trigger cercarial *attachment* responses. This does not, however, detract from

the argument that even a small temperature differential might complement other stimuli when contact with the host is achieved (or is, at least, imminent).

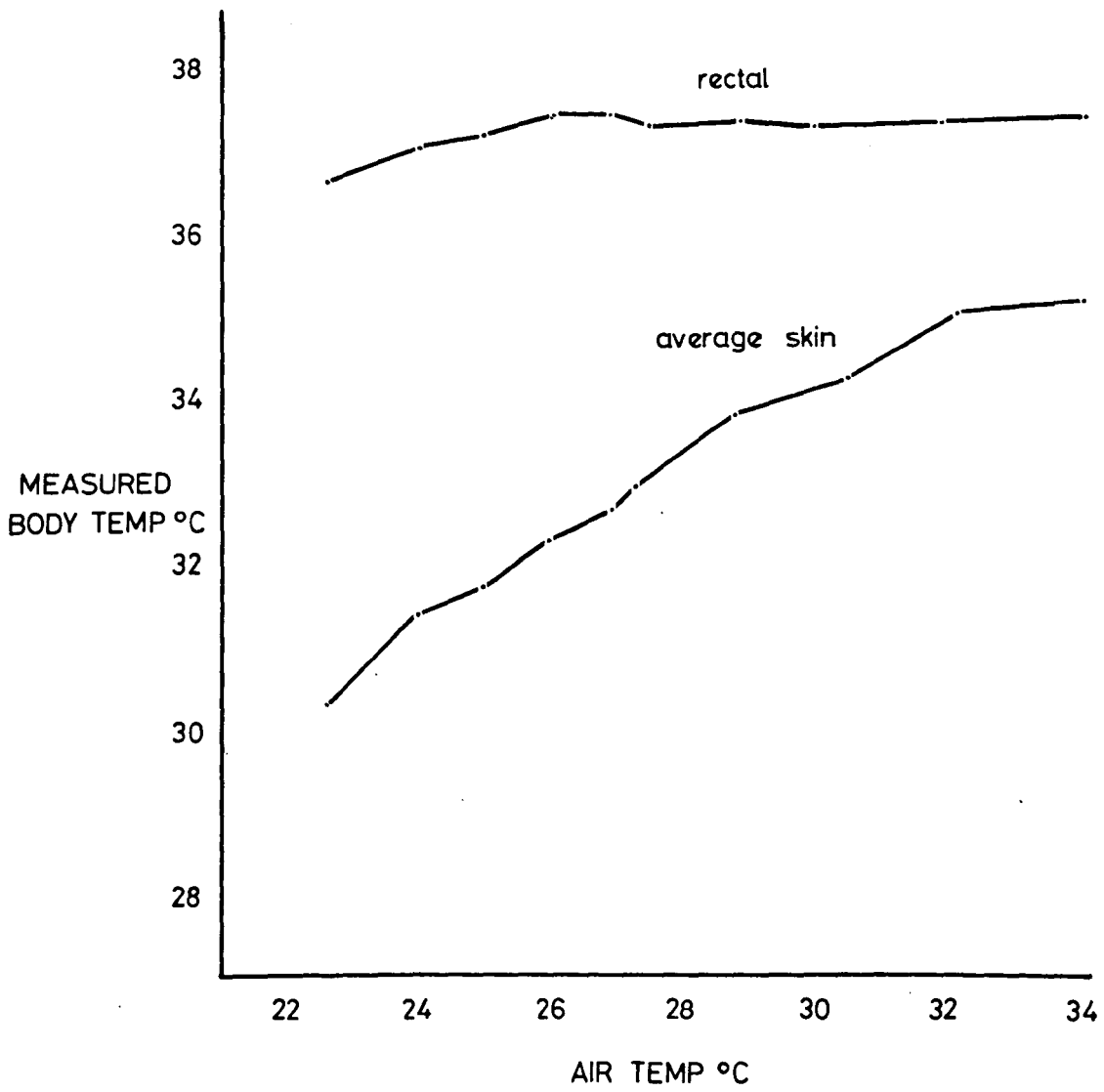
The skin surface temperatures measured (in air) were low compared with those which might be expected in subjects adapted to a warmer environment. Aschoff and Wever (1958) show how, under the latter circumstances, the body core temperature effectively spreads to all but the epidermis. The temperature of the latter is only a little lower (due to evaporation). The skin surface may, therefore, measure as much as 36°C in air. The relationship between skin temperature and air temperature is given in Text Fig. 24, redrawn from Du Bois (1939). Facilities were not available for measuring the temperature gradients in water adjacent to the skin surface of subjects acclimatized to heat. It is doubtful, however, that these gradients would be significantly different from those described above. The efficiency of water as a conductor would probably still ensure that skin surface temperature approximated to water temperature. This would particularly apply if the water was moving over the skin surface (a situation which will almost certainly be more common than that in which exposed skin surfaces and surrounding water are motionless).

In view of the fact that a cercaria would be so close to its 'target' when stimulated thermally and that there is always the distinct possibility of it being swept *away* from the target by water flow, the *speed* of larval reaction assumes some significance. The point has already been made that displacement of cercariae by water currents may be great compared to the distances covered due to locomotory movements alone - Nuttman (1974). In spite of this, the *immediate* responses of cercariae to stimuli, whether chemical or temperature flux, have received little attention: Stirewalt (1970) does not record a short-term response; Stirewalt (1971) allowed 8 minutes to elapse before taking readings;

Text Fig. 24

Graph to illustrate divergence in temp. between the body core
(measured as rectal temp) and skin surface in humans

data from DuBois(1939)



Austin et al (1972) allowed 8 - 20 minutes. Cercariae unable to respond appropriately to temperature (or chemical) gradients associated with the host surface within 1 - 2 minutes, far less 8 - 20 minutes, would surely have an extremely low probability of infecting that host. It was for this reason that, in the experiments described here, the more immediate responses to stimuli were recorded.

The problem was approached in two different ways. Firstly, the responses of individual cercariae to a sudden rise in temperature were observed. About 1 out of every 5 responded by attaching to the glass chamber and crawling for a short period. If the increase in temperature was less than 6°C then no attachment and crawling response was seen, unless there was a concurrent chemical stimulus. Secondly, in the temperature gradient experiment, cercarial distribution was monitored only 1 minute after introduction into the chamber. Consistently more larvae was found on the 'warmer' side of the chamber after this time and the result proved to be statistically significant.

Although some cercariae were seen to crawl when introduced into a temperature gradient this was rare (0 - 4%); where the response was seen, it occurred at the warmer end. This is in general accord with the comment by Stirewalt (1970) who said that "a temperature differential without lipid.... stimulated no cercariae to attempt penetration". She did not define exactly what was meant by "attempt penetration" but it can be assumed that attachment and crawling were probably involved even if glandular activity etc. were not.

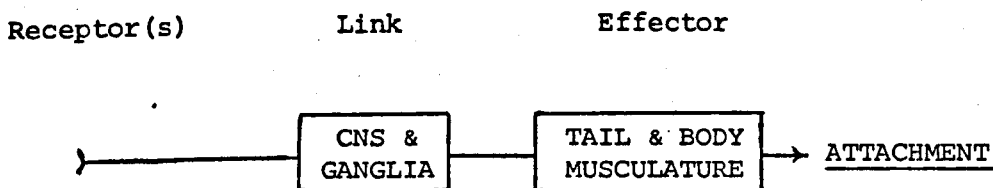
The accumulation of cercariae at the higher end of a temperature gradient is difficult to explain in terms of orientation activity. Cercariae swim faster at higher temperature and so initially one might expect accumulation at the lower end of the gradient. However, in chemically unstimulated cercariae, the horizontal component of swimming

is minimal - larvae tend to swim vertically towards the water surface. A change in larval speed should not, therefore, influence the horizontal distribution. Perhaps a temperature gradient does, in fact, introduce a horizontal component into apparently vertical swimming. There is no direct evidence for this at present and further filming/tracking is necessary to clarify the problem. An alternative to be considered is the possibility that convection currents might have influenced cercarial distribution in the gradient. However, examination of available cine films (taken laterally) does not suggest a lateral drift sufficient to account for the above observations.

The immediate reactions of cercariae to a sudden rise in temperature have not previously been recorded. This kind of stimulus might be encountered naturally if larvae were swept either very close to or into contact with a mammalian host. Results indicated that such a stimulus, even in the absence of a chemical stimulus, could cause more cercariae to attach (albeit still only 21%) than in the 'established gradient' situation. In the presence of a chemical stimulus too, an established gradient of only 5°C was sufficient to cause attachment in about 85% larvae (where $T_1 = 23^{\circ}\text{C}$, $T_2 = 28^{\circ}\text{C}$). As previously argued, however, the temperature differential in natural conditions is probably rather less than this. To sum up, at least some cercariae are able to attach and penetrate within a wide range of temperatures - under experimental conditions - but they are soon inactivated at the higher end of the range. They are more likely to attach in the presence of a temperature *differential*. However, in view of the probability that only a very small differential exists between the human skin surface and surrounding water *under natural conditions*, and that this is restricted to only a narrow boundary layer, I would tend not to agree with the postulate that heat acts as a remotely-detected stimulus, under natural conditions. Its primary significance may be to complement chemical, or other, stimuli when contact is achieved or, perhaps, imminent.

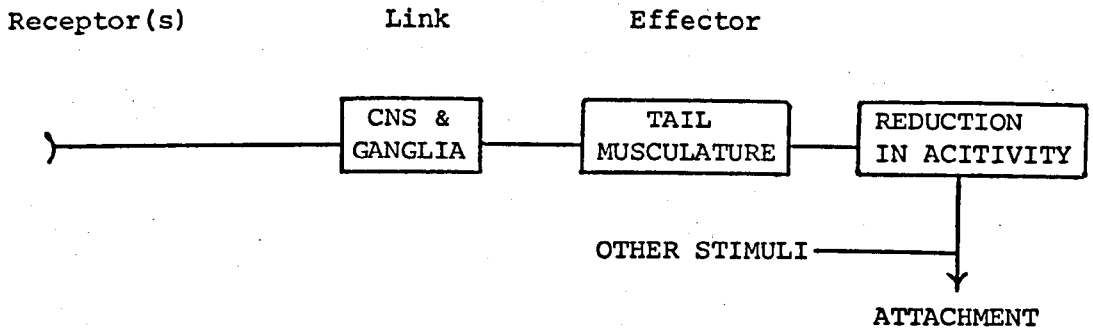
The rapidly established asymmetrical distribution in a temperature gradient might imply the existence of a receptor - effector system distinct from that in which simple triggering of attachment occurs above a threshold temperature. A number of different types of presumed sensory structures have been found in *Schistosoma cercariae* (see Section 1) but the stimulus mode to which each responds must remain a matter for conjecture until appropriate electrophysiological techniques can be brought to bear. Heat reception may in any case be located centrally in the nervous system, if a specialized receptor exists at all. Receptors of all types will be influenced to some extent by ambient temperature as will the musculature which effects locomotory movement (see Section 1). It is, however, the 'link' between receptors and effectors in *Schistosoma mansoni* which I believe is more advanced than that which would only serve a simple 'triggering' system. The latter can be illustrated schematically:

SIMPLE TRIGGERING SYSTEM



The recorded distribution of cercariae in a temperature gradient, could in theory be brought about in a number of ways, e.g. a reduction in locomotory activity with increase in temperature (- ve orthokinesis), analogous to the well-documented response of woodlice in humidity gradients. The question would remain, however, as to whether such a mechanism could bring about a significantly asymmetrical distribution within the 1 minute allowed. Also assumed would be a horizontal component in swimming activity. With this system, further stimuli would then be required before attachment would occur.....

ORTHOKINETIC SYSTEM



In the case of a klinokinetic response, were this to occur, the CNS influence on the effector system would be to modify the probability of turning rather than overall activity. Tactic responses would require correspondingly more complex systems to allow for comparison of input from bilaterally placed receptors, or from one receptor at successive time intervals. As discussed on p. 196 however, it is difficult to see how tactic mechanisms could operate in larvae which rotate while swimming.

3.3 HOST RECOGNITION (2)

THE CHEMICAL STIMULUS

Introduction

The proposal that schistosome cercariae respond in some way to chemical factors associated with the surface of their primary host began to attract more specific investigation during the 1950's. Neuhaus (1952), for example, suggested that chemical as well as thermal and mechanical stimuli caused behavioural modification in *Trichobilharzia szidati*. Bolwig (1955) studied the attachment responses of *Schistosoma bovis* and *Schistosoma haematobium* and found that skin particles or sebaceous secretion would stimulate many cercariae to attach. Negative results were obtained with sweat, lactic acid, cholesterol and CO₂. Bolwig's experimental approach has been described in the previous chapter - the Heat Stimulus.

Wagner (1959) found that very few *Schistosomatium douthitti* cercariae penetrated mouse skin which had been extracted with ether. The residue obtained after evaporation of such ether extracts caused cercariae to shed their tails, discharge penetration glands etc. Addition of free acids such as valeric restored the stimulatory properties of extracted skin. This led Wagner to conclude that such acids are at least partially responsible for the stimulation of *Schistosoma douthitti* under natural conditions. Stirewalt and Uy (1969) demonstrated that whereas no larvae penetrated an "inert" membrane, usually over 50% penetrated rat skin membranes. This point has been reiterated by Stirewalt (1970, 1971).

It has been assumed, therefore, that the chemical properties of the host skin surface play an important part in host recognition in schistosomes.

Mammal (and bird) skin is covered with a thin surface film of lipid, the major source being the sebaceous glands (Nicolaidis, 1963). Their relative contribution depends on the size and number of glands. This 'total glandular volume' depends on the region of skin in question, hormonal balance and environmental factors - notably temperature. The keratinizing epidermis also contributes lipid to the surface film, although there is a degree of anatomical variation. It is also possible that the eccrine and apocrine sweat glands contribute, but the amount would only be small. Micro-organisms may be involved in the breakdown of endogenous lipid, thus forming secondary products in the film.

Lipids from every source effectively mix on the skin surface and so the general term 'skin surface lipids' will be used throughout this report to denote the complete mixture without regard to origins.

Although there may be considerable sample variability from one individual to another, the results of Haahti (1961) serve to illustrate the complex mixture of lipids present:-

TABLE 6

FRACTIONATION OF HUMAN SKIN SURFACE LIPIDS WITH SILICIC ACID CHROMATOGRAPHY.

DATA = % TOTAL ELUTED MATERIAL

	<u>SUBJECT</u>			
	1	2	3	4
Free fatty acids	22.5	2.3	20.1	11.7
Paraffins	1.2	1.2	1.9	1.6
Squalene	7.7	7.5	17.3	13.5
Waxes and sterol esters	24.8	23.1	22.9	24.6
Triglycerides	14.8	43.9	28.1	37.6
Sterols	9.5	4.6	1.5	1.2
Diglycerides	12.4	13.9	5.9	8.0
Monoglycerides and phospholipids	7.1	3.5	2.4	1.8

The proportion of free fatty acids and triglyceride usually have a reciprocal relationship; if a particular sample is rich in free acid it tends to have a low triglyceride content, and *vice versa*. This can be seen in Haahti's data (above).

Recent attempts to specify the particular chemical group responsible for cercarial stimulation have not always resulted in agreement, even for the same species.

In a convincing experiment, Clegg (1969) demonstrated that cercariae of the bird schistosome, *Austrobilharzia terrigalensis*, penetrated a gelatin membrane if it was coated with chicken skin lipid. Significantly fewer larvae penetrated in the absence of the lipid. The free sterol fraction alone (isolated by TLC) had an effect comparable to the whole extract. Cholesterol was found to occur naturally in the skin lipid but, as Clegg pointed out, this may not be the only stimulatory sterol.

MacInnis (1969) examined the attachment responses of *Schistosoma mansoni* cercariae after they had been pipetted onto agar which had been impregnated with one of a variety of chemicals. The presence of butyric acid or certain amino acids significantly increased the number of penetration attempts and to a lesser degree, the number of actual penetrants.

MacInnis quoted the results of an unpublished experiment (Hubbard): when test solutions were confined to one area of the agar plate, significantly more larvae were found in that area.

Using a technique developed by MacInnis (1965), Haas (1971) found that attachment responses were triggered by monocarboxylic acids (C1 - C9) and some amino acids in *Diplostomum spathaceum*. The probability of attachment was comparable to that following cercarial collision with fish, frog or snail epidermis. Experimentally-triggered attachments were not however followed by penetration unless contact had been made

with fish or frog skin. It is also interesting to note that a higher percentage of experimentally-triggered attachments occurred when the agar 'targets' were buffered at a lower pH than the medium, but that attachments were inhibited if the target pH was higher than that of the medium. Haas suggested that reduced pH might be the natural stimulus for attachment and that other stimuli were subsequently responsible for triggering penetration. Similarly, Chapman (1971) in his investigation of the behaviour of *Cryptocotyle lingua* cercariae found that, although attachment and crawling could be induced experimentally, no penetration or encystment occurred. Chapman suggested that the latter responses required a different stimulus under natural conditions which could not be provided in his experimental arrangement.

Three groups of authors (Shiff et al., Gilbert et al. and Austin et al.) published results in 1972 purporting to show which molecular species was responsible for triggering attachment and penetration responses in the human schistosomes. Their respective conclusions are not consistent, however. Much of the experimental detail in these reports warrants a more comprehensive discussion and is dealt with in full on p. 184.

Taking these latter results into account, the proposed attachment/penetration stimulants for cercariae now include cholesterol (or other free sterol), amino acids, low MW fatty acids, high MW unsaturated fatty acids, and phospholipids.

The experiments reported here were carried out with the original aim of identifying the molecular species responsible for triggering attachment and/or penetration responses in *Schistosoma mansoni*. As in several of the above reports, thin layer chromatography was the technique chosen to fractionate crude lipid extracts from the skin surface, although the method of extraction and subsequent bioassay differ.

