THE HOST-LOCATION MECHANISMS OF THE MIRACIDIUM AND CERCARIA OF SCHISTOSOMA MANSONI

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ABSTRACT.

Photographic techniques were used to record the behaviour of schistosome larvae in the presence and absence of host stimuli. A quantitative analysis of the behaviour patterns was performed, not only to provide information about the behaviour of the larvae, but also to enable the techniques of computer simulation modelling to be employed.

In the absence of stimuli, the miracidia swam in relatively straight lines with occasional changes in direction. An unstimulated miracidium, hitting the glass walls of the test cell, usually bounced off at an angle approximately equal to the angle of incidence. A proportion of the miracidia swimming close to a snail showed an increased rate of turning with a possible decrease in speed. These behaviour patterns were incorporated into a model of the host location process. The model predicted that the increase in turning alone was insufficient to improve the chance of contact with the snail and that sensory adaptation or reaction-time might be important in the location of the host. It was also shown that the length of the relatively straight periods of swimming in between intervals of turning, whether the miracidium was stimulated or unstimulated, greatly affected the chance of contact.

In the absence of host stimuli, the cercariae showed a rhythmic pattern of motility comprising of active upward swimming alternating with passive downward sinking. A cercaria reaching the surface usually stopped swimming and started to sink. A cercaria sinking onto the bottom was often stimulated to swim upwards. The cercariae responded

to rapid mixing by an increase in the time spent swimming so that an upward migration of the larvae was observed. A shadow also produced a similar upward migration except that, upon contact with the top of the cell, many of the cercariae continued to swim along the surface. In response to skin surface chemicals, the cercariae were found to swim in all directions with occasional reversals in the mode of swimming. The rapid mixing stimulus increased the effect of the chemicals. The response of the cercariae to skin surface chemicals and the unstimulated swimming pattern were incorporated into a model of the host location process. The model predicted that, in likely infection situations, the increased level of activity made the chance of contact with the host more likely than if no stimulation took place.

GENERAL INTRODUCTION.

It has been estimated that over 250 million people suffer from schistosomiasis throughout the world. This disease is caused by digenean parasites of the genus Schistosoma. The schistosomes that infect man are confined to the tropical and sub-tropical areas and the disease in itself is not necessarily fatal but has a severely debilitating effect on the individual.

There are three main species of schistosomes that infect man. These are Schistosoma mansoni, S.haematobium and S.japonicum. The parasite used in this study is Schistosoma mansoni.

The adult worms live in the mesenteric veins of man where they release their eggs. The eggs either work their way into the lumen of the gut or become lodged in the capillary beds of the host, in particular the liver. The host reactions to the various fates of these eggs cause the main symptoms of the disease. Many of the eggs voided to the outside with the faeces hatch in water to release a free-swimming miracidium larva. A miracidium, if it locates its intermediate host, the aquatic snail Biomphalaria glabrata, penetrates and transforms into the mother sporocyst. An asexual reproductive phase now takes place, daughter sporocysts and then cercariae being produced. The cercariae are shed into the water where the location and penetration of the definitive host, man, takes place. A migration of the parasites within the host to the mesenteric veins completes the life cycle.

This study is concerned with the two free-swimming larval stages, the miracidium and the cercaria.

The first chapter of this thesis is a study of the behaviour of the miracidium. The swimming pattern of unstimulated miracidia and of miracidia swimming close to a snail was photographically recorded and analysed.

Chapter 2 is concerned with the cercaria larva. The activity of the cercariae as they aged, and in response to host generated stimuli were also photographically recorded and analysed.

In Chapter 3, the data collected in the two previous chapters is incorporated into simulation models of the behaviour patterns of the miracidia and cercariae in an attempt to answer fundamental questions about the mechanisms of host location.

TECHNIQUES FOR RECORDING THE MOVEMENTS OF MICROSCOPIC ORGANISMS

The microscopic size of schistosome larvae makes the accurate recording of their activity very difficult and necessitates the use of optics to produce a magnified image. This image can be recorded using photography. Wilson and Denison (1970 a) and Mason and Fripp (1976,1977) have used a dark-ground technique to record the swimming behaviour of miracidia. Only periods of a few seconds can be recorded using this technique and the behaviour of an individual from second to second cannot be followed directly. Chernin (1970) and Etges et al (1975) have used single brief exposures of vessels containing miracidia to illustrate the change in distribution in response to a stimulus, but they failed to use this technique quantitatively. Sponholtz and Short (1975) have used high speed cine photography to record the behaviour of miracidia in an assay system for chemostimulation. However, these records of behaviour were only analysed by categorising the behaviour patterns and the actual swimming movements of the miracidia were not analysed in detail.

These photographic methods all suffer from the limited depth of field resulting from the high degree of magnification used. The movement of the larvae is recorded as if it occurred in only two dimensions and so will obviously introduce artefacts into the recorded behaviour. The sophisticated "Bugwatcher" of Davenport (1969), basically a flying spot microscope connected to a computer, suffers from the same limitations of the depth of field, as a television camera was used to view the organism under study.

At least two devices have been developed in an attempt to track the movements of bacteria in three dimensions (Lovely

et al 1974, Berg 1971). Lovely et al used variable speed stepping motors to drive a test cell in three dimensions in such a way that a single motile bacterium was kept stationary and in focus at the centre of the field of view. The servo control of this system was supplied by an operator using a joy stick to control the X and Y axes and a foot control for the Z axis (focus). Berg used three pairs of pre-focused fibre optics in a system which eliminated the human operator. Each of the three fibre optic pairs was positioned so as to view one of the three axes of the test cell. Each pair was focused at different levels within its axis so that a cube in space was produced within which a bacterium would appear partially in focus to all of the fibre optics. If the bacterium moved, the balance of the degree of focus within fibre optic pairs was upset and a servo mechanism was electronically engaged to reposition the cell and so restore the balance of focus. In this way a free-swimming bacterium could be tracked in three dimensions.

Both of these systems suffer from problems of inertia so that sudden changes in direction would not be recorded accurately. In addition, the behaviour of the organism might be affected by the movements of the test cell which maintain the organism in the field of view. Lovely's system also would suffer from the errors produced by operator reaction time. Neither of the systems is more accurate than one organism length and larvae spinning on their own axis are simply recorded as if they were stationary. These two systems require either sophisticated electronics or a computer for control of the experiment and data storage.

After consideration of all these techniques, I decided that a combination of time-lapse and cine photography would produce information on the behaviour of schistosome larvae with the least technical development and at considerably reduced cost.

Recording of miracidial activity

To prevent miracidia from swimming in and out of focus, a fused glass cell was specially constructed by Optiglass Ltd., London. The cell was 70 mm high, 50 mm wide and had an internal front to back dimension of 1 mm (see Fig. i). The cell was supported horizontally below a vertically mounted Bolex H16 Reflex cine camera and illuminated as shown in Plate 1. The intensity of the light at the level of the test cell was 500 foot candles. The film used was Kodak Plus X negative film and processing was by Filmatic Labs. Ltd., London.

Preparation of miracidia for photography.

The eggs of S. mansoni were separated from the digested livers and intestines of infected mice by sedimentation. The eggs were transferred to fresh tap water and allowed to hatch for one hour. Approximately 100 miracidia were then pipetted into the test cell and the recording of their behaviour followed five minutes later. Only miracidia that were less than two hours old (after hatching) were used for experimentation.

Recording of cercarial activity.

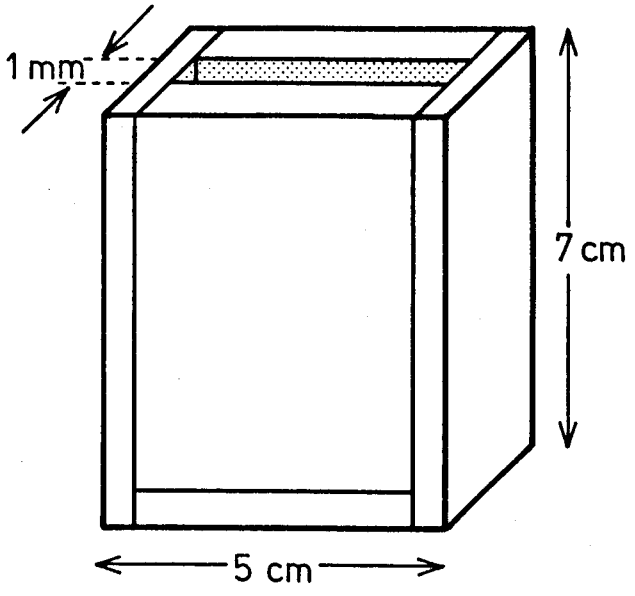
The behaviour of schistosome cercariae was recorded at two levels. Firstly, the distribution of a population of larvae was recorded using time-lapse photography and secondly, the swimming behaviour of individuals was recorded by cine

FIGURE i.

Diagram of the fused glass test cell.

FIGURE ii.

Diagram of the path of light passing through the test cell showing the areas not illuminated.


1 mm
7 cm
5 cm

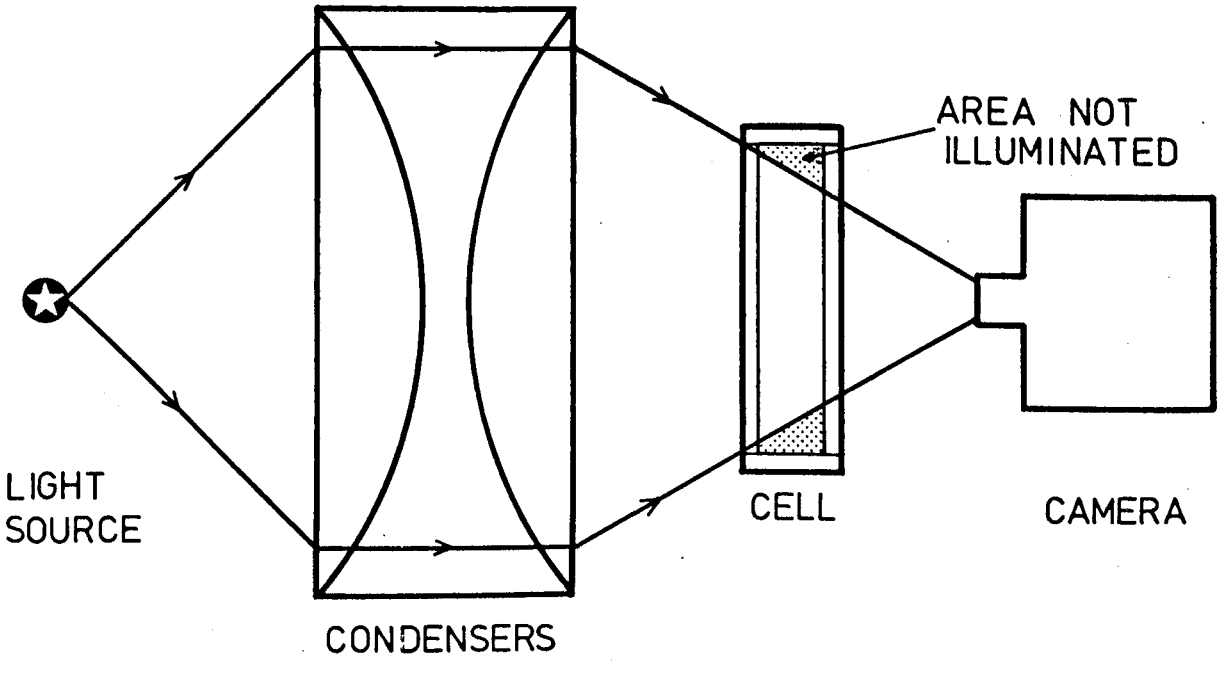
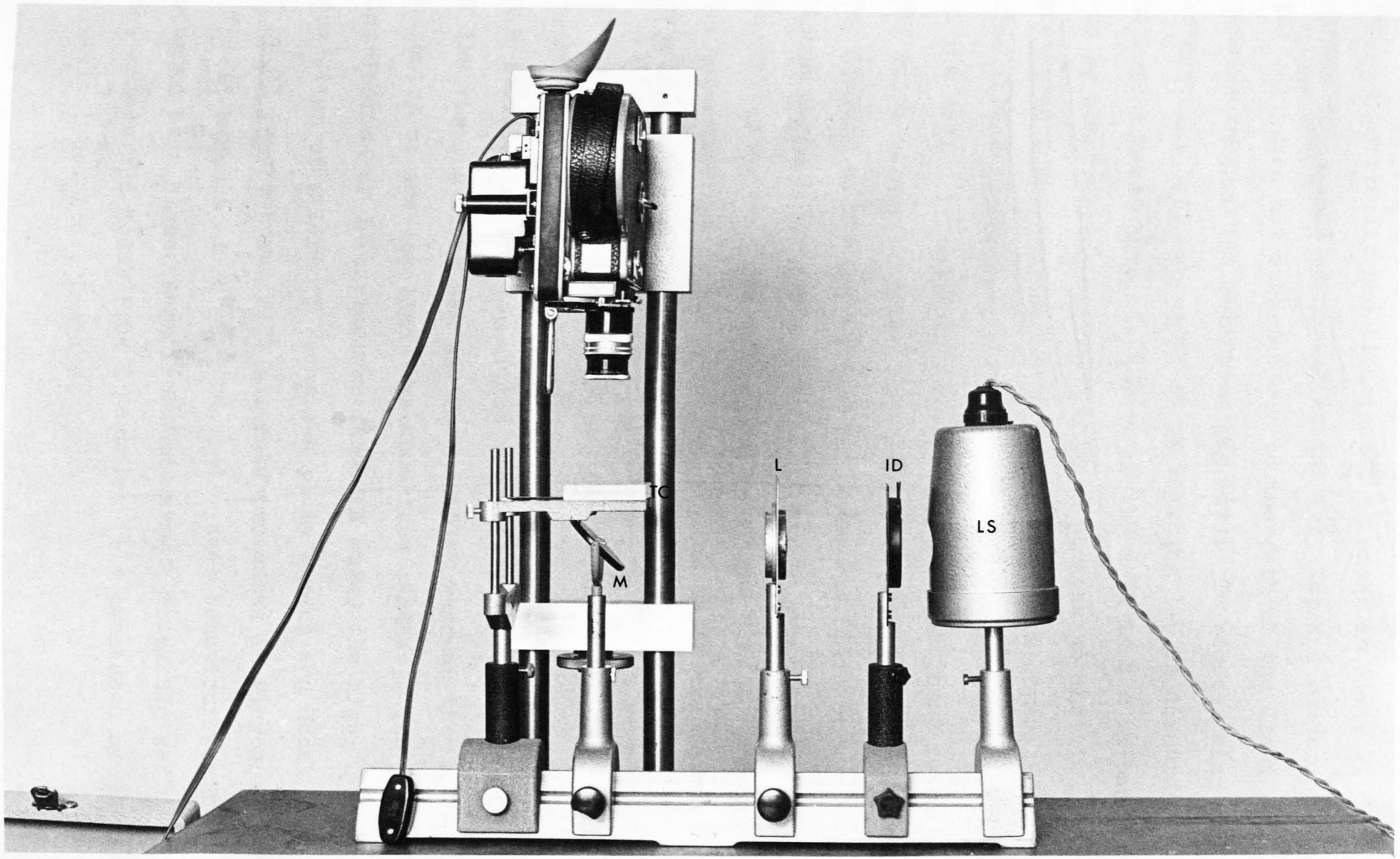

AREA NOT
ILLUMINATED
LIGHT
SOURCE
CONDENSERS
CELL
CAMERA

PLATE 1.