The existence of chemoreceptors is assumed by all authors examining behavioural responses to chemical stimuli. Presumed sensory structures have been described and mapped for a number of species, and information relating to *Schistosoma mansoni* is presented in Section 1 of this report.

Materials and Methods

a) Extraction and storage of skin lipids

All glassware to be used in behavioural experiments was first washed in Analar chloroform and soaked overnight in chromic acid. It was then washed finally in distilled water and air dried. Only by following this procedure could all attachment/crawling behaviour and extended swimming periods be eliminated from control suspensions.

Surface lipids were obtained from male and female human 'volunteers'. Both forearms of each of 10 people were extracted by application of a jet of chloroform which was collected in a glass vessel. Cotton wool swabs were used initially, but it was found that even after being washed several times in solvent they still contributed material to subsequent chromatography samples. The main disadvantage of not using swabs, however, was that a much smaller quantity of lipid could be obtained at any one time.

The solution as collected above was filtered through solvent-washed, glass-fibre filter paper to remove debris such as skin scales, hair etc. Solvent was then removed at 20°C under reduced pressure in a rotary evaporator. The solute was weighed and if stored, the samples were kept dry, under nitrogen at 4°C.

The extraction procedure was then repeated using distilled water as a solvent.

b) Thin-layer chromatography (TLC)

Glass plates (20cm x 20cm) were cleaned with chloroform and acid, as described above. Initially Silica gel G was extracted with chloroform but the report of Clegg (1969) was substantiated in that a compound was found to be present which proved lethal to cercariae. Subsequently, plates were coated with Kieselgel 60 HR (Merck) after it had been extracted with chloroform.

A 60% W/V suspension of Kieselgel in distilled water was shaken vigorously in a conical flask for 1 minute and then applied to a row of glass plates, using a Gallenkamp plate spreader. The plates were left to air - dry before being stored in a covered vessel and were routinely activated prior to use, for 1 hour at 100°C.

Lipids were spotted onto the plates about 2 cms from their bottom edge and several solvent systems were tested for their suitability in separating the extract components. These included:

- SOLVENT No 1 Hexane: Diethyl ether (90:10)
- 2 Hexane: Diethyl ether: Acetic acid (90:10:1)
- 3 Benzene: Diethyl ether: Ethanol: Acetic acid (125:125:2:0.2)
- 4 Chloroform: Methanol: Water (65:25:4)
- 5 Benzene: Diethyl ether: Ether (50:40:2)

None was entirely satisfactory, as a small degree of streaking was invariably found on the developed plates and there was inadequate separation of some fractions. These factors assume some importance when each fraction is to be eluted off separately and without cross-contamination. It is possible that a double-development procedure, using extra-long glass plates, might produce better results.

Solvents similar to 1 and 2 have been used by previous authors to separate a lipid mixture into general classes e.g. Clegg (1969). Very little difference in performance was found between them here, except for a small extra degree of spot movement near the origin in solvent 2. However, in the bioassays following the use of solvents 2 and 3, larvae were found to swim erratically and many were soon inactivated. Contamination due to the presence of acetic acid was suspected and so the use of solvent systems containing it was discontinued. The separation achieved with solvent 5 was not as clear as that with solvent 4 and therefore the latter system was later used in an attempt to separate more effectively the polar constituents of the extract for bioassay.

Preliminary analyses were performed to determine an optimum quantity of extract per plate for preparative TLC and as a result, a streak of 100 μ l, containing 1.6 mg lipid was applied to the plate. Development was in covered glass tanks to which solvent had been added 20 minutes previously to allow time for saturation. Plates were, in fact, developed in pairs, a preparative plate being placed in the same tank as a control plate. The latter was spotted with a (smaller) sample of lipid extract and several standards. Lipids on the control plate were visualized after development by spraying with chromic acid and incubating at 100 $^{\circ}$ C for 10 mins. The corresponding locations on the test plate were then marked with a needle. The advantages of the particular spray used included the fact that sterol and sterol esters could be identified by a temporary reddish colouration. Alternatives also used were iodine vapour; 2, 7 dichlorofluorescein with inspection under UV light; molybdophosphoric acid and incubation at 100 $^{\circ}$ C.

The paired plate technique therefore avoided any chance of contact between the locating agents and the fractions for bioassay.

The Kieselgel containing each of the test fractions was scraped from

the test plate, using an acid-washed microscope slide, and extracted with chloroform. The resulting suspensions were centrifuged to remove all silica particles and each volume of chloroform adjusted to 1 ml. A 'plate control' consisted of a similarly prepared sample but taken from an adjacent, clear section of the chromatography plate, i.e. a section which would contain none of the applied lipid.

c) Bioassay

The apparatus used to observe cercarial responses to chemical stimuli was the same as that described in Section 3.2, with the water supply to the water jacket arranged as in Text Fig. 21 B. A temperature differential of 2°C was set (26-28°C).

Each fraction was tested by applying a 5µl sample (about 80µg lipid) to the centre of the observation chamber and allowing the solvent to evaporate. This process was hastened by directing a jet of nitrogen into the chamber. One ml of dechlorinated tap water was then added and allowed to equilibrate. The lipid sample remained intact as a thin film adhering to the glass. Larvae were pipetted into the equilibrated chamber in a minimal volume of water and the number of individuals crawling on the glass surface was recorded after 1 and 2 minutes. A total of approximately 100 larvae were examined in this way for each test fraction and control, but in samples of only 15-20 larvae at any one time. It was not possible to count the attaching larvae quickly and with sufficient accuracy in samples much larger than this.

A larva was deemed to have attached and started to crawl if at least 1 "step" was taken, (see Section 2). In practice, more than one step was invariably taken in these tests by responding larvae.

RESULTS

A diagram showing the fraction pattern obtained using the hexane: diethyl ether solvent system is given in Text Fig. 25. The density of shading indicates the intensity of the staining reaction and the 'R' indicates a red colouration. The latter is taken to indicate the presence of free or combined sterol. The 10 fractions could be divided into three main groups, A, B, C.

Positive larval responses were obtained only to fractions 2, 3, and 4 from group A near the origin, with a maximal response to fraction 4, (see Table 7).

It is considered likely (from examination of control plates and comparison of results with previously published work) that the three main groups could have included the following skin lipid components:

GROUP A	free fatty acid free sterol phospholipid monoglyceride
GROUP B	triglyceride
GROUP C	hydrocarbon sterol ester

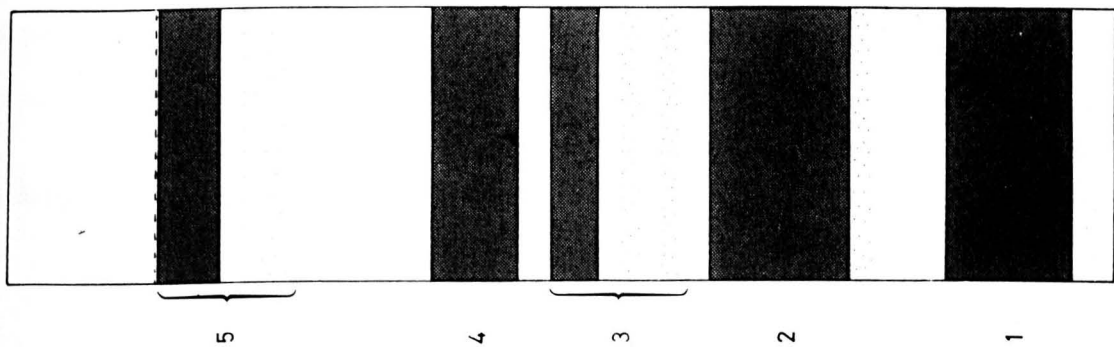
This, however, does not represent a comprehensive list of all the components of skin surface lipid and the true cercarial stimulant may not necessarily fit one of these categories. Most of the stimulant appeared to travel a short distance from the origin (the latter showing no activity at all) perhaps with some degree of 'tailing' back towards the origin.

The use of solvent 4 (chloroform: methanol: water) resulted in the partitioning of the extract into 5 main fractions, (see Text Fig. 25) each of which was subjected to bioassay. Once again, the active area

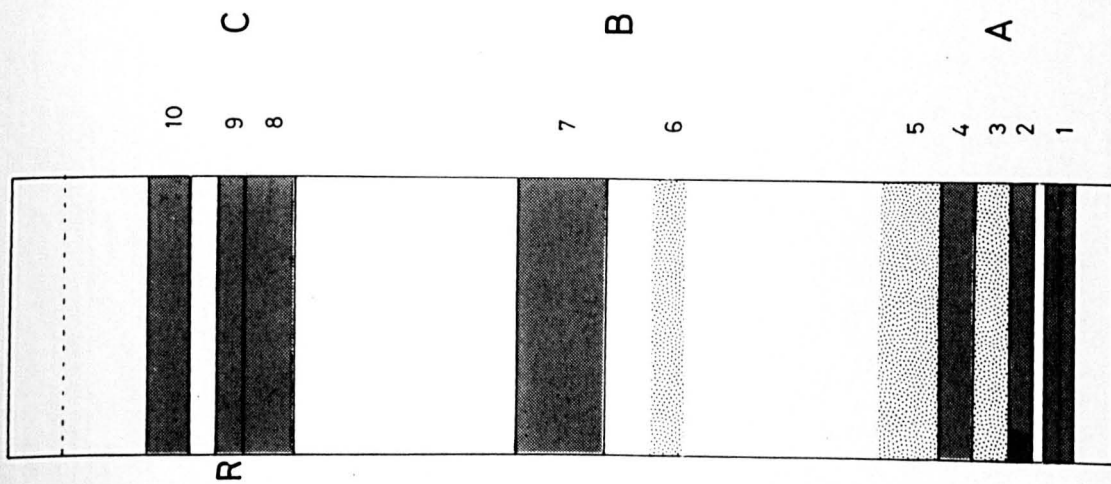
Text Fig. 25

Diagram to show the pattern of TLC fractions obtained in two different solvent systems. The density of shading indicates the intensity of staining reaction. R = reddish colouration.

SOLVENT 4



SOLVENT 1



was highly localized, being confined in this case to fraction 1 close to the origin (and including the latter). An important feature to note is that all fatty acid standards (including stearic, oleic, linoleic, linolenic and arachidonic) i.e. whether saturated or unsaturated, had Rf values of 0.8 or more in this solvent.

Bioassay results are presented in Table 8.

TABLE 7

Positive attachment responses to TLC fractions (hexane: diethyl ether: ether solvent system)

<u>FRACTION NO</u>	<u>NO OF ATTACHMENTS</u>		<u>TOTAL LARVAE</u>
	<u>@ 1 MIN</u>	<u>@ 2 MINS</u>	
1	-	-	120
2	-	2	90
3	9	16	141
4	30	73	87
5	-	-	110
6	-	-	100
7	-	-	100
8	-	-	104
9	-	-	150
10	-	-	75
PLATE CONTROL	-	-	114
CHAMBER CONTROL	-	-	124

TABLE 8

Positive attachment responses to TLC fractions (chloroform: Methanol: water solvent system)

<u>FRACTION NO</u>	<u>NO OF ATTACHMENTS</u>		<u>TOTAL LARVAE</u>
	<u>@ 1 MIN</u>	<u>@ 2 MINS</u>	
1	30	44	94
2	-	2	96
3	-	-	93
4	-	-	114
5	-	-	100
PLATE CONTROL	-	-	121
CHAMBER CONTROL	-	-	120

DISCUSSION

A complementary effect of thermal and chemical stimuli was noted by Stirewalt (1970), larval response to either stimulus alone being limited. She found that skin lipids alone were a weak stimulus for cercariae to penetrate a dried rat skin membrane. Schistosomule collections were only 5% at 25°C and observed cercarial responses rare. A temperature differential of 28-35°C to 38-39°C (exact limits unspecified) increased the schistosomule harvest to 60% and observed penetration responses to 100%. I have found that a relatively small temperature differential is adequate and consequently a 2°C differential was routinely used for tests described in this chapter. It has been demonstrated here that 'control' suspensions should not include any larvae which have attached to a surface and started to crawl. Such behaviour may be taken to suggest that a positive stimulus is, in fact, present and this could consist, for example, of traces of chemical contaminant. These trace quantities, however, may be sufficient to complement an experimentally applied stimulus (such as a small temperature differential, or other chemicals) to produce a marked attachment response.

It is possible that the results of some previously reported experiments may have been influenced, in part at least, by such contamination - see for example Austin et al (1972) Fig. 3. Seven main fractions of rat tail surface lipids were obtained by thin layer chromatography. In their sample No 1, each of the seven fractions stimulated at least some larvae to penetrate an experimental membrane; in one fraction approximately 40% cercariae penetrated, whereas in their third sample this same fraction had no effect. The authors attributed this variability to the fact that sample 3 was tested at 35°C and sample 1 at 39°C; an increased level of response to most fractions was observed in sample 1. This interpretation is not entirely consistent with other

published results, such as those of Purnell (1966), De Witt (1965) and Stirewalt and Fregeau (1965), which suggest that temperatures above 35°C are associated with a reduced percentage penetration. Neither is it consistent with the results presented in the previous chapter of this report. There are, in addition, other experimental variables to be considered. Firstly, the time of storage of Austin's samples 3,2,1 were 15 minutes, 24 hours and 1-14 days respectively; the increasing level of responsiveness to most fractions follows this pattern. Secondly, the only experimental membranes to be used in sample 3 were those found in preliminary tests to be completely non-stimulatory. This precautionary selection was not carried out for samples 1, 2. Thirdly, the fractions in sample 3 were eluted from the chromatography plate without direct contact with iodine vapour. In other words, when appropriate precautions were taken (short storage time, careful selection of membranes, no contact with iodine) only 10-15% cercariae were stimulated to penetrate - and then only within 8 minutes - by the most active fractions A, B. Under these conditions no larvae were stimulated by fractions C-G. Other authors, e.g. Clegg (1969) have accepted this relatively low level of stimulation in plain, non-lipidized, control membranes as an indication of a lack of positive response.

Taken alone, the results of Austin et al ought not, therefore, to be taken as conclusive evidence of the stimulatory role of unsaturated fatty acids which they assumed to be present in their stimulatory fractions A, B. This conclusion has, however, been reached independently by Shiff et al (1972) who did provide more positive evidence that the unsaturated acids of human skin surface lipids stimulate attachment/penetration in *Schistosoma mansoni* cercariae. In the light of Stirewalt's (1971) results it seems surprising that nearly 60% cercaria should penetrate in the presence of crude lipid extract.

It must be assumed that, although not mentioned by Schiff et al, a temperature differential existed to complement the chemical stimulus. As in Austin's experiments, Schiff's initial separation of lipid classes (by TLC) produced fractions which all stimulated cercariae to a certain degree, but responses were most marked to those fractions with similar Rf values to sterol and free fatty acid standards. The control sample showed no activity. As fraction overlap on the chromatography plate was suspected, further purification was carried out and, in the subsequent bioassay, the proportion of stimulated larvae in the presence of free fatty acids and free sterol were 33.9% and 0.6% respectively; the sterol effect found by Clegg (1969) for *Austrobilharzia* apparently not being present in *Schistosoma mansoni*.

In order that the stimulant molecules could be characterized more precisely, the fatty acid fraction was itself partitioned by Schiff et al. Although no significant difference was found between the effects of branched and unbranched acids, such a difference was seen between saturated and unsaturated acids. Only the unsaturated group produced a response (36.6%) comparable to that of the total fatty acid fraction. The mono-di- and triunsaturated acids of dermal origin were all similar in their action to one another and to pure oleic, linoleic and linolenic acids.

Unfortunately, Schiff et al do not state clearly the time allowed for cercarial responses to occur. They used the bioassay technique of MacInnis (1969) but the latter author used two different time limits - 10 mins at 23°C and 1 hour at 37°C. This must therefore be taken into account in a comparison of Schiff's results with those of others using short time limits. The importance of the time factor has already been discussed.

The chemicals tested by MacInnis (1969) included glucose, several amino acids, and low MW acids such as butyric. The latter were found to trigger penetration responses and the author suggested that the same compounds were responsible under natural conditions. This conclusion had been reached by Wagner (1959) who observed the responses of *Schistosomatium douthitti* cercariae. This is open to question in view of the small quantities of low MW fatty acids in mammalian skin surface lipid. I did not find that 1-2 mM butyrate caused attachment in *Schistosoma mansoni*, but a significant increase in swimming activity was observed. This discrepancy may be due to bioassay technique: MacInnis pipetted cercariae directly onto agar plates incorporating test chemicals and it was therefore not possible to dissociate the mechanical stimulus inherent in pipetting from the test chemical stimulus.

MacInnis (1969), Austin et al (1972) and Shiff et al (1972) have therefore all come to the conclusion that the free fatty acid fraction of mammalian skin surface lipids is the chemical stimulus involved in host recognition by *Schistosoma spp* cercariae, as has Wagner (1959) for *Schistosomatium*. To be contrasted with these results are those of Gilbert et al (1972) who said that "lipid fractions containing hydrocarbons, triglycerides, cholesterol and free fatty acids were much less active than fractions containing the more polar phospholipids." I found the latter group (presumed to be at the origin in my first separation) to be inactive. Unfortunately the temperature at which Gilbert's experiments were conducted was not defined, apart from a reference to "room temperature". A long time limit of between 20 minutes and 1 hour was also allowed. A further result obtained by Gilbert et al was that pure oleic acid stimulated 10-20% cercariae to attach (Shiff reported that over 50% responded in this way to oleic). No such response to pure oleic acid was found in this investigation.