The lighting system for the photography of miracidia.

LS	Light source
TC	Test cell
M	Mirror
L	Lens
ID	Iris diaphragm and heat filter


L
ID
TC
LS
M

photography. The test cell, previously described, was mounted vertically and illuminated as shown in Plate 2. It was found that cercariae at the very top and bottom of the cell were not recorded due to the path of the light passing through the cell (see Fig. ii). To reduce this effect, the cell was modified by layering 145 µm diameter ballotini beads onto the bottom of the cell in a 2 mm band and by constructing a coverslip from perspex so that it protruded 2 mm into the top of the cell. This coverslip also served to reduce the evaporation of water from the top of the cell. It was this evaporation that was found to be the major source of convection currents. Distilled water was applied to the top of the cell (see Fig. iii) by a siphon arranged to replace the loss due to evaporation (distilled water rather than pond water was used to prevent the concentration of salts in the cell as water evaporated from the surface).

A flourescent light tube was placed above the test cell in order to produce a more natural vertical light gradient. At a position at the top of the test cell, the intensity of of the viewing light was 500 foot candles whereas the intensity of the top light was 4000 foot candles. The distribution of cercariae was recorded using the high resolution of a Linhoff Technicha plate camera and Kodak 5" by 4" Pancro Royal 7174 cut film processed in Microdol X for 15 minutes at 20° C. The individual behaviour was recorded at a higher magnification using a Bolex H16 Reflex cine camera and Kodak Plus X negative film processed by Filmatic Labs. Ltd., London.

PLATE 2.

The lighting system for the photography of cercariae.

PC	Plate camera
TC	Test cell
LS	Light source
C	Condenser lenses and heat filter
R	Reservoir and siphon system
TL	Top light (fluorescent)

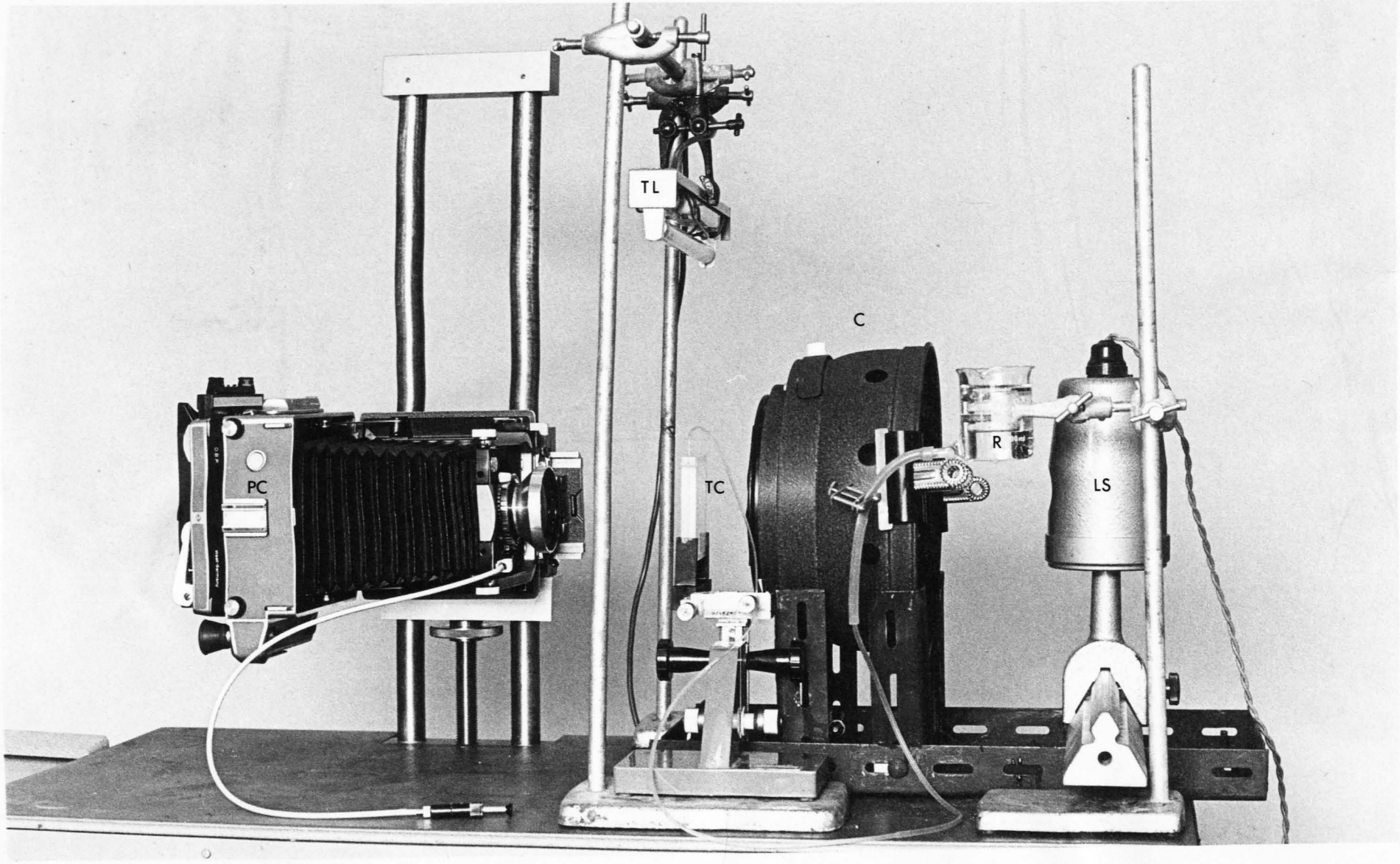
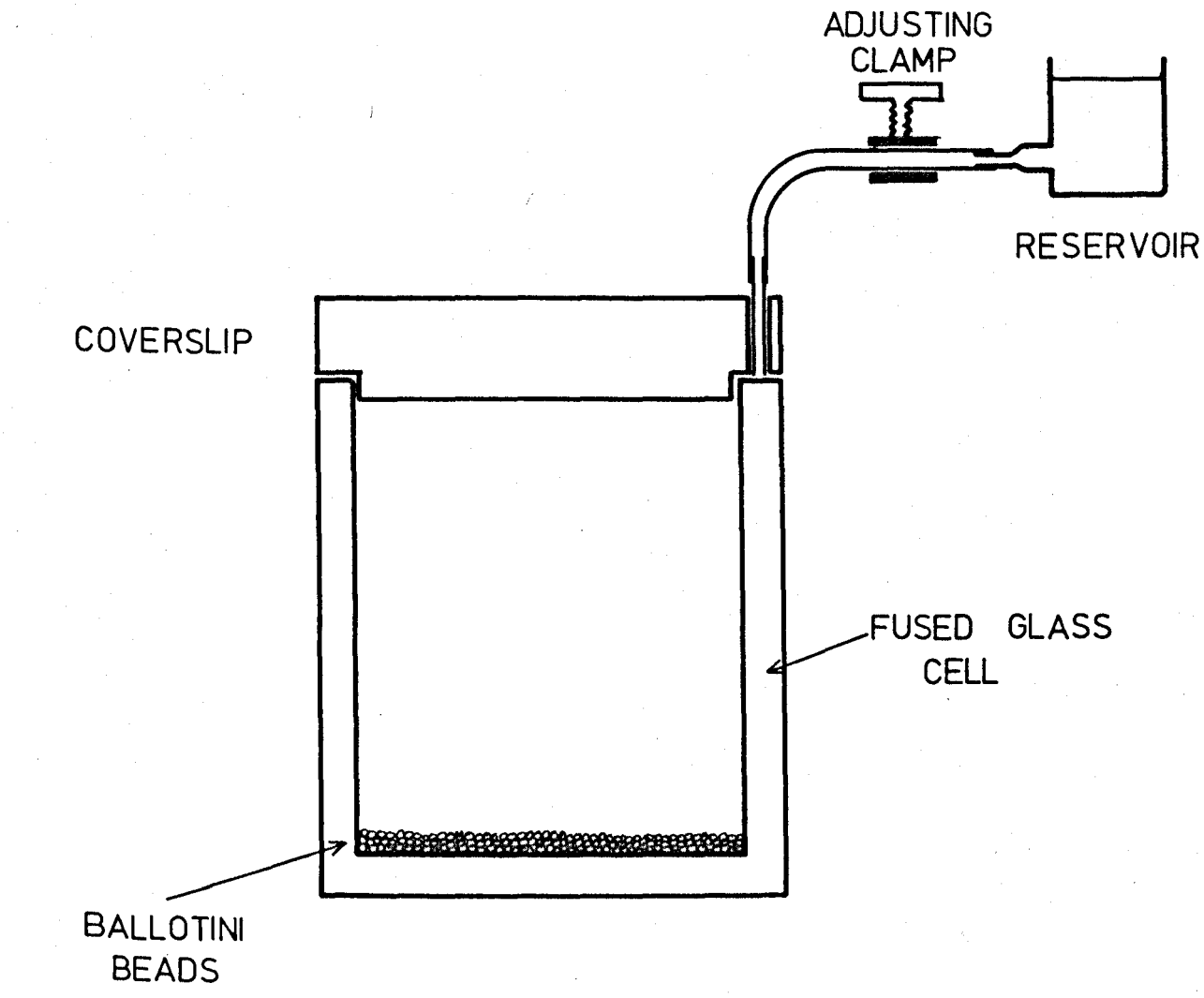

TL
C
PC
TC
R
LS

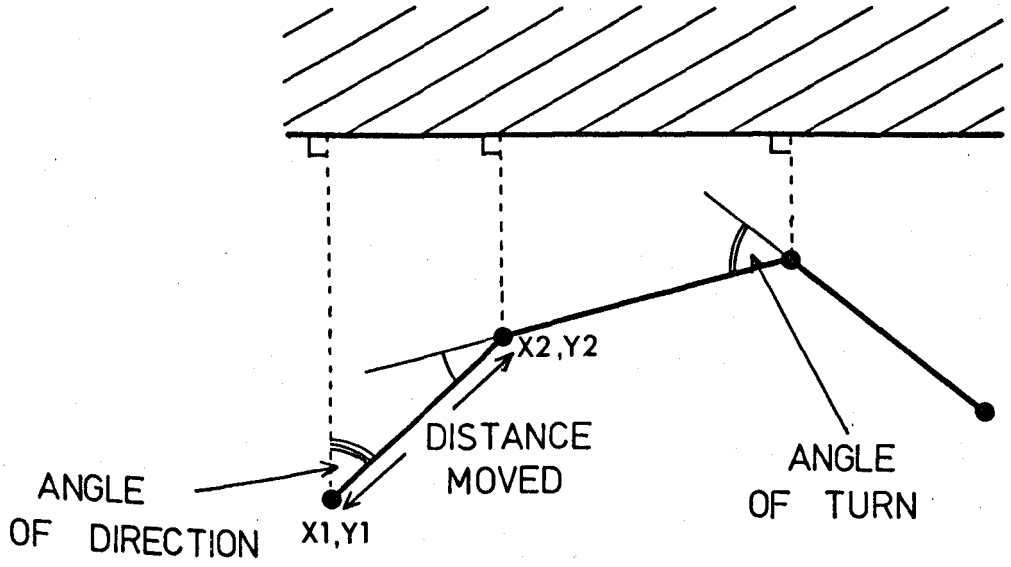
FIGURE iii.

Diagram of the test cell arrangement used for filming cercariae.

FIGURE v.

Diagram showing the angles of turn and direction and the distance between points calculated by track analysis.


ADJUSTING
CLAMP
RESERVOIR
COVERSLIP
FUSED GLASS
CELL
BALLOTINI
BEADS


X2,Y2
DISTANCE
MOVED
ANGLE
OF TURN
ANGLE
OF DIRECTION
X1,Y1

In most cases, cine photography is more than adequate to to record the activity of the cercariae. However, as the cine camera has a limited field of view, only cercariae that show the particular pattern of behaviour that retains them in the field of view are properly recorded. Cercariae exhibiting long swimming or sinking periods will swim in and out of the cine frame and will be lost from view. This situation is only a considerable problem when old cercariae are being followed or when the cercariae are highly stimulated. To overcome this biased sampling, direct observation of the cercariae over the entire cell was also used. The activity pattern of individual cercariae was recorded on a simple event recorder consisting of a morse key connected in series with a 9 volt battery and a Wantanabe WRT751 Miniwriter. The duration of swimming periods was marked onto the paper trace by depressing the morse key and so producing a comparable "step" on the trace. Sinking periods were recorded as the intervals between swimming "steps". The duration of periods in seconds was obtained by dividing the length of the "step" by the speed of the pen recorder trace. In this way extended swimming or sinking periods could be recorded. This method, however, will overestimate short periods due to operator reaction time. The selection of individuals when using an event recorder cannot be easily randomised. There is thus a possibility that a non-random sample of the population might be selected.

Preparation of cercariae for photography.

Two days prior to experimentation, the tanks containing the infected snails were cleaned and the water changed. Care was taken to ensure that the snails were maintained with a slight excess of food. On the day of experimentation, three

hours after the lights of the constant temperature room were switched on, the infected snails were washed and transferred to a glass beaker containing 5 ml per snail of aerated tap water. The snails were left to shed for one hour and then the water containing the cercariae was decanted into a glass filter containing a 4 µm pore cellulose filter. As the water containing snail metabolites and other impurities drained from the filter, it was replaced with aerated tap water until five times the original volume of suspension had been added. The suspension was then poured off.

The amount of dissolved oxygen in the cell affects the concentration of cercariae that can be supported over long periods. Bruce et al (1971) report that cercariae three hours after collection use oxygen at a rate of 6 µl of oxygen per 1000 cercariae per hour. The volume of water in the cell at 25° C contains 6 µl of oxygen. Thus 100 cercariae will have enough oxygen for 10 hours activity. This value assumes that there is no mortality of the cercariae and that no oxygen will diffuse into the cell. Thus approximately 100 cercariae were used in the cell for all experiments where the cercariae were allowed to age. The volume of the cercarial suspension was thus adjusted until there were approximately 30 cercariae per ml (the volume of the cell is 3.5 ml). The test cell was then filled with the cercarial suspension, the coverslip fitted and the water flow of the siphon adjusted. The cell was allowed to equilibrate for at least 15 minutes before any reading was taken. The mean age of the cercariae was calculated from the mid-point between the start and end of the shedding period.