The results of my first separation (using a hexane: diethyl ether solvent system) are in accordance with the results of Austin et al (1972) and Shiff et al (1972) as free fatty acid standards had similar Rf values to my active fractions. However, further analysis showed the active region to contain a number of separate components and a more polar solvent system was used to separate them more effectively. The results of this second separation (chloroform: methanol: water solvent) suggest that a molecular species other than free fatty acid is involved. Activity was highly localized in a fraction with a very low Rf value whereas both saturated and unsaturated fatty acids travel more than half way up the plates in this solvent system. If larvae had responded to free fatty acid in the extract then a positive response should have been elicited by one of the high Rf fractions.

Clegg (1969) demonstrated clearly that *Austrobilharzia* penetration responses were triggered by free sterol (including cholesterol). These are probably not involved in the response of *Schistosoma*, however; pure cholesterol did not stimulate cercarial attachment and free sterol does not remain near the active origin in the chloroform: methanol: water solvent system.

The divergence in results between authors may reflect a difference in experimental temperature conditions. Some degree of inaccuracy might also be expected in Gilbert et al's technique of observing 200 cercariae simultaneously in 2 mls of suspension, but this alone could not account for their unique results. Glassware cleaning technique prior to the testing of chemical stimuli might also contribute to the variability of results. The low threshold of the chemical responses of these larvae makes it necessary that stringent precautions be taken against contamination throughout the experiment. It is, nevertheless, difficult to see how routine practical details such as these can alone account for the contradictory results outlined in this section.

3.4 DISCUSSION OF SECTION 3

Most trematode species possess a free-living cercarial stage. These cercariae may reach their next host in one of several different ways. For example, they sometimes encyst on vegetation which is subsequently ingested by the final host (*Fasciola hepatica*); they may penetrate and encyst in a second intermediate host which is itself ingested by the final host (*Cryptocotyle lingua*). Sometimes a third intermediate host is involved in the cycle. In contrast, the life cycles of certain species involve fewer hosts as cercariae are either ingested by their host or penetrate its surface. *Schistosoma mansoni* is, of course, an example of an actively penetrating larva.

One might expect all parasites with such complex life cycles to possess appropriate physiological control mechanisms to ensure that development from one larval stage to the next only occurs when environmental parameters are within suitable limits. The 'requirements' of successive larval stages may vary significantly and this change in requirement may occur very rapidly, e.g. the metamorphosis from cercaria to schistosomulum in *Schistosoma mansoni* involves a rapid loss of the ability to live in a fresh water environment. Many instances are now known in which specific environmental stimuli trigger processes such as hatching or metamorphosis in parasites, e.g. *light* in the hatching of *Fasciola hepatica* miracidia, *bile salts* in the activation of *Hymenolepis diminuta* cysticercoids and both *thermal* and *chemical* stimuli in the attachment/penetration responses of *Schistosoma mansoni* cercariae. The original purpose of Section 3 of this study was to identify not only those factors which trigger the attachment/penetration responses of schistosome cercariae but also to examine the reversible modifications in locomotory behaviour which occur prior to attachment.

The investigation as a whole has been carried out in three main phases: firstly, a survey was made of cercarial muscle systems and sensory receptors (Section 1); secondly, the propulsive mechanism was examined, using a photographic technique (Section 2) and the results correlated with the morphological data obtained in Section 1. The final phase has included two rather different experimental approaches. The first of these was the monitoring and quantitative analysis of cercarial displacement - i.e. a track analysis. Technical problems arose, however, in the development of the monitoring system; the analytical technique and equipment tested are discussed below. The second experimental approach in this final phase largely involved the observation of frequencies of cercarial attachment under defined chemical and thermal conditions. An attempt has been made to assess the adaptive significance of the various types of locomotory movement and of the attachment responses.

Many previous authors have contributed to the field of cercarial behaviour, but have concentrated on the process of penetration. Larval displacement, attachment and crawling behaviour have received rather less attention; no track analysis for any cercarial species has yet been published.

The 'unstimulated' cercaria of *Schistosoma mansoni* spends most of its time swimming, tail-first, in short bursts of activity which alternate with passive, sinking periods. It maintains a vertical primary orientation with its tail pointing upwards, towards the water surface. In relatively young larvae at least, this behaviour results in a population distribution biased towards the surface. This distribution is enhanced after mechanical disturbance when even older, inactive larvae swim up from the substratum. In the absence of water currents and of potential 'stimulants' (thermal, chemical etc) there is very

little lateral displacement. I have consequently designated this behaviour as *vertical swimming*.

As it is generally accepted that cercariae are a dispersive stage in the life cycle, why is it that their normal swimming behaviour does not bring about any significant lateral dispersion? The answer, in my view, is that although vertical swimming does not directly achieve lateral dispersion, it maintains the cercarial population in a position, clear of the substratum, where it is subject to dispersive water currents. A minimum of energy expenditure is probably involved in this behaviour. When cercariae respond to certain host-related stimuli, a major horizontal component appears in their swimming; they no longer stop on contact with the surface film but, instead, their active periods become very extended and they follow a three-dimensional path through the water. I have called this *horizontal swimming*. As described in this Section, stimulated cercariae may then attach to the substratum and crawl for some time. This happens, for example, in response to a sudden rise in temperature or to vigorous mechanical disturbance. With the additional presence of skin surface lipids, crawling culminates in penetration behaviour, i.e. expulsion of glandular contents, tail shedding etc. It is likely, however, that such behavioural modification can only occur when cercariae are very close to, or in contact with, the host surface. The proposal that body heat is monitored by cercariae at some distance from the surface to act as an attractant (Austin et al, 1972) does not appear tenable, at least under natural conditions. No evidence has yet been offered to suggest that any significant temperature differential exists beyond a very narrow boundary layer adjacent to the skin surface. The relative weakness of skin lipids as stimulants in the absence of any temperature differential suggests that chemicals diffusing beyond the boundary layer are unlikely to trigger attachment responses. What

remains to be investigated, however, is the possible influence of these diffusing chemicals on cercarial displacement, speed, directionality etc. and the problems associated with such analyses are discussed here.

The first simplification often introduced is that only movements in two dimensions are considered. This is because of the necessity to focus optical systems on such small organisms. As a result, experimental vessels such as the type illustrated in Section 2 are used, in which the larvae do not swim completely out of focus. An alternative technique is that of Wilson and Denison (1970): miracidia were photographed while swimming in a small, cylindrical vessel, filled to a depth of only 2mm. The extent to which this restriction of movement might influence experimental results is not yet clear but would have to be taken into account in any future tracking of stimulated *Schistosoma mansoni* cercariae.

One limitation of the technique of Wilson and Denison (1970) is that only a relatively short exposure time is possible (these authors generally using an exposure of 1 sec.). If longer times are attempted the dark ground effect is progressively lost due to overexposure of the background and consequent loss of definition. As cercariae swim with alternating active/inactive periods, longer exposure times are considered necessary to obtain a useful representation of displacement. For quantification of recorded tracks it is preferable to pinpoint the position of a larva at successive time intervals rather than obtain a continuous track. For both cercariae and miracidia this interval may be as short as 0.2 sec. It was in order to satisfy these requirements and to overcome some of the limitations of the photographic technique that an alternative system was planned in the York laboratory. Development of this system, however, took rather longer than planned, and

consequently it was not fully operational before the termination of this project. An outline description of the system is included in Appendix 13.

During the above period of development, an *Algol* computer program was devised to aid in the analysis of tracks. The data input for this program consists of a series of X,Y coordinates. These were to have been measured from tracks obtained on the scanning equipment as larval positions at successive time intervals. Any number of tracks and number of coordinates per track can be accommodated by the program which is therefore suitable for investigating the movements of many types of organism. The program output consists of:

1. Angles turned at each point on the track and the mean angle turned.
2. Distances between each point on the track and the mean distance.
3. The number of left-and right-handed turns.
4. The directionality at each point on the track and the mean directionality.
5. Histogram representations of the above variables.
6. Standard deviations and standard errors for each variable.

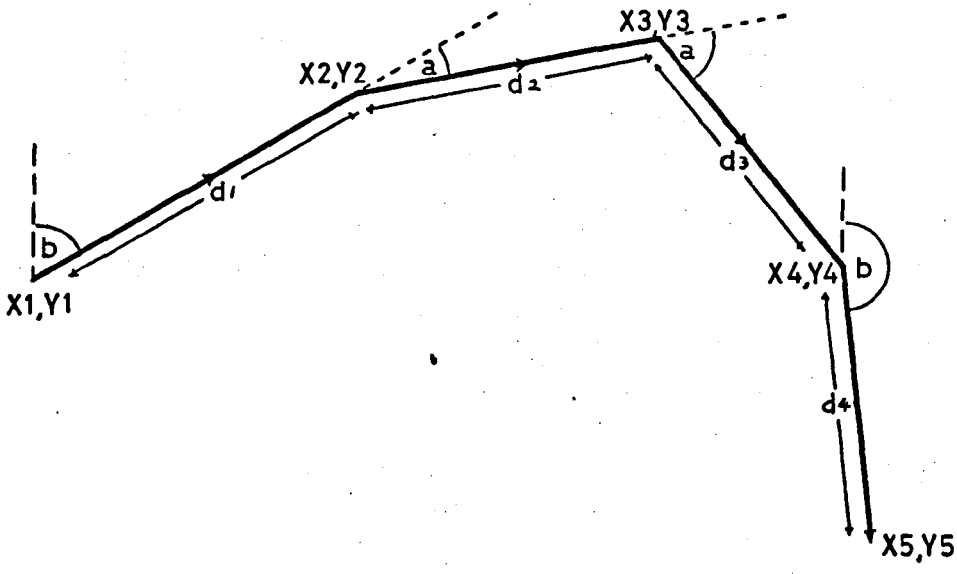
The way in which these angles were measured is shown in Text Fig. 26.

A flow chart, giving an outline of the working program, is included in Appendix 14. The calculations for angles and distances were based on simple trigonometrical formulae.

Although there is, as yet, no published account of cercarial behaviour being analyzed in this way, other larval stages have occasionally been examined. Wilson and Denison (1970a,b) investigated the activity of individual *Fasciola hepatica* miracidia. The parameters they used as an index of activity were:-

Text Fig. 26

Diagram to illustrate the angles/distances calculated in the analysis of cercarial tracks.



- x, y = coordinates
- a = angle turned
- b = directionality
- d = distance travelled

The analysis, of course, includes the calculation of a , b and d for each step, but not all are labelled on this diagram, for clarity.

Distance moved per second.

Cumulative angle turned per mm OR angle turned per second.

These authors ascribed an increase in the rate of turning in the presence of the snail host to short-chain fatty acids and suggested... "One possibility is that the increase in turning results in the restriction of the area traversed by the miracidium, with a consequently greater possibility of it contacting the host." This may well be the case, but the precise adaptive nature of observed larval movements has yet to be clarified.

Other organisms for which a type of track analysis has been attempted include the nematodes. Monitoring does not always present the same type of technical difficulty, however. A commonly used method is to photograph the tracks inscribed by nematodes on an agar surface, (e.g. Sandstedt et al, 1961; Croll, 1971). An interesting observation by Croll (1972) was that individuals of *Trichonema* spp. were sometimes found to produce their own idiosyncratic paths in repeated tests. Croll and Blair (1973) considered that the tracks could be a function of species, developmental stage, age and physiological status. Croll (1972) also discussed the mechanism of orientation in nematodes: when moving over a solid surface, nematodes effectively 'swim on their sides'. Paired, lateral sense organs are therefore functionally dorsoventral. Croll appeared critical of previous authors who had assumed klinotactic orientation in nematodes but who had overlooked this fact. There does not appear to be any real problem over terminology, however, as, by definition, klinotaxis is possible when only a single, median intensity receptor is present.

The question of a mechanism of orientation in cercariae cannot be tackled effectively until positive evidence for some kind of orientation is established. It is hoped that the type of analysis presented

here will contribute something to this field. If such evidence is forthcoming, however, an important feature to bear in mind will be the mode of propulsion of schistosoma cercariae. I refer particularly to the rotation about the longitudinal axis during tail-first swimming; any paired, lateral receptors which may be operating are continually switching position (as are the muscle blocks on left and right sides of the tail stem). It is difficult to determine how, in view of this rotation, a cercaria could respond (positively or negatively) to directional stimuli by means of a taxis. I suspect that if swimming orientation to stimuli is eventually demonstrated, a kinetic rather than tactic mechanism will be involved.

Investigation of the chemicals which stimulate cercarial responses have involved two types of test. Firstly, natural complexes such as skin lipids have been applied, before and after fractionation. Secondly, pure compounds, such as amino acids, fatty acids or sterols, have been applied. It has not always been possible to reconcile the results of these two approaches, however.

Previous authors have not always used techniques which allow straightforward comparisons between their respective results. One important variable is the time allowed between the application of a stimulus and measurement of the response. Another is the temperature conditions of their experimental arrangement. The complementary effect of heat and chemical stimuli has already been stressed.

Chemical stimulation need not necessarily result in orientation up or down the chemical gradient *per se* although subjective assessments may lead to this conclusion. An alternative is that the chemicals may trigger a rheotactic response. The latter has been demonstrated in free-living flatworms which move against the prevailing current in the presence of food molecules. The total absence of convection

or other currents will therefore have to be ensured in investigations of cercarial orientation. A further cautionary note is that some chemical species might damage receptor membranes. Even at low concentration this might manifest itself in 'false' positive responses and subsequently affect the behaviour of the larva in its response to other stimuli. Steinhardt (1967), for example, discussed the action of straight-chain hydrocarbons on the chemoreceptors of the blowfly, *Phormia regina*, in terms of injury effects.

A factor stressed in this section, especially with regard to the heat stimulus, is that constant reference should be made to the *natural* environment of cercariae. The value of laboratory experiments lies to a large extent in the isolation of stimuli, of the same or different modes. However, the selection pressures responsible for the evolution of cercarial receptors and response patterns can only operate through naturally occurring types and levels of stimulus. Therefore, experimental results obtained in the presence of particularly large temperature differentials, for example, should be viewed in this light.

An example of an author who has tried to relate his results in this way to natural conditions was Dönges (1964). He found that *Posthodiplostomum cuticola* cercariae were stimulated to swim by a sudden fall in light intensity (a positive shadow response). This was thought to be associated with shadowing by passing fish and the predominantly upward swimming was presumed to increase the chance of contact with the fish. Periodic shading caused only a transient response and Dönges related this type of stimulus to the flickering of light passing through waving vegetation. Haas (1969) was more cautious in his interpretation of similar responses in *Posthodiplostomum spathaceum*, saying that they could not be directly ascribed to efforts to locate a new host; the mere shadowing of a passing fish scarcely

appeared to bring the cercariae closer to their objective since only very short bursts of swimming activity were triggered. Haas concluded that positive selection pressure had brought about the development of these responses but did not speculate as to the nature of this selection pressure. *Schistosoma mansoni* also responds positively to shadowing but the response has not yet been quantified.

There are, therefore, a range of stimuli to which schistosome cercariae respond. Specific chemical species are evidently involved in attachment and penetration (although different species respond to different chemicals) and may be associated with a heat stimulus. General mechanical disturbance is a non-specific stimulus causing an increase in active swimming periods and some attachment behaviour. Host specificity is only broadly defined by the sensory capabilities and behaviour of these infective larvae. Host responses against post-penetration stages are probably much more important in this respect.

Very little is known of the behaviour of parasites after entry into a host. In many instances, migration routes are assumed without clear experimental support. Moreover the precise methods by which larvae move through different types of tissue have not yet been reported. Michaels et al (1969) have investigated the pairing behaviour of male and female adult schistosomes and demonstrated that even anterior or posterior halves of the body could 'recognise' the corresponding halves of the opposite sex. Whether any chemoreceptive (or other) mechanism is involved in the location of a partner by unpaired worms is not known.

Miracidia and cercariae appear to provide an interesting and useful model for investigations of simple behaviour patterns: receptor/effector systems are adaptable yet relatively simple morphologically; their behavioural repertoire has not yet been described adequately but

consists of relatively low-order processes and seems amenable to quantification. The mechanistic approach to behaviour still has a place, when applied to organisms at this level of organization. With regard to schistosome cercariae in particular, specific areas in which current behavioural investigations might be extended include: track analyses under various conditions; the effects of pH levels; ablation of specific receptors or other parts of cercarial anatomy (perhaps by microlaser); combinations of physical/chemical/thermal stimuli.

APPENDIX 1

Components of Tyrodes saline used in the collection of schistosome eggs.

NaCl	8.00 gms
KCl	0.20
CaCl ₂ .2H ₂ O	0.20
NaHCO ₃	1.00
MgCl ₂ .6H ₂ O	0.10
NaH ₂ PO ₄ .H ₂ O	0.05
Glucose	1.00
	Make up to 1l

APPENDIX 2

Infection of Mice

Mice of about 6 weeks of age (15 - 20gms in wt.) were used. The anaesthetic was Nembutal (Abbot Laboratories 60mg/ml). It was diluted 1:1 in 10% ethanol in distilled water, and injected intraperitoneally with a 0.25ml tuberculin syringe and narrow gauge needle. The dose was 1.0ml per 100gm, i.e. 0.2mls for a 20gm mouse. The response to anaesthetic was quite variable, some animals recovering after an hour while others could still be unconscious after 2 hours. The fur was shaved from the abdominal region with electric clippers and the mouse placed on its back on a grooved board. (Size of grooves 2cms deep, 3cms wide so that mice are supported in position). The stomach was then moistened with water and a heavy nickel-plated brass ring (approx. 1.5cm in diameter) placed onto the stomach surface. The rings held just over 1 ml of suspension which was pipetted into the ring chamber with an automatic dispenser. After about 20 minutes the rings were removed and placed in disinfectant and the mice returned to their cages.

APPENDIX 3

Preparation of fixative solutions for electron microscopy

A. Glutaraldehyde fixative

A 0.2M solution of sodium cocodylate was first prepared (42.8g $\text{Na}(\text{CH}_3)_2 \text{AsO}_2 \cdot 3\text{H}_2\text{O}$ per litre). To 50 mls of this, 2.7 mls 0.2M HCl was added and the resultant mixture diluted to 200 mls, (pH 7.4).

25% glutaraldehyde in water was then mixed with the buffer to give a final concentration of 2% glutaraldehyde.

B. Zetterquist's osmium tetroxide fixative

Veronal acetate buffer was prepared by dissolving 2.94g sodium veronal and 1.94g sodium acetate (hydrated) in 100 mls distilled water.

Ringers solution was prepared by dissolving 8.05g sodium chloride, 0.42g potassium chloride and 0.18g calcium chloride in 100 mls distilled water.

A glass vial containing 0.1g osmium tetroxide was scored with a glass cutter and then broken inside a glass bottle; this was carried out in a fume cupboard. 5mls distilled water were then added before the glass stopper was firmly replaced.

The final fixative solution comprised:-

5 mls osmium tetroxide
2 mls veronal acetate buffer
0.68 mls Ringers solution
2.2 mls HCl

APPENDIX 4

A. Formvar support films

Glass microscope slides were cleaned in detergent (but not in acid) and dried with a clean cloth. They were then placed into 0.3% solution of Formvar in ethylene dichloride for 30 secs. After removal they were drained onto filter paper and the film surface cut close to the edge of the slide with a razor blade. The slide was then held in spade-tipped forceps and lowered slowly into a water reservoir. The Formvar film floated from the slide which was allowed to fall to the bottom of the vessel. Separation of the film was more easily achieved if it was gently breathed on prior to immersion. 20-30 grids were then placed, matt side down, on to the floating film. A piece of filter paper was lowered on to the film, then withdrawn. The film, with grids, adhered to the paper which was placed into a petri dish to dry.

B. Carbon films

After the plastic-coated grids were dry (normally left overnight) the carbon film was applied with an NGN vacuum coating unit. Two graphite rods were trimmed and mounted in the insulated holders of the coating unit such that light pressure was maintained between them. On the floor of the vacuum chamber were placed both the Formvar-coated grids and a piece of white, glazed porcelain on which a drop of vacuum oil had been placed. The time of evaporation was about 0.5 sec. The thickness of the carbon layers deposited on the grids was estimated by the contrast between the oil-covered and clear areas of porcelain. A just-discernible difference was the aim, representing a theoretical carbon-film thickness of about 5nm.

After removal from the apparatus, grids were placed in grid holders and stored in a dessicator until required.

APPENDIX 5

Stains used for electron microscopy

Lead citrate stain was prepared by dissolving 1.33g lead nitrate and 1.76g sodium citrate in 30 ml distilled water. The mixture was shaken vigorously in a 50 ml volumetric flask for 1 minute and then left to stand for 30 minutes, with occasional shaking. 8.0 ml N Caustic soda were then added and the mixture diluted to 50 ml with distilled water. The solution was stored at 4°C and always filtered prior to use.

The *uranyl acetate stain* consisted of a 1% aqueous solution, freshly prepared before use. Vigorous shaking was required to dissolve all of the solute. A 1% solution in 70% ethanol was also tested but the results were not improved.

APPENDIX 6

Preparation of stock and substrate solutions used in the method for localization of acetylcholinesterase.

Stock solution. 0.3g copper sulphate, 0.375g glycerine, 1.0g magnesium chloride and 1.75g maleic acid were added to 50 mls sodium sulphate solution saturated at 100°C. The mixture was stirred continuously while 20 ml N caustic soda and a further 120 mls saturated solution were added. It was then allowed to cool to room temperature (approx 21°C). The pH was adjusted to 6.8 by the addition of N caustic soda and the final solution was stored at 4 °C. It was allowed to warm to room temperature before use.

Substrate solution. 80 mg acetylcholiniodide were dissolved in 10 mls stock solution. This was prepared fresh for each incubation.

**THE FINE STRUCTURE OF CILIATED NERVE ENDINGS IN
THE CERCARIA OF *SCHISTOSOMA MANSONI***

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THE FINE STRUCTURE OF CILIATED NERVE ENDINGS IN THE CERCARIA OF *SCHISTOSOMA MANSONI*

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ABSTRACT: The fine structure of ciliated nerve endings located in the tegument of *Schistosoma mansoni* cercariae is described. These include a previously undescribed multiciliate type as well as relatively simpler, unciliated endings similar to those found in the adult. Comparison is made with similar structures seen in other animal groups.

Specialized, unciliated nerve endings in the adult of *Schistosoma mansoni* have been described by Morris and Threadgold (1967), Smith et al. (1969), and Silk and Spence (1969). These are generally assumed to have a sensory function. Little is known, however, about the nervous system of the cercaria of this species. Wagner (1961) reported large numbers of "papillae" distributed over the body and tail; Morris and Threadgold (1967) referred to the presence of a cercarial process similar to that found in the adult.

The present study describes three distinct types of ciliated nerve endings in the cercaria, and their possible role as sensory receptors is discussed.

MATERIALS AND METHODS

Cercariae were obtained from infected snails maintained at this laboratory and fixed within 2 hr of emergence. Initial fixation was carried out at 4 C for 1 hr in cacodylate-buffered 2% glutaraldehyde, and postfixation was in Zetterquist's buffered osmium tetroxide (Glauert, 1965). Cercariae were dehydrated in a series of alcohols from 30 to 100% and finally embedded in an araldite-epon mixture. Sections were cut on an LKB 4800A ultratome, mounted on copper grids coated with formvar and carbon, and stained with uranyl acetate and lead citrate. Material was examined in a Hitachi HS8 electron microscope operating at 50 kv.

RESULTS

Ciliated cavities

Ciliated cavities (Figs. 1, 2) have been found only on the cercarial body, situated laterally, adjacent to the longitudinal nerve cords. Each is basically flask-shaped, up to 1.5 μ across, derived from a single nerve, and possesses five or six cilia. The latter are 0.20 to 0.25 μ in

diameter, at least 1 μ long, and project into the central cavity. In the nerve bulb, at the base of each cilium, is a basal body which is continuous with peripheral ciliary microtubules. Also present in the bulb are numerous electron-lucent, membrane-bound vesicles 500 to 1,000 Å in diameter.

The cavity, which is packed with small, dense vesicles approximately 400 Å in diameter, opens to the exterior via a 0.2- μ pore (Fig. 2). In the region of the pore, attachment of the nerve cell to the surrounding tegument is by means of a septate desmosome.

Unciliated nerve endings

(a) Unsheathed (Fig. 3). These are widely distributed over the body, tail, and furca. Each consists of a bulbous nerve ending, 1.0 to 1.5 μ in diameter, attached to the tegument by means of a circular, septate desmosome. The terminal cilium, which contains both central and peripheral microtubules, has been seen to project up to 7 μ beyond the tegument.

The basal body is composed of several elements. The largest is essentially cylindrical, the electron-dense wall being continuous with peripheral ciliary tubules. A dense basal plate is situated centrally at the level of the cell surface and, distal to this, a second, smaller plate is continuous with central tubules.

Adjacent to the nerve cell membrane, below the desmosome, is a ring of osmiophilic material which may be attached to a further, incomplete ring, angled towards the base of the bulb.

Large, clear vesicles 500 to 1,000 Å in diameter are present in the bulb, together with microtubules which are continuous with those of the nerve axon.

(b) Sheathed (Fig. 4). These have been found only at the anterior end of the body,

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adjacent to the openings of the gland cell ducts. Nerve bulbs are similar in size and contents to those of unsheathed organs, but the terminal cilia are only 1 to 2 μ long. The tegument surrounding each organ extends to form a tubular sheath which encloses the cilium except at its apex. Basal body structure has not yet been determined.

The nerves, from which all the organs described above are derived, can be traced to nerve cords situated beneath the circular and longitudinal musculature in both tail and body (Fig. 5). The axons in these nerve cords frequently appear to be packed with small, clear vesicles and small, dense vesicles about 300 and 400 Å in diameter, respectively.

DISCUSSION

The results of recent behavioral studies of motile trematode larvae (Chernin, 1970; MacInnis, 1969; Wilson and Denison, 1970) suggest the presence of specialized sensory receptors in these organisms. Examination of a number of species at the electron microscope level has confirmed that modified nerve endings exist, and these are generally assumed to be sensory. However, the small size of these "receptors" and, indeed, of the larvae themselves precludes experimental characterization, and function can only be inferred on comparative morphological grounds.

It is difficult to envisage the ciliated cavities of the schistosome cercaria functioning as mechanoreceptors except in the event of general compression of surrounding tissue. The pore does provide contact with the exterior, however, and although it is overlaid by the pericercarial envelope a chemoreceptive function seems possible.

The presence of large numbers of small, dense vesicles is problematical. Identical vesicles are commonly present in central and peripheral nervous tissue in invertebrates, fre-

quently associated with areas of high catecholamine concentration. None have been seen beyond the pore despite the fact that no obvious barrier has been identified. It is possible that they are held in a highly viscous medium, or broken down soon after their formation, or both.

The small clear vesicles (Fig. 5), present in the cercarial nervous system generally, seem identical to synaptic vesicles described in many invertebrate nerves. They are thought to contain synaptic transmitters, and it is relevant to note that acetylcholinesterase has been demonstrated in *Schistosoma mansoni* cercariae by Lewert and Hopkins (1965).

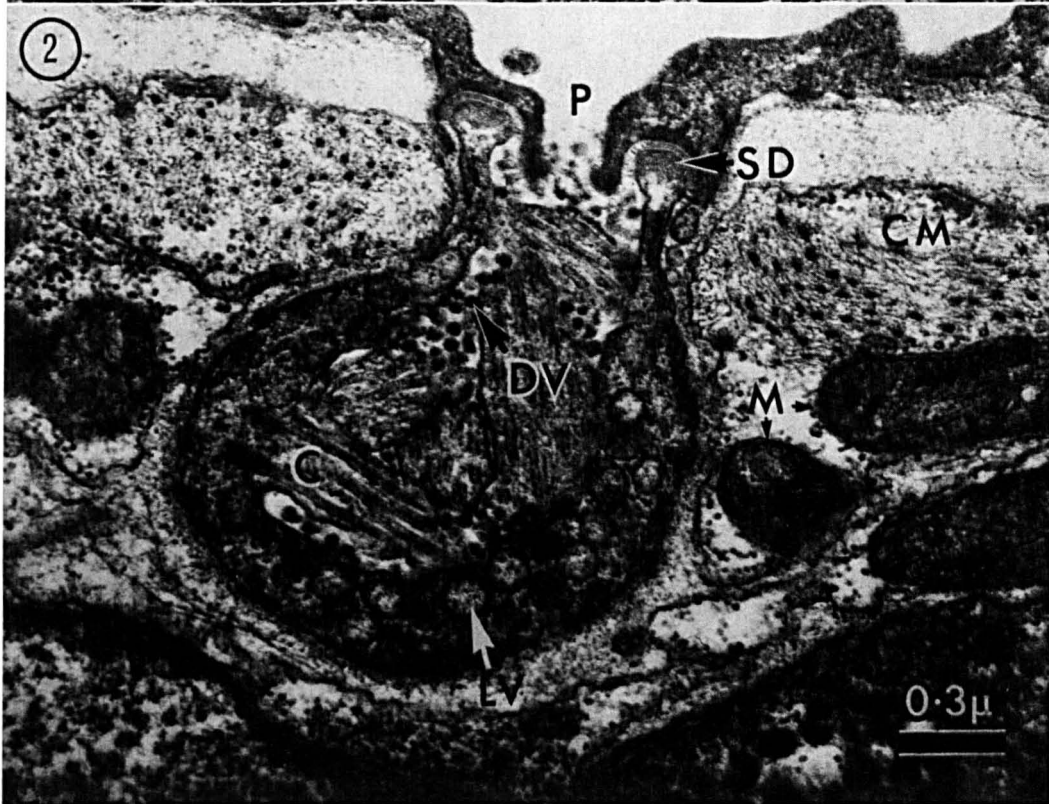
Organs similar to the unsheathed, unciliate endings described here are present in *Himasthla secundum* as reported by Chapman and Wilson (1970), and Wilson (1970) has shown that sheathed, unciliate endings are present in the miracidium of *Fasciola hepatica*. These authors consider mechanoreception to be the most likely function of structures of this type.

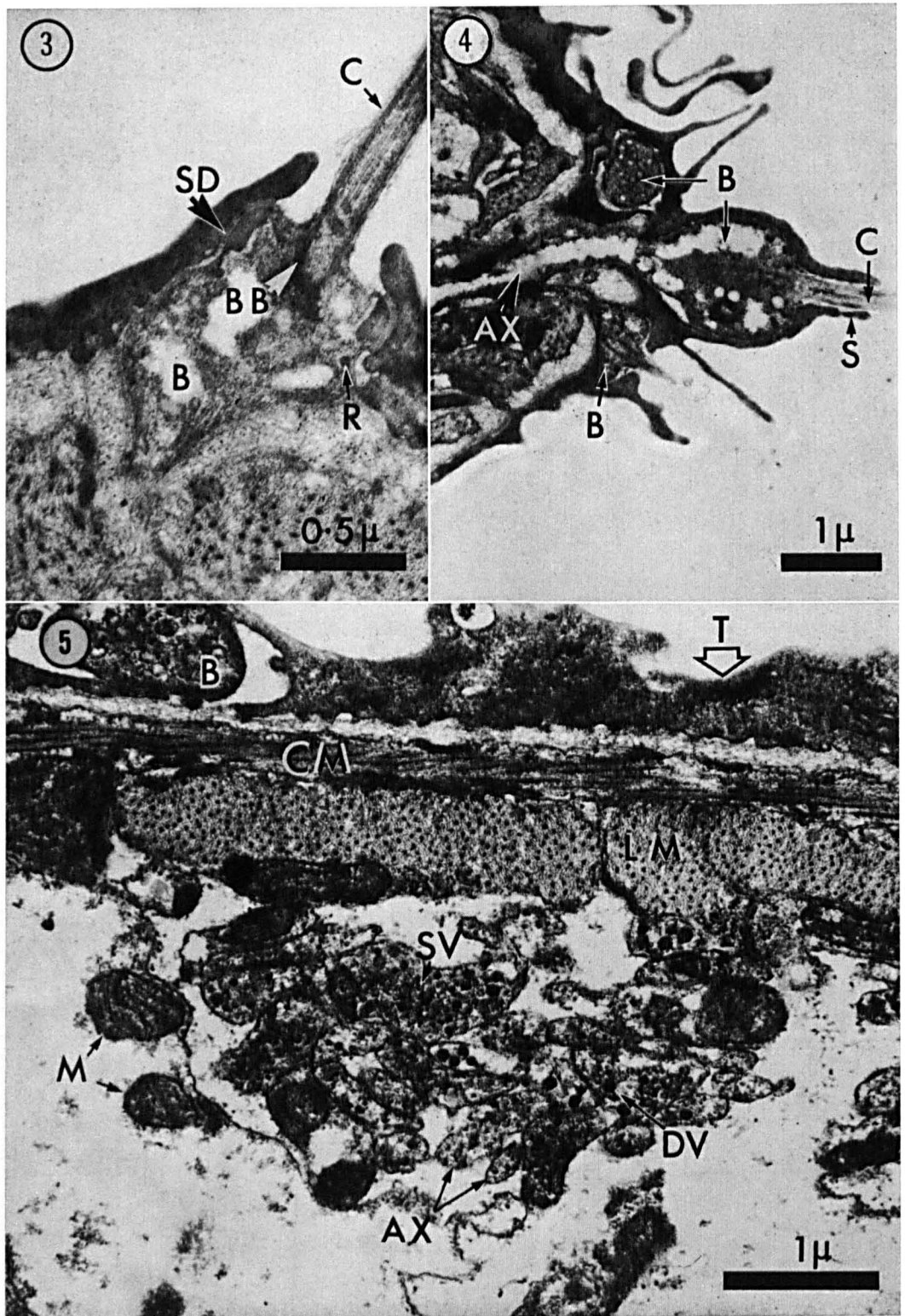
Morris and Threadgold (1967) have speculated that the sheathed nerve endings of the schistosome adult function as directional mechanoreceptors, although no polarization in nerve cell structure is visible at the electron microscope level. Directionality in mechanoreceptors may also be related to modifications of accessory structures: this is the case in, for example, the hair plate sensillum of the honey bee, where a radially symmetrical nerve cell is associated with an asymmetrical joint at the base of the hair (Thurm, 1965). There is no evidence, so far, of asymmetry in the tegumental sheaths surrounding nerve cell cilia in either adult or larval trematodes.

Laverack (1968) discusses an unsheathed, unciliate nerve cell which has been shown experimentally to be chemoreceptive: this occurs on the sensory hillock of gemmiform pedicellariae in *Echinus*, and apart from modifica-

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 FIGURES 1-2. Cercaria of *Schistosoma mansoni*. 1. Longitudinal section through body: general view of a ciliated cavity and surrounding tissues. The associated nerve axon can be seen, packed with small vesicles, to the left of the large muscle nucleus. 2. Ciliated cavity. Shafts of several cilia seen projecting into the central cavity which is loosely filled with small dense vesicles.

Abbreviations: AX, nerve axon; B, bulbous nerve ending; BB, basal body; C, cilium; CM, circular muscle; DV, small, dense vesicles; F, fibrous layer of body wall; LM, longitudinal muscle; LV, large, clear vesicles; M, mitochondria; NU, nucleus; P, pore of ciliated cavity; R, osmiophilic ring; S, lateral sheath; SC, secretory cell; SD, septate desmosome; SV, small, clear vesicles; T, tegument.





tion of the basal body-rootlet system it seems identical in structure to adjacent mechanoreceptive cells. Also, in *Helix* statocyst sensory cells, there is no projecting ciliary shaft, only basal bodies being present. Further evidence to implicate the basal body system in transduction of chemical stimuli is provided by Tucker (1967), who removed 99% of the cilia from olfactory cells in the box turtle by a process which left basal bodies intact, and the cells still chemoreceptive.