TRACK ANALYSIS.

Ullyott (1936 a,b) tracking the movements of planaria manually with paper and pencil, has used two basic parameters to describe the movements. Firstly, he simply measured the speed of the organism and secondly, he measured a parameter he called "the rate of change of direction (rcd)". The rcd is the summation of all the angles turned by the organism between the points on the track divided by the total time of the track. Wilson and Denison (1970 a) and Mason and Fripp (1976) have used similar analyses of the movements of trematode larvae recorded using dark-ground photography. These techniques provide a good estimation of the way the organism is reacting over a period of time but the changes in speed and direction that are occurring dynamically throughout this period are not recorded. The analysis of a cine film record of the movements of an organism using developments of the methods of Ullyott (1936 a,b) comes much closer to the quantification of these dynamic aspects of the animal's behaviour.

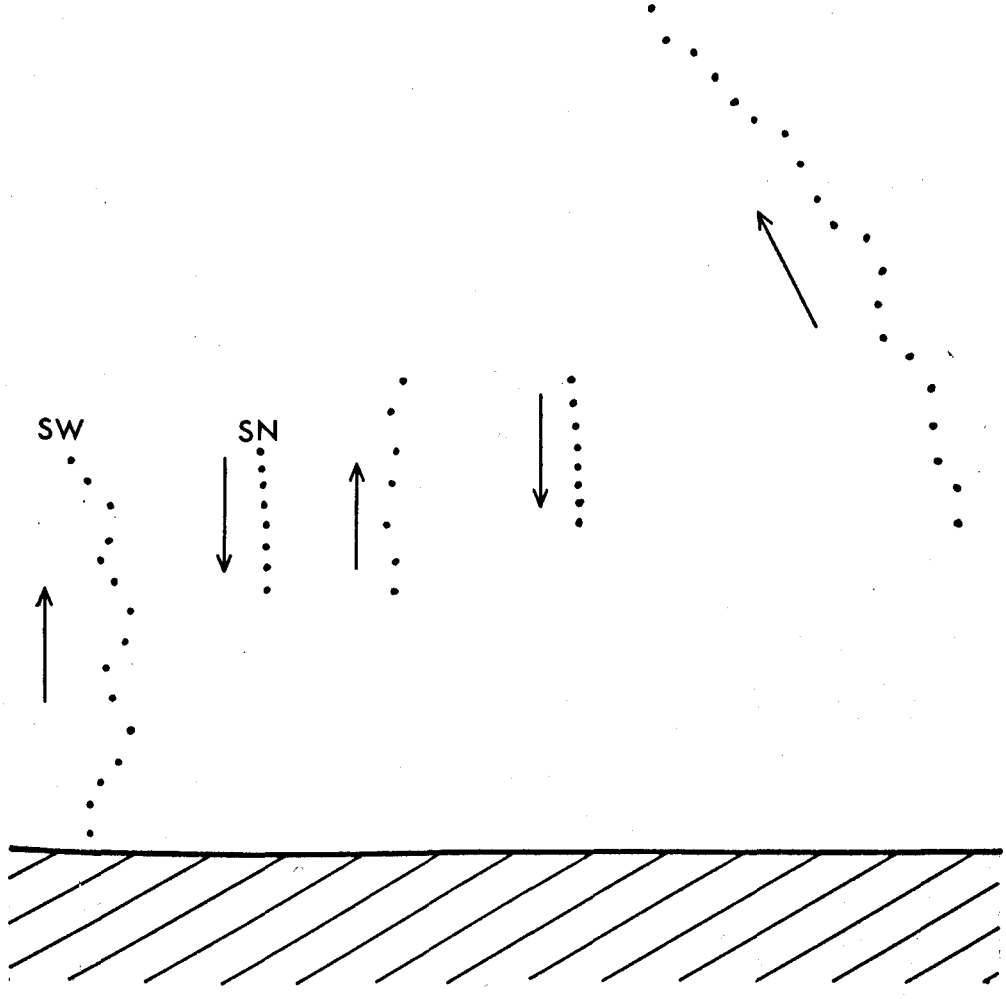
The following techniques were employed to obtain selected parameters of the motility of schistosome larvae by the analysis of high magnification cine films. A film of larval activity was loaded into a Specto Mk III Motion Analysis projector and projected onto a screen. Advancing the film frame by frame, the position of a larva on the screen was marked onto paper so that its passage was recorded as a series of points. Figure iv shows a series of these tracks for a single cercaria at the bottom of a test cell. Because cercariae exhibit two distinct activity patterns (ie. swimming and sinking) the movement of the cercaria has

FIGURE iv.

Sample tracks of a cercaria swimming at the bottom of the cell

SW swimming

SN sinking


SW
SN

been split into a series of alternate swimming and sinking tracks. To prevent tracks from being superimposed, the paper was moved parallel to the bottom of the cell between successive swimming and sinking periods. The bottom or side of the test cell was used as a reference line to allow different records to be compared. In addition, the position of a routinely filmed reference cross, 5 mm wide and long, was marked onto the paper to give a measure of the magnification in both axes. The face of a stop watch was also routinely filmed so that the speed of filming could be calculated by counting the number of frames between second intervals.

Each point of the tracks marked on paper was converted into a pair of Cartesian coordinates using the coordinate plotter shown in Plate 3. Two movable arms at right angles to each other are connected by miniature chains to two calibrated potentiometers. The voltage drop across the potentiometers is proportional to the position of the arms and was measured on a digital voltmeter with an Ascii coded paper tape output. The positions of the ends of the reference cross were also recorded in this way.