However, the functional relationship between basal bodies and stimulus reception (if any exists) cannot be universal: the olfactory bipolar cells in elasmobranchs are devoid of cilia and basal bodies (Reese and Brightman, 1970).

To conclude, it is considered that the observed structural differences between cercarial nerve endings imply a distinction between the types (or intensities) of stimuli to which the respective organs respond optimally, but it is not yet possible to ascribe specific functions to each.

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FIGURES 3-5. Cercaria of *S. mansoni*, cont'd. 3. Unsheathed, uniciliate nerve ending in the tail. Several elements of basal body structure can be distinguished. 4. Sheathed, uniciliate nerve endings at anterior tip of body. Two ridges each with 5 nerve endings present in this region: parts of 3 such endings in one ridge are seen in this section. 5. Transverse section through the dorsal part of the tail showing a nerve bulb and the underlying dorsal nerve cord. Axons in this cord contain both dense and clear vesicles.

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With 6 plates and 1 figure in the text

**The fine structure and organization of the tail musculature
of the cercaria of *Schistosoma mansoni***

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(Received 10 September 1973)

SUMMARY

The fine structure and organization of schistosome tail musculature has been investigated by transmission electron microscopy. A three-dimensional reconstruction is presented and the relationship between morphology and cercarial behaviour is discussed.

INTRODUCTION

The structure and function of cercarial musculature have been the subject of a number of previous investigations. Pearson (1956), using light microscopy, was able to describe not only the transverse striations of longitudinal muscle cells in the tail of two species of *Alaria* but also the transverse muscle fibres of the furcae.

The development of electron microscopy has, of course, facilitated study in greater depth of the overall organization of muscle fibres and of their subcellular constituents. For example, Lumsden & Foor (1968) compared the ultrastructure of body and tail muscle in *Heterobilharzia americana* cercariae. While the body fibres did not differ significantly from those found previously in adult platyhelminths, the longitudinal, caudal fibres possessed transverse striations which the authors attributed to a regular arrangement of dense bodies associated with the thin myofilaments. A similar conclusion was reached by Chapman (1973) in his detailed description of the organization of the tail musculature in *Himasthla secunda* and *Cryptocotyle lingua*. However, no clear indication of cross-banding was found by Rees (1971) in the tail of *Parorchis acanthus*.

The rate of tail oscillation seen in *Schistosoma mansoni* cercariae was found by Graefe, Hohorst & Drager (1967) to be more than 20 cyc/sec, enabling the larvae to develop a speed of 1.4 mm/sec. One might, therefore, expect that the tail muscle would show a related structural adaptation.

The purpose of this investigation was to examine the ultrastructure of the *Schistosoma mansoni* tail musculature responsible for this movement.

MATERIALS AND METHODS

A Puerto Rican strain of *Schistosoma mansoni* maintained in this laboratory was used throughout the investigation. Cercariae were collected by allowing infected

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Biomphalaria glabrata to shed into a small volume of fresh water from which the larvae could be separated by gentle centrifugation.

They were prepared for examination in the transmission electron microscope by initial fixation in cacodylate-buffered 2% glutaraldehyde at 4 °C and postfixation in Zetterquist's buffered osmium tetroxide (Glauert, 1965). Dehydration was followed by embedding in an araldite-epon mixture. The appropriate orientation for sectioning was accomplished by cutting out small cubes of embedding medium each containing only one cercaria and mounting these on pieces of perspex dowel before final trimming.

Sections were cut using a LKB 4800A ultratome and mounted on copper grids coated with formvar and carbon. Both uranyl acetate and lead citrate stains were used.

RESULTS

Present along the entire length of the tail stem are closely spaced, subtegmentary muscle fibres, arranged in outer, circular and inner, longitudinal layers. Circular fibres each measure approximately $1.40 \mu\text{m} \times 0.24 \mu\text{m}$ in transverse section and are consistently smaller in this respect than corresponding fibres in the body. They are very closely packed and there appears to be minimal intercellular space between adjacent fibres which are attached to the subtegmentary fibrous zone. No indication of regular, transverse striation was found (Pl. 1A).

There are six distinct blocks of longitudinal muscle in the tail stem, lying immediately beneath the circular muscle layer. These do not pass directly through the tail base into the body: the excretory bladder is situated centrally at the tail-body junction and is partially enclosed by the terminal region of body longitudinal muscle which contains, at this point, almost exclusively thin myofilaments. Caudal longitudinal myofibres are attached to the inside of the posterior end of the resulting 'cylinder' of thin filaments (Pls. 1B, 2A).

Mid-dorsal and mid-ventral blocks each comprise two closely adjacent myofibres which are rounded anteriorly but become flattened dorso-ventrally towards the distal end of the tail (Pl. 2B). There is no evidence of transverse striation.

The four lateral blocks may each appear in transverse section to consist of up to five fibres. However, the longitudinal muscle in furcocercariae does not necessarily lie parallel to the long axis of the tail. In *S. mansoni* each fibre lies at an angle of approximately 10–12° to that axis (Fig. 1A, B), with its anterior extremity situated laterally and its most posterior insertion close to the mid-dorsal or mid-ventral muscle block. This 'herringbone' pattern may be seen under the light microscope in whole mounts of cercariae which have been stained in, for example, 1% methylene blue.

A complete lateral muscle block therefore contains many more fibres than any one transverse section (Pl. 3A) might indicate. Where a fibre is flanked on both sides by others, it appears U-shaped in transverse section. The corresponding appearance of the anterior and posterior extremities of a fibre is L-shaped. Pl. 3A and Pl. 3B illustrate two clearly defined regions within these fibres: the contractile region lies near the circumference of the tail and is sheathed by a fenestrated

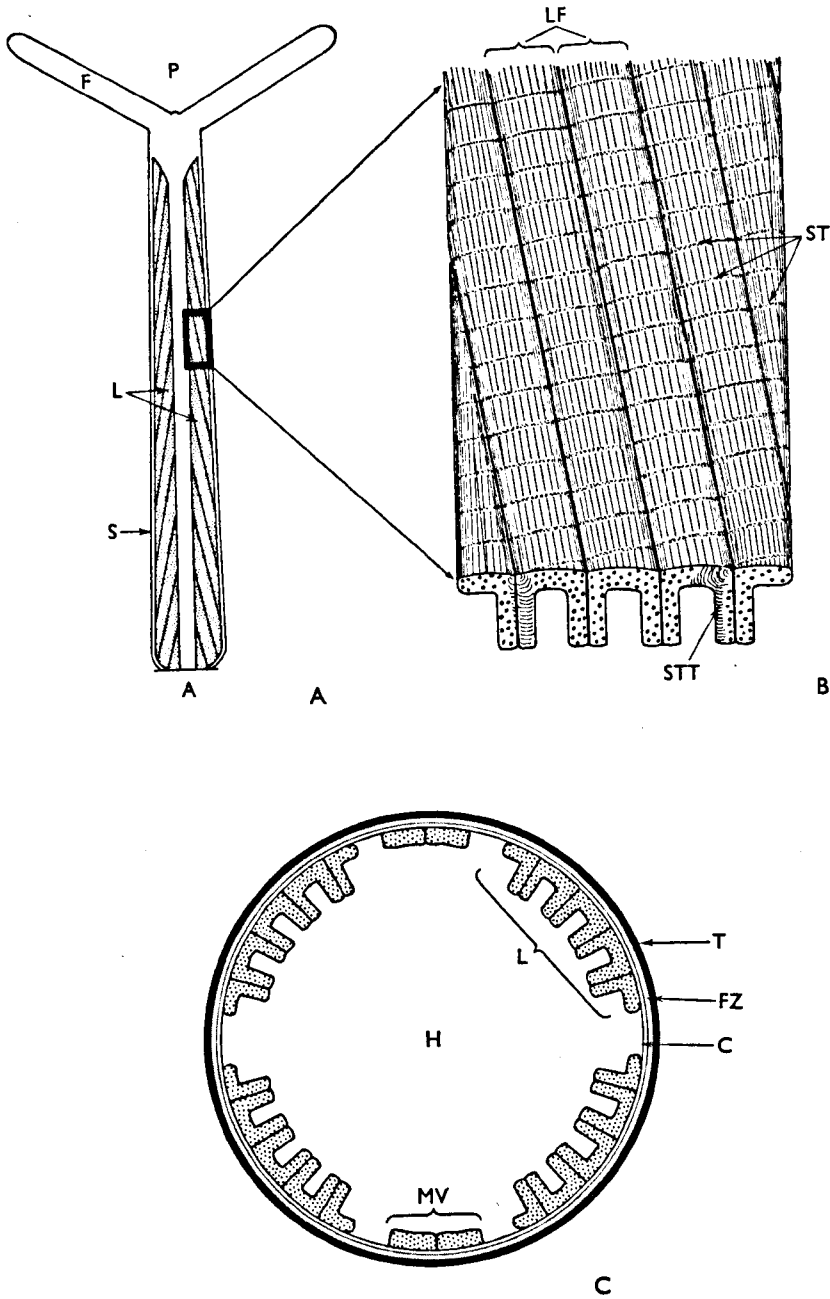


Fig. 1. A diagrammatic reconstruction of the organization of the tail musculature of *S. mansoni*. (A) Dorsal view of tail to illustrate location of dorsolateral muscle blocks and orientation of myofibres. (Not all myofibres shown, for clarity.) B. Enlargement of area indicated in A, showing the contractile regions of myofibres in surface view and in transverse section. C. Transverse section of central region of tail stem. (Sarcoplasmic regions of myofibres, excretory system etc. are omitted.)

sarcoplasmic reticulum. An inner region contains a nucleus, mitochondria, which are frequently closely associated with the contractile region, and glycogen granules. Together the inner regions contribute most of the volume of the tail core (the hydrostatic skeleton).

The lateral fibre contractile regions are distinctive in their structural periodicity: transverse striations occur at 0.8–1.0 μm intervals. Pl. 3B shows three such fibres in a longitudinal section of one lateral block and might appear to illustrate branching of the contractile region.

Closer examination of the striations reveals that each is composed of a series of electron-dense rods associated with branches of tubular sarcoplasmic reticulum, traversing at right angles to the fibre axis (Pl. 4A, B). As the plane of the section in Pl. 4B does not pass only through the centre of the striation, the arrangement of filaments between striations may also be seen. The centre is indicated by the asterisk and both dense bodies and sarcoplasmic tubules are present. To the right of this, immediately adjacent to the striation, only tubules and thin filaments are seen. Most of the sarcomere area, however, contains both thick and thin filaments.

The dense rods appear to be continuous with the thin filaments and are also attached, at least in part, to the sarcolemma (Pl. 5A, B). Striations of adjacent fibres are usually aligned across the width of each muscle block.

The relative number of thick and thin filaments appears to vary slightly, but there are frequently up to 10 or 12 thin filaments surrounding each thick filament (Pl. 4A).

Axons have not yet been traced across the tail-body junction, although nerve tissue has been identified immediately anterior to the bladder and a bilobed nerve mass is situated posteriorly. From this arise dorsal and ventral nerve cords which lie immediately beneath the mid-dorsal and mid-ventral muscle blocks and extend posteriorly to the furcae. Although axons have been traced which link these nerve cords to ciliated nerve endings associated with the tegument, a positive identification of a neuromuscular junction has not yet been made.

A diagrammatic representation of the tail stem musculature is given in Fig. 1.

The furcae are somewhat flattened laterally (Pl. 5C) and myofibres are not grouped into discrete blocks as in the tail stem but are more widely spaced. In addition to circular and longitudinal fibres there exist narrow, transverse ribbons of muscle (Pls. 5D, 6). No furcal fibres are striated.

DISCUSSION

Some of the most detailed reconstructions of cercarial tail musculature to be published before very much information was available from electron microscope studies were those of Pearson (1956, 1961). In the latter paper Pearson proposed that the longitudinal fibres in the four main muscle blocks of *Neodiplostomum intermedium* did not lie parallel to the long axis of the tail but that they formed a 'herringbone' configuration when viewed from the dorsal or ventral side. It is proposed that the musculature in *Schistosoma mansoni* cercariae is similarly arranged.

Cardell & Philpott (1960) claimed that, in *Himasthla quissetensis*, a continuous sheet of longitudinal muscle lay beneath the cuticle and that longitudinal fibres were orientated spirally around the tail. However, the evidence presented did not support this. In his more recent interpretation, Chapman (1973) found that the longitudinal muscle in *Himasthla secunda* had a greater cross-sectional area dorso- and ventro-laterally, to produce four main muscle blocks and that there was no lateral longitudinal muscle at all. The same general arrangement was found in *Cryptocotyle lingua*. Chapman, however, further proposed that each myofibre within each muscle block could be traced along the length of the tail, but it is difficult to reconcile this with the variable number of myofibres visible in the serial transverse sections presented.

Could it be that, as in *Schistosoma mansoni*, *Himasthla* provides another example of longitudinal fibres making an acute angle with the longitudinal axis of the tail such that, although all transverse sections still indicate approximately the same number of fibres per section, the same fibres are not necessarily seen? In addition, exact orientation of the tail during longitudinal sectioning would preclude similarly exact orientation of the fibres within. Unusual fibre patterns (e.g. in Chapman's Pl. 2A) might be explained in the above way.

Lumsden & Foor (1968) described the axial musculature of *Heterobilharzia americana* as being positioned obliquely to the tail axis but did not attempt a three-dimensional reconstruction. These authors also described branching in myofibres (see their Fig. 7). Pl. 3A of this paper appears to show the same feature in *Schistosoma mansoni* but branching is not considered to be responsible. What appear to be branches are, in fact, the two sides of the one fibre contractile region which is U-shaped in transverse section.

The presence of four main dorso- and ventro-lateral longitudinal muscle blocks appears, therefore, to be common to *Neodiplostomum intermedium* (Pearson, 1961), *Himasthla secunda* and *Cryptocotyle lingua* (Chapman, 1973) and *Schistosoma mansoni* (this paper), but opinions differ as to the precise arrangement of myofibres within each block.

Locomotion is effected by rapid, lateral vibration of the tail. This is presumably due to the contraction of the main (striated) blocks on the left and right sides of the tail alternately. Rotation about the longitudinal axis exhibited by swimming *Schistosoma mansoni* cercariae might be explained by slight differences in the degree, or timing, of tension developed in the two blocks of one side. In their analysis of tail movement by high-speed cinematography Graefe *et al.* (1967) did not mention this rotation. This study has demonstrated that, although transverse striations are present in the four main longitudinal muscle blocks of *Schistosoma mansoni*, they are absent from all circular fibres, from dorsal and ventral longitudinal fibres and from the furcae. Kruidenier & Vatter (1958) postulated that these striations were due to regular transverse arrays of sarcoplasmic tubules. Pearson (1961) compared them (in *Neodiplostomum*) with the Z-lines of vertebrate muscle. Lumsden & Foor (1968) demonstrated that an intimate association of sarcoplasmic tubules and dense bodies was responsible in *Heterobilharzia* and this would also appear to be the case in *Himasthla* and *Cryptocotyle* (Chapman, 1973).

It is interesting to note that in a recent study (Rees, 1971) of *Parorchis acanthus* there was no comparable demonstration of any striation. This could be associated with the relatively slow rate of oscillation of the tail of this larva (about 3/sec) compared with that of the other species discussed.

In *Schistosoma mansoni* striations reflect the presence of regular transverse arrays of sarcoplasmic tubules, interdigitating with transversely extended dense bodies, or rods, which are continuous with the thin myofilaments. Dense bodies are the functional equivalent of the Z-disk of vertebrate muscle. Franzini-Armstrong & Porter (1964) considered that the Z-disks might be important in the co-ordination of myofibril movement.

No true transverse tubule (T) system has been found in *Schistosoma mansoni*. Pearson (1961) found that striated tail muscle extended into the furcae, but this was not found to be the case in *Schistosoma*. The posterior terminations of the striated lateral muscle blocks were in the region of the bifurcation of the main excretory duct, just anterior to the tail stem-furcae junction. The presence of transverse furcal fibres may be related to the normally flattened appearance of the furcae. During normal (tail-first) swimming, the furcae are spread out laterally and the flattening would, therefore, have the effect of increasing the lateral surface area and presumably also the thrust.