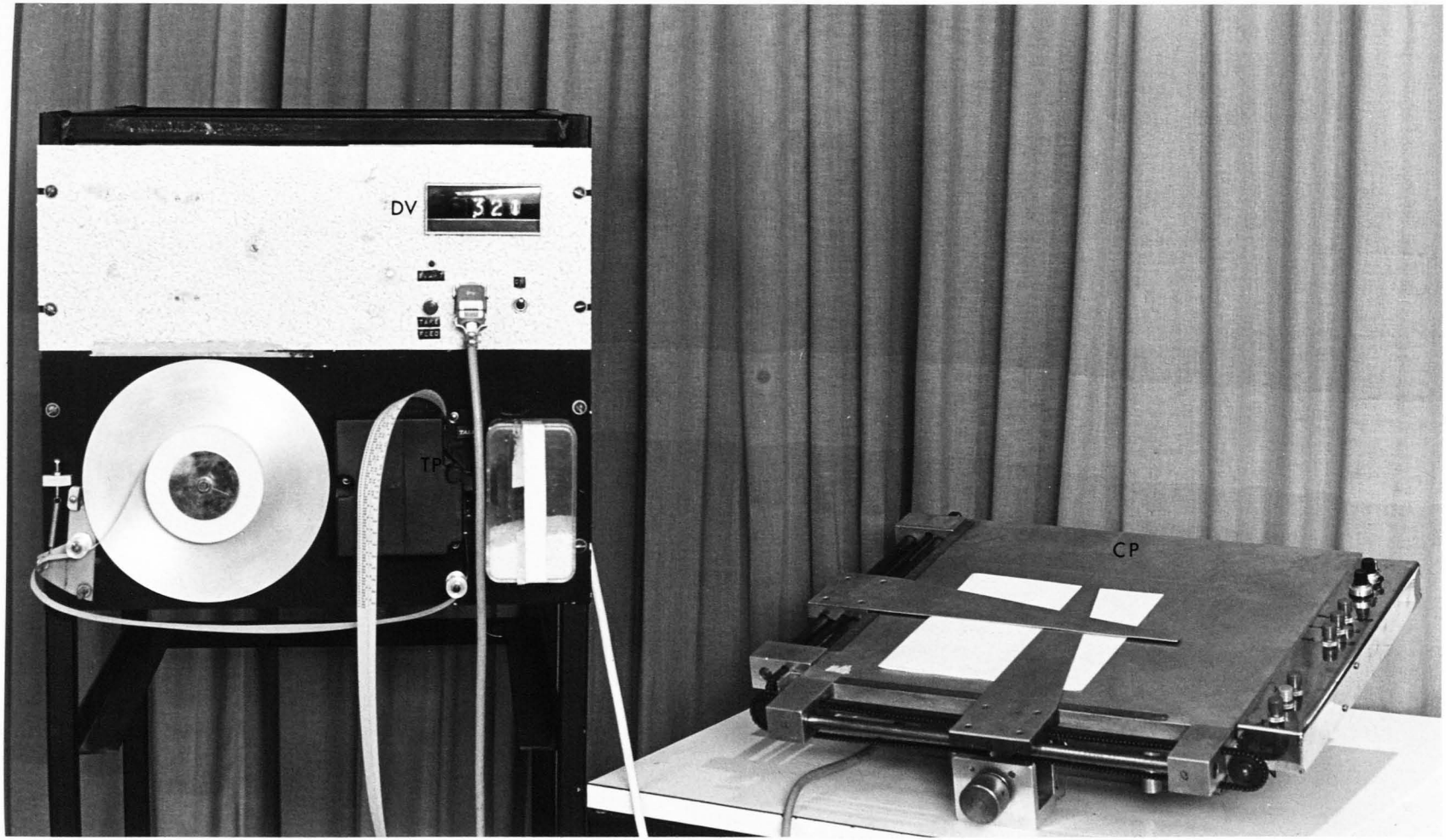
Data tapes of series of tracks were fed into a DEC 10 computer and each of the coordinates was corrected for the magnification of the cine camera, the projector, and the plotter using the values obtained for the reference cross. The corrected coordinates were then displayed on a Tektronix storage oscilloscope and the tracks were visually checked against the original tracks marked on paper. The following parameters were then calculated (see Fig. v)

a) the distance between points on the track (referred to as a "step") calculated by $\sqrt{(X2-X1)^2 + (Y2-Y1)^2}$

PLATE 3

The coordinate plotter, digital voltmeter and paper-tape punch.

CP Coordinate plotter
DV Digital voltmeter
TP Tape punch


DV
321
TAPE FEED
TP
CP

b) the direction of movement between points on the track measured relative to a target (eg. the top of the cell) and calculated by Arctan((X2-X1)/(Y2-Y1))

c) the angle turned between successive distance steps on the track. This was calculated by the difference between successive angles of direction.

d) the sign of the angle turned (ie. whether the angle turned relative to the preceding step is to the right (+ve) or to the left (-ve)).

e) the number of successive turns of the same sign. This parameter is referred to as the string length.

From the distance between points and the speed of filming, the speed of the larva at time intervals on the track can be calculated. For a series of tracks, these analyses result in frequency distributions for each of the selected parameters.

LABORATORY MAINTENANCE OF THE PARASITE.

A Puerto Rican strain of Schistosoma mansoni was used for experimentation. The original parasites were obtained from Dr. S.R.Smithers (N.I.M.R., Mill Hill, London, NW7). The parasite life cycle was maintained basically by the methods of Lawson (1977). The infected snails were kept in alternating periods of 12 hours light and 12 hours dark.

CHAPTER 1

THE ACTIVITY OF THE MIRACIDIA

1.1 INTRODUCTION

The schistosome miracidium becomes free-swimming when it hatches from the egg. The main stimulus promoting hatching is the dilution of the surrounding medium (Faust and Meleney 1924, Okamoto 1962, Chernin and Bower 1971, Kassim and Gilbertson 1976). As the osmotic pressure of the medium decreases, the number of miracidia hatching increases. Many authors have reported the longevity of schistosome miracidia (Faust 1924, Faust and Meleney 1924, Lampe 1927, Faust and Hoffman 1934, Penner 1939, Maldonado and Acosta-Matienzo 1948, Schreiber and Schubert 1949, Maldonado *et al* 1950, Singh 1950, Sugiura *et al* 1954, Oliver and Short 1956, Farley 1962, Asch 1975, Prah and James 1977). There appears to be general agreement that the half-life of miracidia is somewhere between 5 and 9 hours and that few miracidia survive until 12 hours after hatching.

The free-swimming miracidia respond to many environmental stimuli. The relationship between the swimming speed of the miracidia and temperature is basically linear (Takahashi *et al* 1961, Mason and Fripp 1976). This result suggests that the increase in the swimming speed as temperature rises, is a direct consequence of increased metabolic activity. Phototactic and geotactic responses of schistosome miracidia vary from species to species. *S. mansoni* and *S. japonicum* are generally positively phototactic and negatively geotactic (Takahashi *et al* 1961, Chernin and Dunavan 1962 Upatham 1972a,b, Sturrock and Upatham 1973, Chernin 1975, Mason and Fripp 1976, 1977). In contrast, *S. haematobium* is negatively phototactic and positively geotactic (Faust

1924, Shiff 1969,1974). As a result, S. mansoni and S. japonicum are most frequently found near the surface of the water, whereas S. haematobium is usually found near the bottom. The significance of these results is apparent when the distribution of the different host snails in the water is considered. The hosts of S. mansoni and S. japonicum are found near the surface while the host of S. haematobium is mainly a bottom dweller.

The similarity of snail and miracidial behaviour is even more complete in the case of S. haematobium. Shiff (1966), reported that the snail host, Bulinus globosus, is a bottom dweller in the summer months but that during the winter the snails could be found "basking at the upper surface of a pond" in the sunlight. Later he showed (Shiff 1974), that as the temperature fell, the orientation of the miracidia to light changed from negative to positive so that the miracidia tended to move from the darkened, cooler regions at the bottom to the illuminated and warmer regions near the surface. The reversal of a response to light as the temperature changes has also been reported for S. japonicum by Takahashi et al (1961).

Although many authors have reported no evidence of the chemo-stimulation of schistosome miracidia resulting in an aggregation of the larvae around the source of stimulation, (Cowper 1947, Stirewalt 1951, Chu and Cutress 1954, Najim 1956, Barbosa 1960, Sudds 1960, Chernin and Dunavan 1962, Wajdi 1964), the weight of evidence is now in favour of some sort of "chemo-attraction".

Three basic investigation methods have been employed to study the responses of miracidia to snails, snail products

or specific chemicals. Firstly, the distribution of miracidia in choice chambers containing snails or snail conditioned water (SCW), and around sources of test chemicals has been determined (eg. Shiff and Kriel 1970, Wright and Ronald 1972, MacInnis et al 1974, Etges et al 1975). An increase in the number of miracidia found around the source of stimulation was generally found when compared with control areas. Secondly, direct observation has been used to categorise the responses of miracidia to snails, SCW or test chemicals (eg.França and Almeida 1922, Faust 1924, Sudds 1960, MacInnis 196 Wright 1966, Chernin 1970, Sponholtz and Short 1975, Etges et al 1975). Lastly, the behaviour of the miracidia has been studied indirectly by measuring the percentage infection of sentinel snails in different experimental situations (Chernin and Dunavan 1962, Shiff 1968,1970, Etges et al 1975).

In most of these studies, when the specific nature of the orientation mechanism involved (if one exists) has been reported, it has been done without a detailed analysis of the behaviour patterns of individuals being undertaken. This has understandably led to some controversy. However, I feel that many of the authors referring to a "chemotaxis" (eg. Sudds 1960, Wright 1966, Shiff 1968,1970) have applied the term rather loosely and are simply referring to the response which leads to the aggregation of miracidia around the source of stimulation. Chernin (1970) suggests that, as the response of miracidia was found to be non-specific to the source of stimulation, it is more likely to be a chemokinesis that is operating rather than a chemotaxis. This view is supported by the observations of Wilson and Denison (1970 a) on the miracidium of Fasciola hepatica. Using photographic

methods to record the swimming pattern of the miracidia, they were able to quantify the responses of the larvae to several factors. The miracidia showed an increased rate of turning in the presence of the snail or snail mucus. They considered that this response was probably a chemoklinokinesis. Mason and Fripp (1976) have used a similar photographic technique to record the behaviour of S.mansoni miracidia. They also found an increased rate of turning when the miracidia were swimming in Snail Conditioned Water (SCW) and concluded that this response was a "klinokinesis with adaption". The more directed responses of miracidia described by MacInnis (1965) appear to take place over a short range, close to the snail. Some of these short range responses might indeed be chemotaxes.

The nature of the stimulant chemical has also been subject to some controversy. Short-chain fatty acids (MacInnis 1965), amino acids (Wright and Ronald 1972, MacInnis et al 1974), peptides (Mason 1977) and serotonin (Etges et al 1975) have all been reported as the stimulant for different schistosome species. Recently, an increase in the ratio of magnesium to calcium ions in the water (Sponholtz and Short 1976) and magnesium ions alone (Stibbs et al 1976) have been reported as miracidial stimulants (host snails absorb calcium and excrete magnesium). However, Mason (1977) found that ability of SCW to increase the turning of miracidia was not decreased if the calcium and magnesium ions were chelated with EDTA. Thus the role of calcium and magnesium in the behaviour of miracidia is somewhat unclear.

The host-location mechanism of schistosome miracidia would appear to be as suggested by Wright (1959). Firstly, the responses of the miracidia to environmental stimuli bring the larvae into areas likely to harbour snails. In the vicinity of a snail, the behaviour of the miracidia changes, possibly in a chemoklinokinetic way. This response to the presence of the snail has generally been assumed to increase the chance of contact with the host. Much nearer to the snail, tactic responses might be important in the actual attachment of the miracidia to the snail surface.

No reports have been found where the value of the responses of the miracidia to the presence of the snail have been assessed. It is to this problem that this chapter and part of Chapter 3 are addressed. The need to separate the individual stimuli of the host-location process has been eliminated by the use of a live snail as the source of stimulation. The behaviour of the miracidia in the presence and absence of a snail is recorded and analysed in a quantitative way such that the data produced can be used in a simulation model. The need to use a model of the host-location process is fully discussed in Chapter 3.

1.2 UNSTIMULATED BEHAVIOUR - EXPERIMENTS AND RESULTS.

1.2.1 TRACKS OF NORMAL SWIMMING

Miracidia were prepared for filming as previously described and approximately 100 were pipetted into the test cell. Only miracidia which were less than two hours old were used for filming. The test cell filled with miracidia, was placed horizontally beneath the cine camera and was illuminated as previously described. A coverslip was not needed as the miracidial suspension was kept in the cell by surface tension. All experiments were carried out in a constant temperature room set at 25° C.

One hundred feet of film were exposed as the miracidia swam in and out of the field of view. The processed film was projected onto a screen and individual miracidia were selected for analysis. Normal random sampling techniques were not used to select the miracidia, as many of those selected rapidly left the field of view. Instead, a circle of radius 100 mm was drawn onto the screen and its perimeter divided into 30 equal sections. The first miracidium to enter each of the sections was then tracked. In this way most of these miracidia swam right across the circle and so could be tracked over considerable distances. The positions of the miracidia in each frame of the cine film were recorded onto paper and the tracks subjected to track analysis. Typical miracidial tracks are shown in Fig. 1.2.1.

Speed of swimming.

The frequency histogram of the distance between points on the track was produced by track analysis and was found to be normally distributed about a mean of 0.20 mm with a standard error of 0.001 (n=934). This distribution is shown in Fig. 1.2.2. The mean of the distance between

FIGURE 1.2.1

Typical tracks of unstimulated miracidia

The time interval between points on the track is 0.091 seconds.