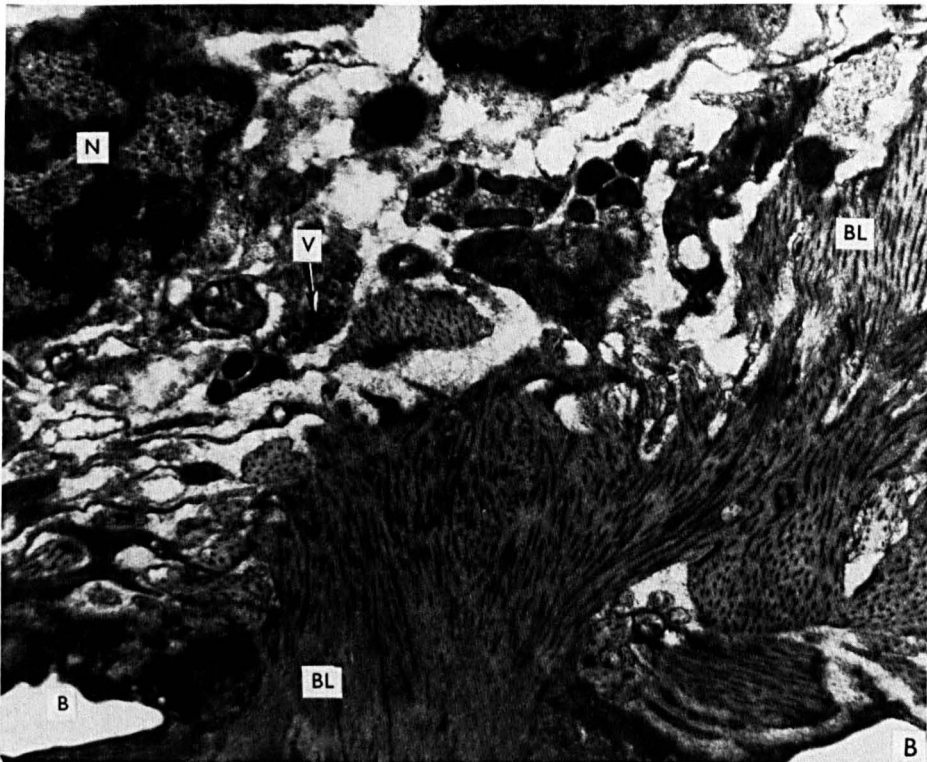
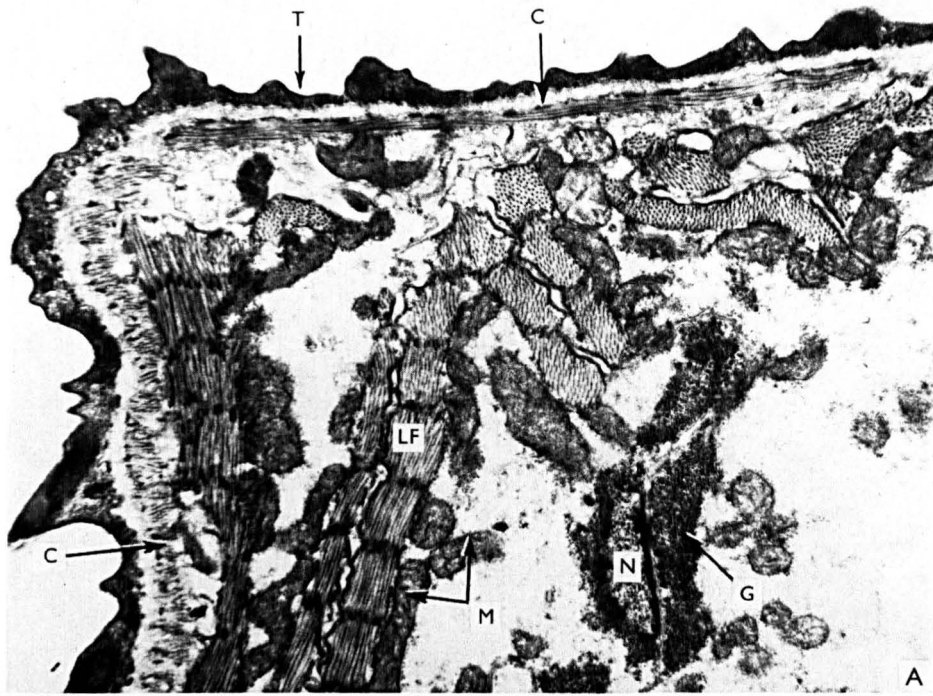
The ability of *Schistosoma mansoni* cercariae to swim at a speed corresponding to several times their own length per second is evidently an adaptation correlated with their dispersive function. However, when not subjected to potentially host-related stimuli such as mechanical disturbance, temperature gradients or appropriate chemicals in solution, they do not disperse laterally in the water. In these circumstances, swimming consists of alternating active/inactive periods when the larvae first swim almost vertically upwards then sink passively. Extended periods of horizontally orientated activity (which may culminate in crawling behaviour) occur only after appropriate stimulation and even then the horizontal distance travelled is negligible compared with the displacement caused by drift in even small water currents.

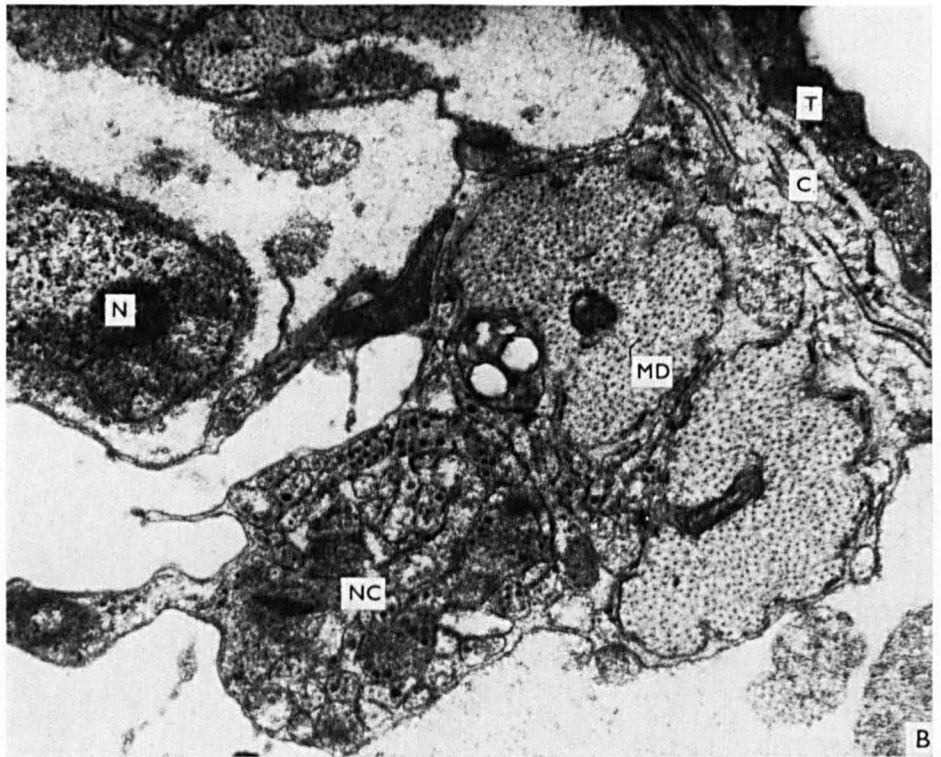
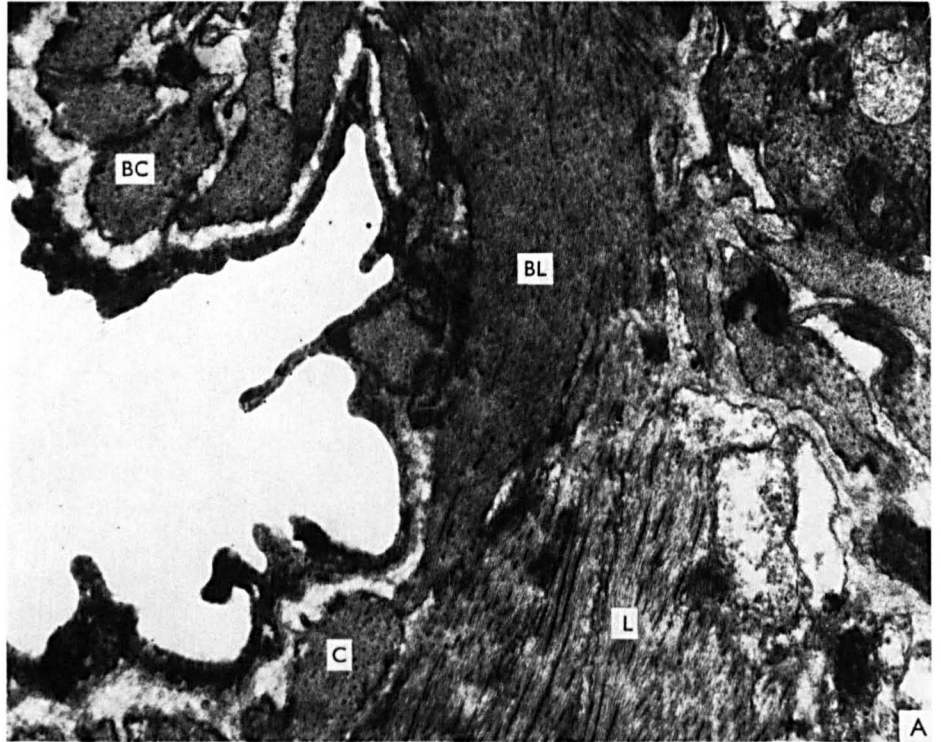
Swimming is considered to be important in enabling the cercariae first to maintain a position, clear of the substratum, where they will be subject to dispersive currents and secondly to react rapidly to the close proximity of a potential host by horizontal swimming which may then increase their chance of contact with the host.

I am grateful to Professor M. H. Williamson for providing the excellent research facilities and to Dr R. A. Wilson for his advice and encouragement. The work was carried out during the tenure of a Wellcome Trust research scholarship.

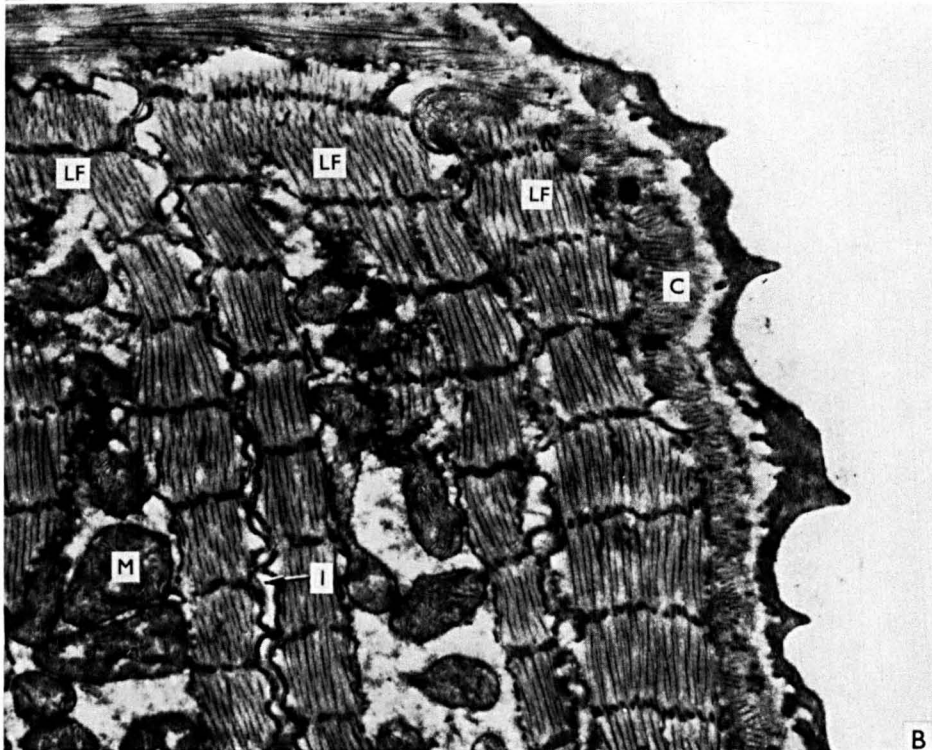
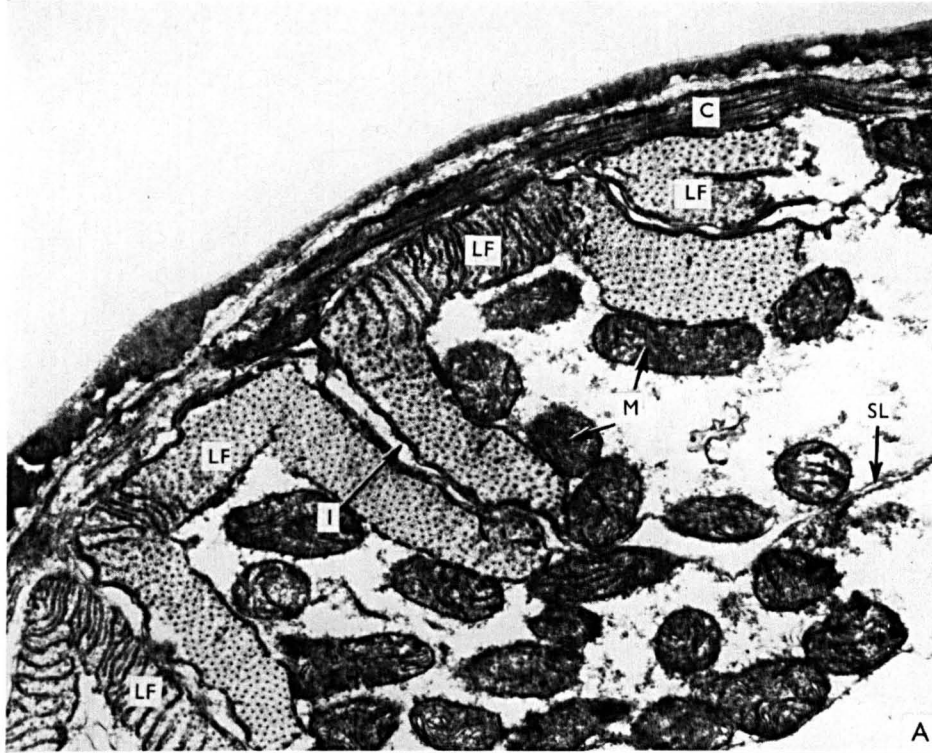
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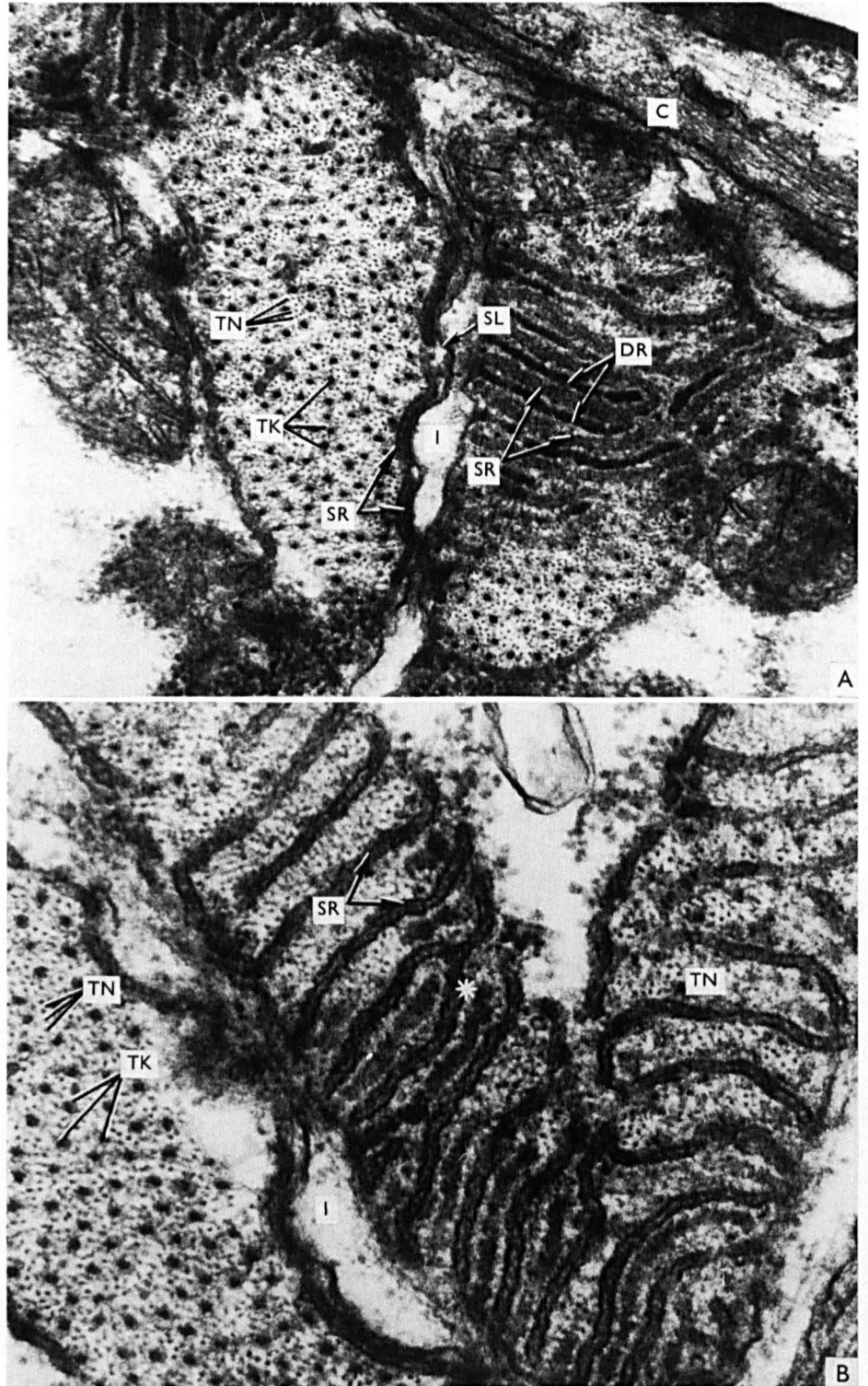
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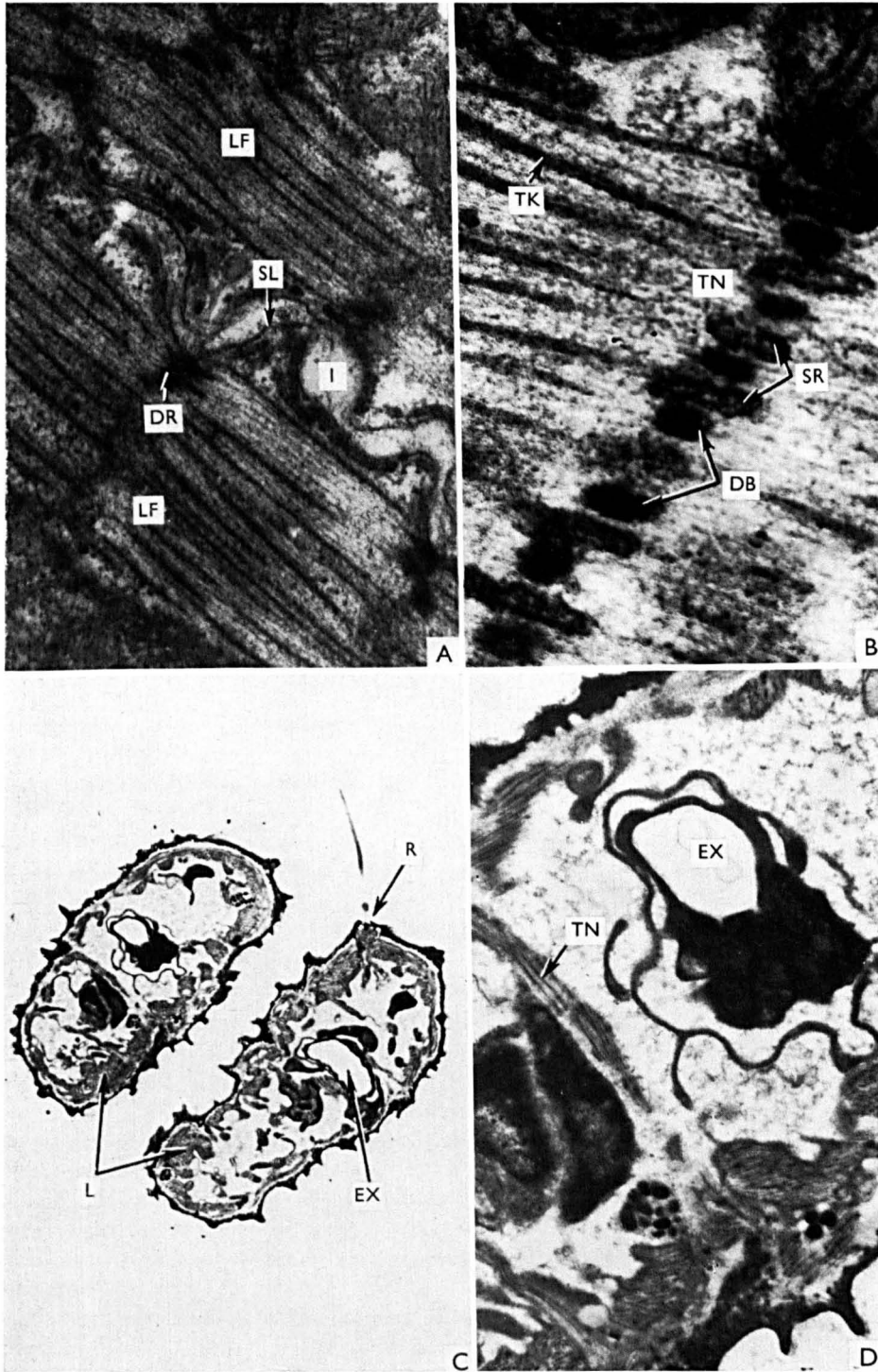


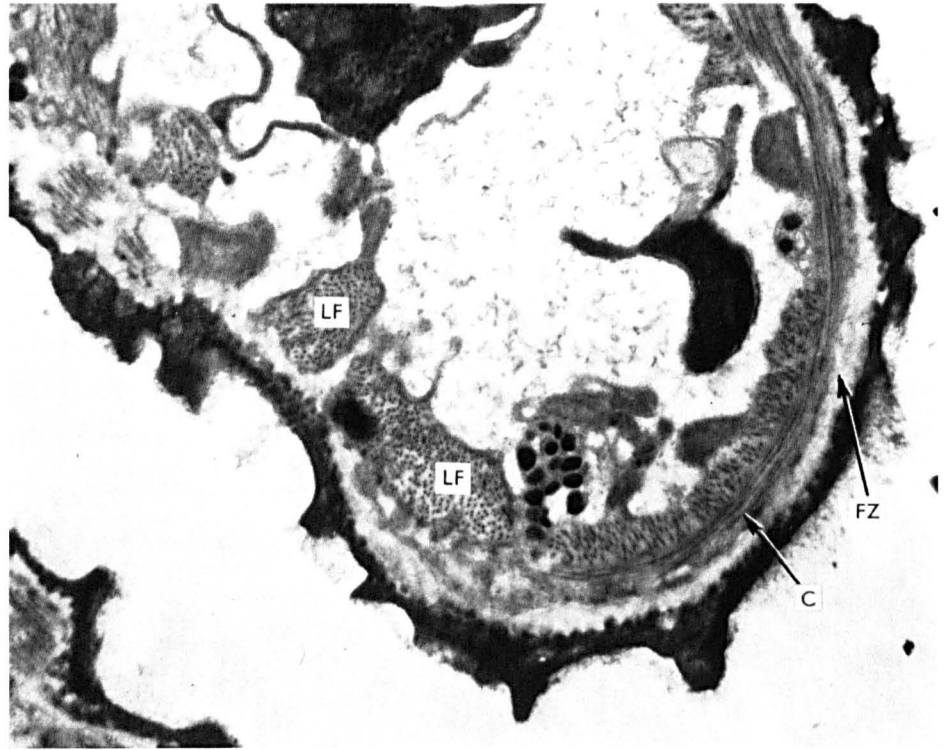
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EXPLANATION OF PLATES

PLATE 1

- A. Longitudinal section of base of tail. Circular myofibres seen in transverse and longitudinal section. Longitudinal myofibre nucleus, mitochondria and glycogen. ($\times 9600$.)
- B. Longitudinal section of posterior of body to show longitudinal fibres. ($\times 16800$.)

PLATE 2

- A. Longitudinal section of tail/body junction to show union of two sets of longitudinal musculature. ($\times 24000$.)
- B. Transverse section near base of tail. Mid-dorsal muscle block and associated nerve cord. ($\times 45600$.)

PLATE 3

- A. Transverse section of tail. Four fibres of one main (striated) longitudinal muscle block. ($\times 18400$.)
- B. Longitudinal section near base of tail. Parts of three fibres in one main (striated) longitudinal muscle block. ($\times 14400$.)

PLATE 4

- A. Transverse section of striated myofibres. Dense rods clearly shown. ($\times 47500$.)
- B. Transverse section of striated myofibre to show transverse tubules of sarcoplasmic reticulum in striation. ($\times 77000$.)

PLATE 5

- A. Longitudinal section of striated myofibre showing attachment of dense rods to sarcolemma. ($\times 42500$.)
- B. Longitudinal section of striated myofibre showing dense rods and sarcoplasmic tubules in transverse section. ($\times 133300$.)
- C. Transverse section of furcae to show overall shape and the peripheral distribution of muscle. ($\times 2800$.)
- D. Transverse section of furca. Excretory canal and transverse ribbons of muscle. ($\times 11300$.)

PLATE 6

- Transverse section of furca. Circular and longitudinal myofibres. ($\times 13200$.)

KEY TO LETTERING OF PLATES AND FIGURES

A	anterior	MD	mid-dorsal longitudinal muscle block
B	excretory bladder	MV	mid-ventral longitudinal muscle block
BC	body circular muscle	N	nucleus
BL	body longitudinal muscle	NC	nerve cord
C	tail circular muscle	P	posterior
DB	dense bodies	R	unciliate receptor
DR	dense rods	S	tail stem
EX	excretory canal	SL	sarcolemma
F	furca	SR	sarcoplasmic reticulum
FZ	subtegumentary fibrous zone	ST	striation
G	glycogen	STT	striation in transverse section
H	tail core - the hydroskeleton	T	tegument
I	intercellular space	TN	thin myofilaments
L	longitudinal muscle block	TK	thick myofilaments
LF	longitudinal myofibre	V	dense vesicles of nerve cell
M	mitochondria		

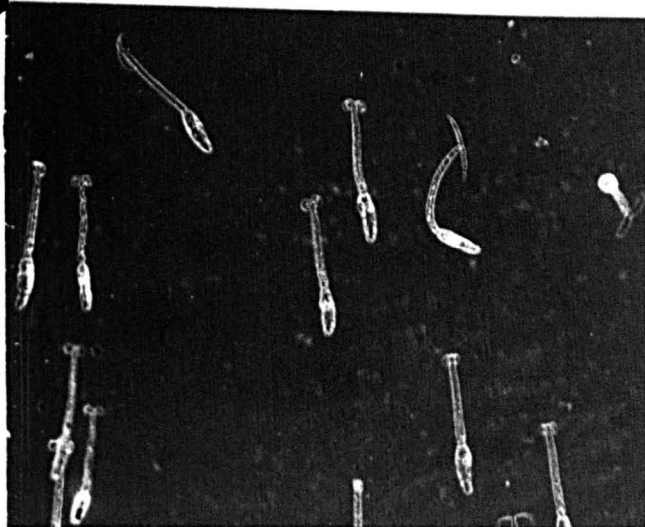
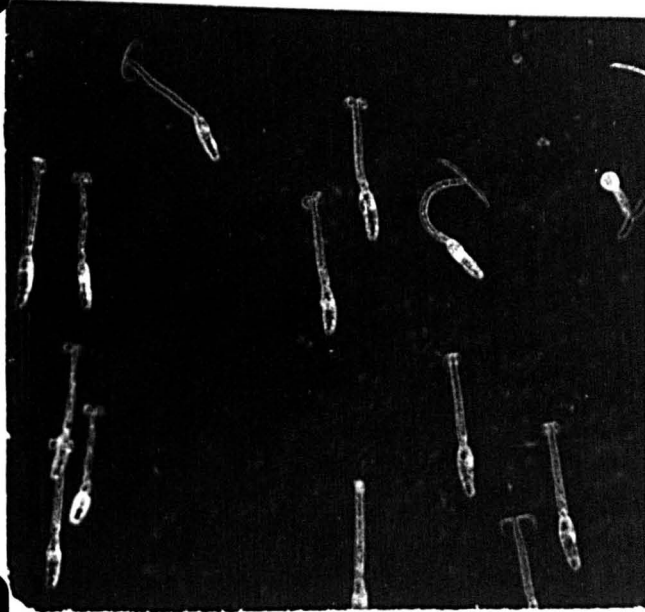
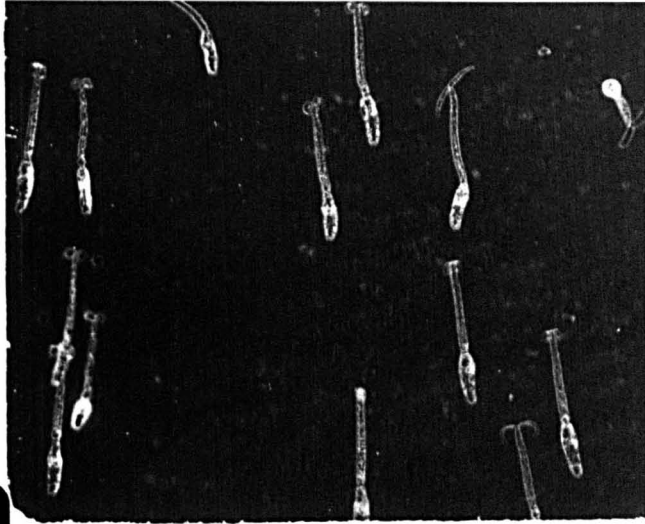
APPENDIX 8

Enlarged prints of cine frames used in the analysis of cercarial movements.

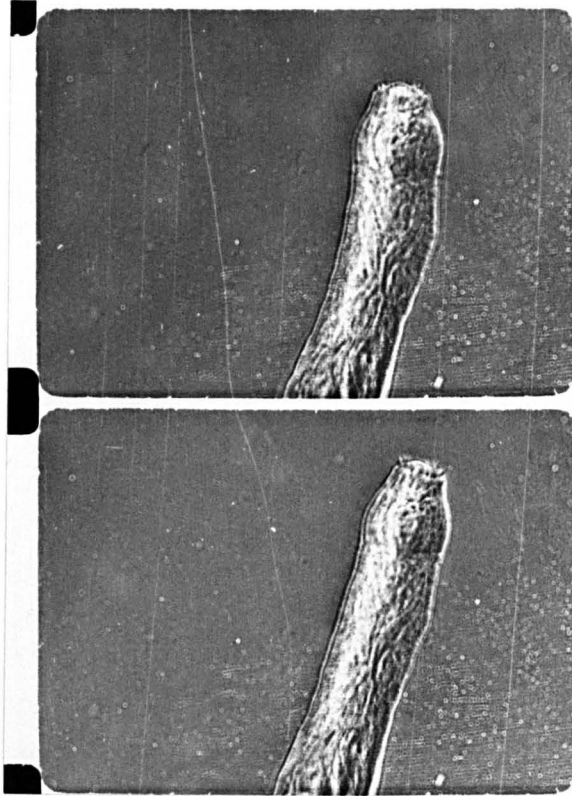
A tail first swimming

B body flexure.

A.



B.



APPENDIX 9

Tables to show the angular deviations of body, tail and furcae from their 'resting' position and the (cumulative) distance moved along the locomotory axis.

This data is presented graphically in Text Figs. 13-16.

BODY	}	DEGREES DEVIATION (as defined in Materials and Methods, Section 2)
TAIL		
FURCAE		
d		VERTICAL DISTANCE in mms from lower edge of cine frame, as measured on enlarged photographic prints.. This represents <u>x 45 the actual distance travelled.</u>

CERCARIA 1 (a)

<u>FRAME No.</u>	<u>BODY</u>	<u>TAIL</u>	<u>FURCAE</u>	<u>d</u>
1	56	155	110	27
2	35	34	80	25
3	-34	-76	-26	28
4	-73	-126	-100	28
5	-47	-24	-79	26
6	36	90	39	30
7	63	145	110	31
8	72	58	100	29
9	-20	-48	2	31
10	-67	-127	-92	32
11	-80	-73	-115	30
12	6	49	-41	32
13	51	127	87	34
14	62	90	107	33
15	4	-10	17	34
16	-37	-118	-84	36
17	-68	-79	-105	34
18	5	14	-45	35
19	47	101	76	38
20	61	71	94	36
21	6	11	26	37
22	-41	-116	-80	39
23	-80	-70	-101	42
24	-8	10	-24	39
25	53	100	73	41
26	72	119	90	40
27	17	26	49	41
28	-50	-111	-68	43
29	-85	-98	-80	41
30	-31	-16	-27	43
31	48	80	66	45
32	66	132	108	44

CERCARIA 1 (b)

<u>FRAMENO.</u>	<u>BODY</u>	<u>TAIL</u>	<u>FURCAE</u>	<u>d</u>
1	18	25	21	41
2	47	133	77	43
3	41	90	91	41
4	2	-37	-32	44
5	-32	-97	-99	45
6	-13	3	-70	43
7	42	82	41	46
8	72	103	94	45
9	27	-10	48	45
10	-32	-103	-66	48
11	-70	-114	-113	47
12	-11	-10	-41	47
13	50	100	54	49
14	86	104	94	48
15	17	101	28	49
16	-38	-105	-73	51
17	-66	-108	-104	50
18	7	12	-26	51
19	60	110	76	53
20	88	92	97	50
21	-1	-46	6	52
22	-45	-118	-78	54
23	-66	-112	-96	52
24	4	4	-34	54
25	53	90	87	56
26	93	93	100	54
27	1	-24	35	47
28	-35	-118	-71	58
29	-61	-74	-90	57
30	-11	-7	-70	58

CERCARIA 2 (a)

<u>FRAME NO.</u>	<u>BODY</u>	<u>TAIL</u>	<u>FURCAE</u>	<u>d</u>
1	96	73	80	15
2	29	-20	24	15
3	-15	-121	-95	19
4	-57	-103	-74	18
5	8	-6	-38	18
6	65	84	53	20
7	96	117	92	19
8	41	-2	35	18
9	-26	-82	-61	21
10	-65	-116	-105	21
11	-7	-27	-44	20
12	58	89	39	22
13	93	70	88	21
14	21	-21	11	21
15	-44	-125	-83	24
16	172	-142	-130	24
17	-23	-18	-70	23
18	53	64	26	25
19	100	99	98	25
20	72	0	69	23
21	-19	-100	-46	26
22	-55	-128	-98	27
23	-33	-29	-70	26
24	20	48	27	28
25	90	103	93	28
26	24	-3	48	27
27	-26	-105	-60	30
28	-53	-130	-96	30
29	-18	-22	-72	29
30	30	42	20	32
31	100	133	72	31
32	15	14	26	30
33	-18	-68	-60	33
34	-34	-140	-75	33

CERCARIA 2 (b)

<u>FRAMENO.</u>	<u>BODY</u>	<u>TAIL</u>	<u>FURCAE</u>	<u>d</u>
1	-84	-108	-72	37
2	-74	-10	-74	35
3	-11	74	52	38
4	32	155	113	40
5	38	62	102	39
6	-37	-31	6	40
7	-82	-73	-74	40
8	-104	-69	-89	38
9	-12	42	-15	40
10	41	123	85	42
11	74	122	119	42
12	1	6	47	41
13	-68	-94	-39	43
14	-92	-117	-95	43
15	-38	-9	-47	41
16	37	104	51	45
17	69	120	83	45
18	15	42	50	44
19	-57	-92	-46	46
20	-88	-80	-87	45
21	-1	30	-8	45
22	46	129	75	48
23	61	71	90	47
24	-4	12	38	48
25	-79	-101	-85	49
26	-100	-31	-90	48
27	7	62	11	49
28	40	134	68	52
29	45	68	85	50
30	15	0	21	52
31	-72	-96	-72	53
32	-80	-67	-60	52

APPENDIX 10

Parameters derived from data in Appendix 9 and from Text Figs.13-16.

CERCARIA 1a

a) Maximum angular displacement of tail (degrees) ... (T max.)

	+ve	-ve
	155	126
	145	127
	127	118
	101	116
	119	111
Mean:	129	120
S.D.	21	7

b) Maximum angular displacement of body (degrees) ... (B max.)

	+ve	-ve
	56	73
	63	80
	62	68
	61	80
	72	85
Mean:	63	77
S.D.	6	7

c) Maximum angular displacement of furcae (degrees) ... (F max.)

	+ve	-ve
	110	100
	110	115
	107	105
	94	101
	90	80
Mean:	102	100
S.D.	10	13

CERCARIA la (continued)

d) Maximum angular velocity of tail (degrees per 0.01 sec) ... (V max.)

+ve to -ve	-ve to +ve
74	58
58	54
67	47
67	46
51	49
Mean: 63	51
S.D. 9	5

e) Time tail displaced to each side (secs.) ... (t)

+ve	-ve
0.048	0.047
0.053	0.047
0.052	0.045
0.052	0.044
0.052	0.047
Mean: 0.051	0.046
S.D. 0.002	0.003

CERCARIA lb

a) Maximum angular displacement of tail (degrees) ... (T max.)

	+ve	-ve
	133	97
	103	114
	104	108
	110	118
	93	118
Mean:	109	111
S.D.	15	9

b) Maximum angular displacement of body (degrees) ... (B max.)

	+ve	-ve
	47	32
	72	70
	86	66
	88	66
	93	61
Mean:	77	59
S.D.	19	15

c) Maximum angular displacement of furcae (degrees) ... (F max.)

	+ve	-ve
	91	99
	94	113
	94	104
	97	96
	100	90
Mean:	95	100
S.D.	3	9

CERCARIA lb (continued)

d) Maximum angular velocity of tail (degrees per 0.01 sec.) ... (V max.)

	+ve to -ve	-ve to +ve
	60	58
	66	68
	67	69
	67	64
	67	66
Mean:	65	65
S.D.	3	4

e) Time tail displaced to each side (secs.) (t)

	+ve	-ve
	0.042	0.032
	0.045	0.052
	0.044	0.047
	0.044	0.050
	0.045	0.050
Mean:	0.044	0.046
S.D.	0.001	0.008

CERCARIA 2a

a) Maximum angular displacement of tail (degrees) ... (T max.)

	+ve	-ve
	117	121
	89	116
	99	142
	103	128
	133	130
Mean:	108	127
S.D.	17	10

b) Maximum angular displacement of body (degrees) ... (B max.)

	+ve	-ve
	96	57
	93	65
	100	72
	90	55
	100	53
Mean:	96	60
S.D.	4	8

c) Maximum angular displacement of furcae (degrees) ... (F max.)

	+ve	-ve
	92	95
	88	105
	98	130
	93	98
	72	96
Mean:	89	105
S.D.	10	15

CERCARIA 2a (continued)

d) Maximum angular velocity of tail (degrees per 0.01 sec.)... (V max.)

	+ve to -ve	-ve to +ve
	56	60
	64	63
	63	66
	64	56
	67	57
Mean:	63	60
S.D.	4	4

e) Time tail displaced to each side (secs.) (t)

	+ve	-ve
	0.047	0.052
	0.041	0.050
	0.044	0.053
	0.041	0.053
	0.044	0.053
Mean:	0.043	0.052
S.D.	0.003	0.001

CERCARIA 2b

a) Maximum angular displacement of tail (degrees) (T max.)

	+ve	-ve
	155	108
	123	73
	120	117
	129	92
	134	129
Mean:	114	104
S.D.	14	22

b) Maximum angular displacement of body (degrees) ... (B max.)

	+ve	-ve
	38	84
	74	104
	69	92
	61	88
	45	100
Mean:	57	94
S.D.	15	8

c) Maximum angular displacement of furcae (degrees) ... (F max.)

	+ve	-ve
	113	74
	119	89
	83	95
	90	87
	85	90
Mean:	98	87
S.D.	17	8

CERCARIA 2b (continued)

d) Maximum angular velocity of tail (degrees per 0.01 sec.) ... (V max.)

	+ve to -ve	-ve to +ve
	59	58
	69	61
	68	71
	55	67
	53	53
Mean:	61	62
S.D.	7	7

e) Time tail displaced to each side ... (t)

	+ve	-ve
	0.056	0.048
	0.053	0.047
	0.050	0.048
	0.053	0.038
	0.058	0.034
Mean:	0.054	0.043
S.D.	0.003	0.007

APPENDIX 11

'Lag' correlation coefficients obtained by comparing various combinations of B, T, F as described in the text (p. 105). Each column shows the 'lag' followed by the corresponding coefficient value.

	<u>(H-T) v (T-F)</u>		<u>(T-F) v (T-F)</u>
0	-0.7228	0	1.0000
1	0.2141	1	0.4094
2	0.7483	2	-0.3738
3	0.6789	3	-0.8558
4	-0.0901	4	-0.5147
5	-0.6941	5	0.2583
6	-0.7399	6	0.8596
7	0.0419	7	0.5190
8	0.6552	8	-0.1790
9	0.7253	9	-0.7631
10	0.0563	10	-0.6106
11	-0.6900	11	0.1514
12	-0.8644	12	0.7610
13	-0.1649	13	0.6179
14	0.5190	14	0.0422
15	0.8309	15	-0.7274
16	0.2570	16	-0.7040
17	-0.4683	17	-0.1103
18	-0.7684	18	0.5642
19	-0.4015	19	0.6697

	<u>H v T</u>		<u>H v H</u>
0	0.9250	0	1.0000
1	0.2741	1	0.5016
2	-0.6802	2	-0.4414
3	-0.9545	3	-0.9736
4	-0.3799	4	-0.5877
5	0.6001	5	0.3363
6	0.9550	6	0.9521
7	0.4670	7	0.6421
8	-0.5148	8	-0.2830
9	-0.9505	9	-0.9249
10	-0.5381	10	-0.6785
11	0.4469	11	0.2081
12	0.9356	12	0.9155
13	0.5931	13	0.7229
14	-0.3835	14	-0.1674
15	-0.9430	15	-0.9121
16	-0.6523	16	-0.7618
17	0.3055	17	0.0995
18	0.9443	18	0.8811
19	0.7324	19	0.8474

CERCARIA la (continued)

	<u>T v T</u>		<u>H v F</u>		<u>(H-T) v (H-T)</u>
0	1.0000	0	0.9029	0	1.0000
1	0.5096	1	0.8025	1	0.3266
2	-0.4433	2	-0.0742	2	-0.3089
3	-0.9745	3	-0.8554	3	-0.8708
4	-0.6087	4	-0.8457	4	-0.4277
5	0.3443	5	-0.0200	5	0.2083
6	0.9609	6	0.8017	6	0.9119
7	0.6833	7	0.8898	7	0.4425
8	-0.2350	8	0.1285	8	-0.1155
9	-0.9195	9	-0.7526	9	-0.8059
10	-0.7569	10	-0.9151	10	-0.5802
11	0.1626	11	-0.2009	11	0.0493
12	0.8953	12	0.7299	12	0.8196
13	0.7952	13	0.9240	13	0.6121
14	-0.0756	14	0.2210	14	0.0183
15	-0.8746	15	-0.7003	15	-0.7988
16	-0.8624	16	-0.9540	16	-0.7018
17	-0.0334	17	-0.3269	17	-0.1559
18	0.8244	18	0.6705	18	0.7137
19	0.8933	19	0.9667	19	0.6905

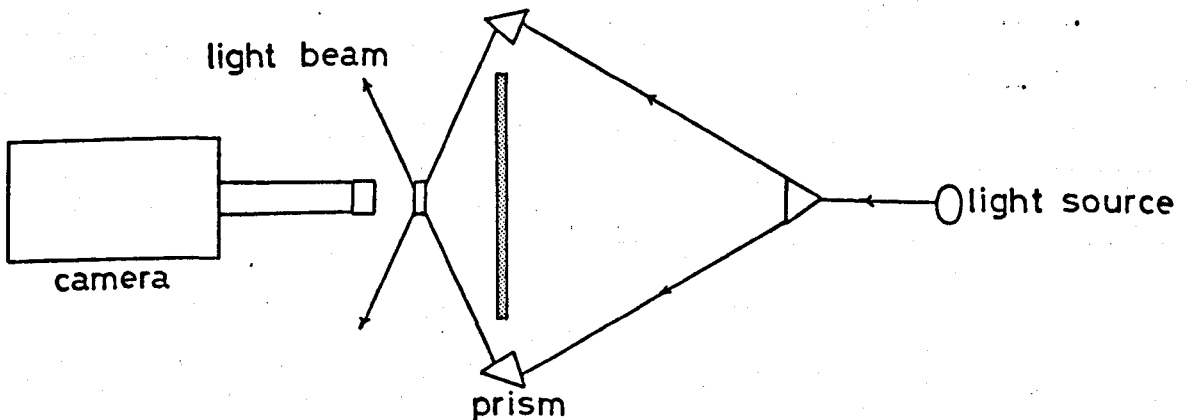
APPENDIX 12

The proportion of larvae 'active' at each of a range of constant temperatures. The figures show the number active and the corresponding sample size.

SAMPLE	TEMPERATURE °C								
	20°	22°	24°	26°	28°	30°	32°	34°	34°
1	19/20	18/20	26/26	22/22	18/20	24/26	12/14	14/22	21/27
2	19/20	20/23	19/20	24/24	18/18	26/28	24/28	12/28	19/27
3	25/25	18/18	25/25	20/20	26/28	17/20	28/30	5/19	8/15
4	20/22	21/21	30/31	23/24	19/19	18/21	27/29	9/24	15/25
5	19/20	17/19	22/26	12/12	21/21	10/13	27/27	5/24	15/34
6	29/30	24/26	19/21	18/19	20/22	26/30	31/31	8/16	10/24
7	24/24	15/17	22/25	20/20	18/20	28/30	42/44	8/23	16/28
8	23/26	18/19	22/22	22/22	21/22	23/26	26/32	7/14	9/28
9	20/20	17/17	28/35	14/18	30/30	21/27	29/29	5/19	16/23
10	20/25	15/17	25/27	17/18	21/23	27/28	28/33	9/21	8/38
TOTALS	218/ 232	183/ 197	238/ 258	192/ 199	212/ 223	220/ 249	274/ 297	82/ 210	137/ 279

APPENDIX 13

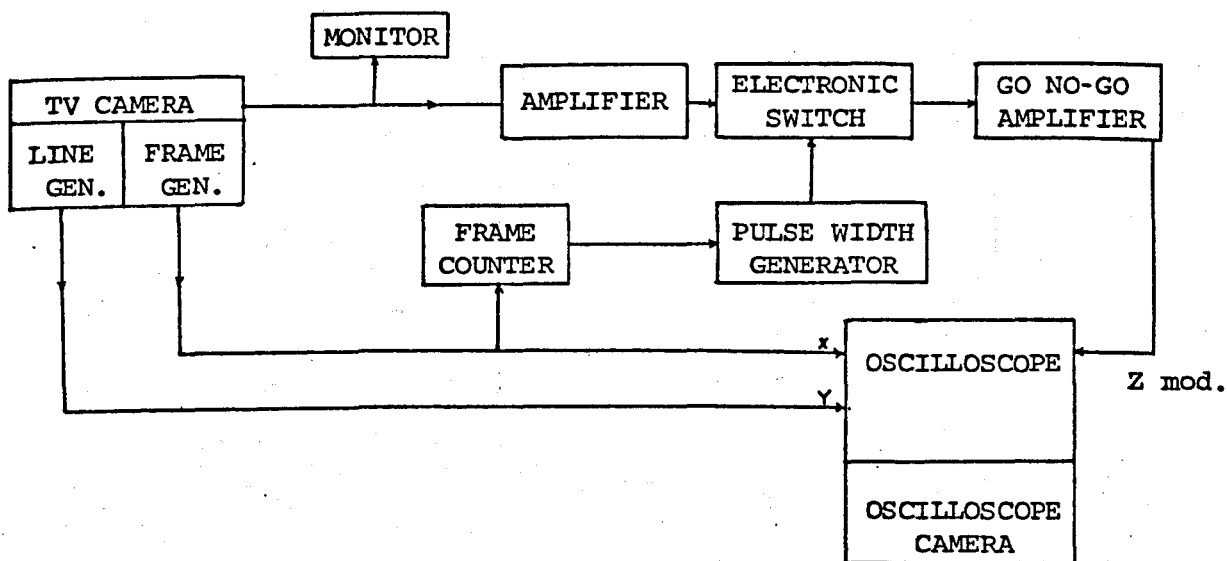
Outline description of scanning system for recording cercarial displacement. The electrical equipment was assembled by B. Adamson. Cercariae were monitored with a Pye 'Lynx' TV camera while swimming in a glass vessel of the type shown in Text Fig.7. The camera was fitted with an extension tube and 25mm lens. Optimum illumination was obtained with a dark ground system in which a beam-splitting prism directed the light from a single source to two laterally-placed prisms. The latter refracted the two light beams across the optical axis of the camera, through the cercarial suspension. An opaque screen was also placed between the camera and beam-splitter:-



The video signal from the camera was fed to a TV monitor and to an amplifier. Line and frame synchronisation pulses were taken from the camera to the X and Y inputs of an oscilloscope to produce a TV raster synchronised to the camera scan. The signal from the frame generator was also taken to an adjustable frame counter. This counted a pre-determined number of frame pulses before allowing a frame signal to pass. This signal was then lengthened to the duration of a complete frame in a pulse width generator and the signal from the latter used to operate an electronic switch. The latter therefore allowed the amplified

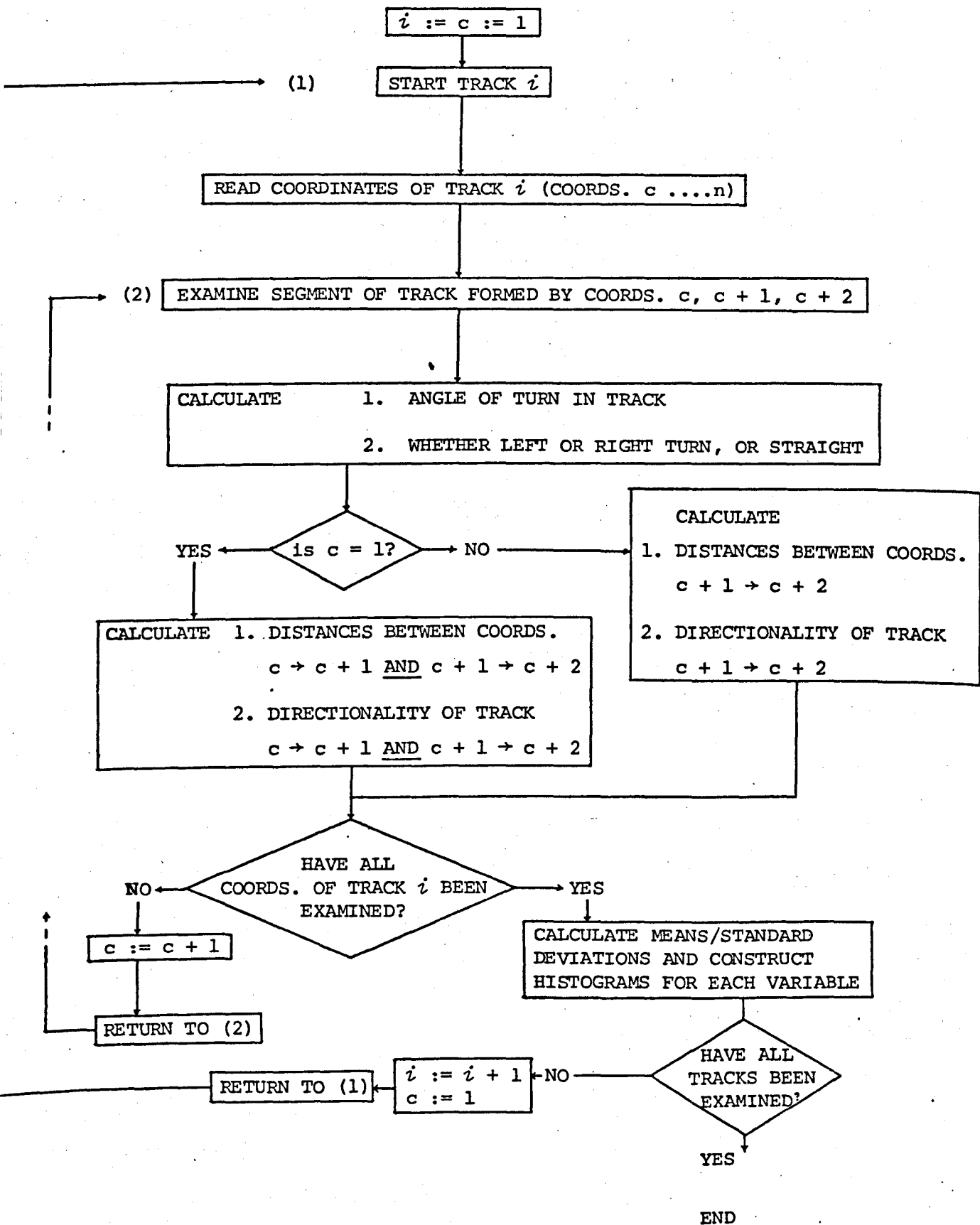
video signal to pass for the duration of one complete frame. A 'Go no-Go' amplifier was used to enhance the image contrast on the oscilloscope; this type of amplifier reacts only to the 'peaks' in the video signal. If the peak height is sufficient, a square wave pulse of fixed height is generated by the amplifier. This is fed to the Z-modulation of an oscilloscope. By this means, low intensity 'background' light is edited out, a cercarial position being marked by a bright spot on a black background. The operation of the frame counter produces an image which flashes onto the screen at the pre-determined rate, so that cercarial positions at successive intervals of time can be pinpointed. These positions were recorded with a Polaroid oscilloscope camera, using long exposure times (about 10 seconds). The positions could be converted to co-ordinates for use in a quantitative analysis of cercarial tracks.

DIAGRAM OF SCANNER SYSTEM



APPENDIX 14

Flow chart giving outline of program devised in ALGOL to analyse the tracks of motile organisms such as cercariae.



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