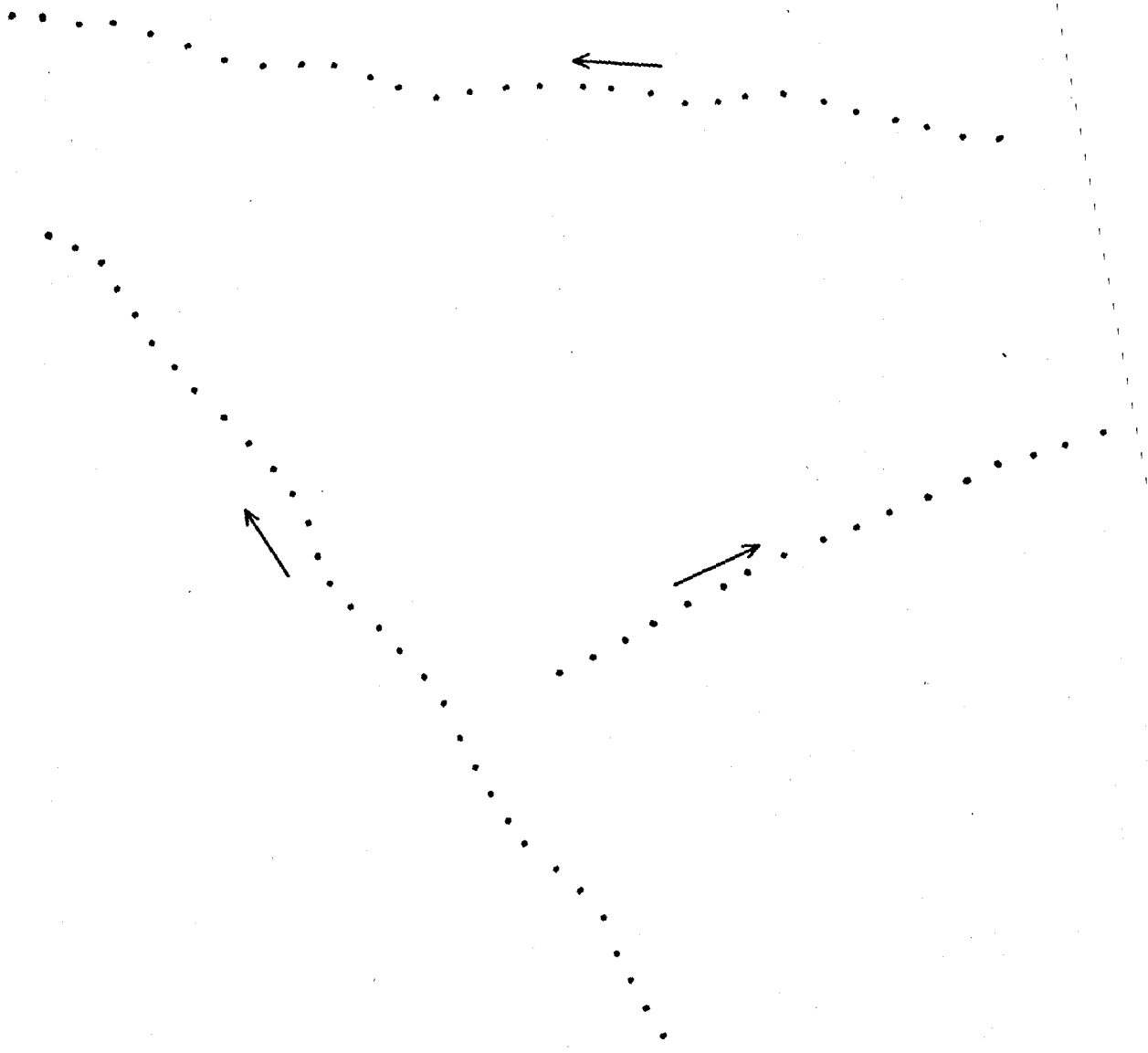


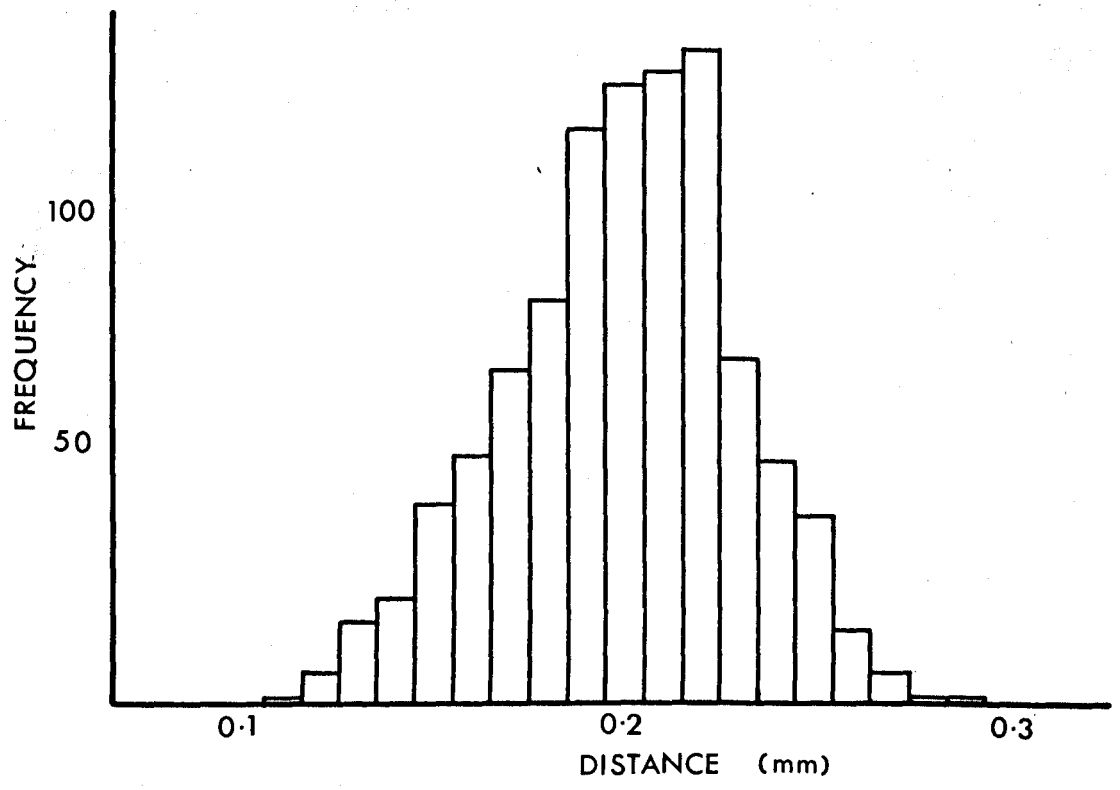
FIGURE 1.2.2

Frequency histogram of the distance between points on the tracks of unstimulated miracidia.

FIGURE 1.2.3

Frequency histogram of the directionality of unstimulated miracidial tracks.

0^{o} directly upwards in the cine frame
180^{o} directly downwards


FREQUENCY
100
50
0·1
0·2
0·3
DISTANCE (mm)

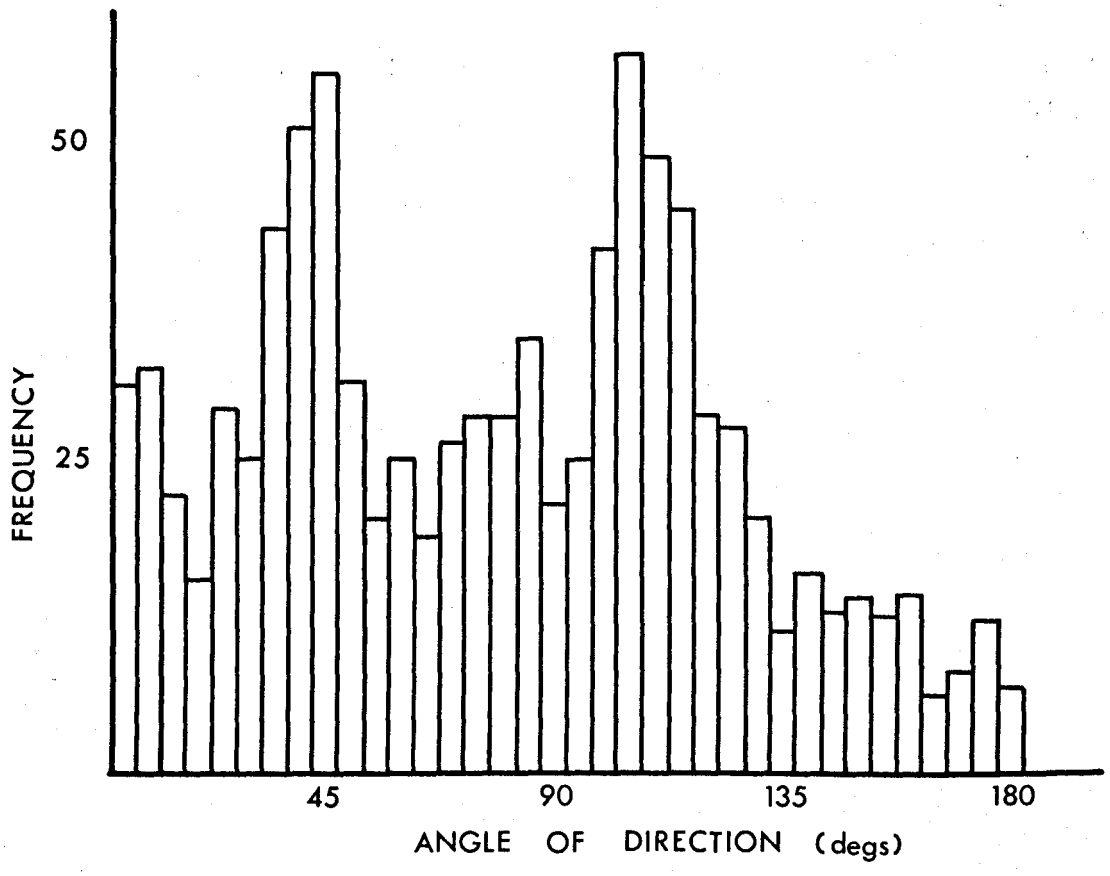

FREQUENCY
50
25
45
90
135
180
ANGLE OF DIRECTION (degs)

TABLE 1.2.A Analysis for angle of direction.

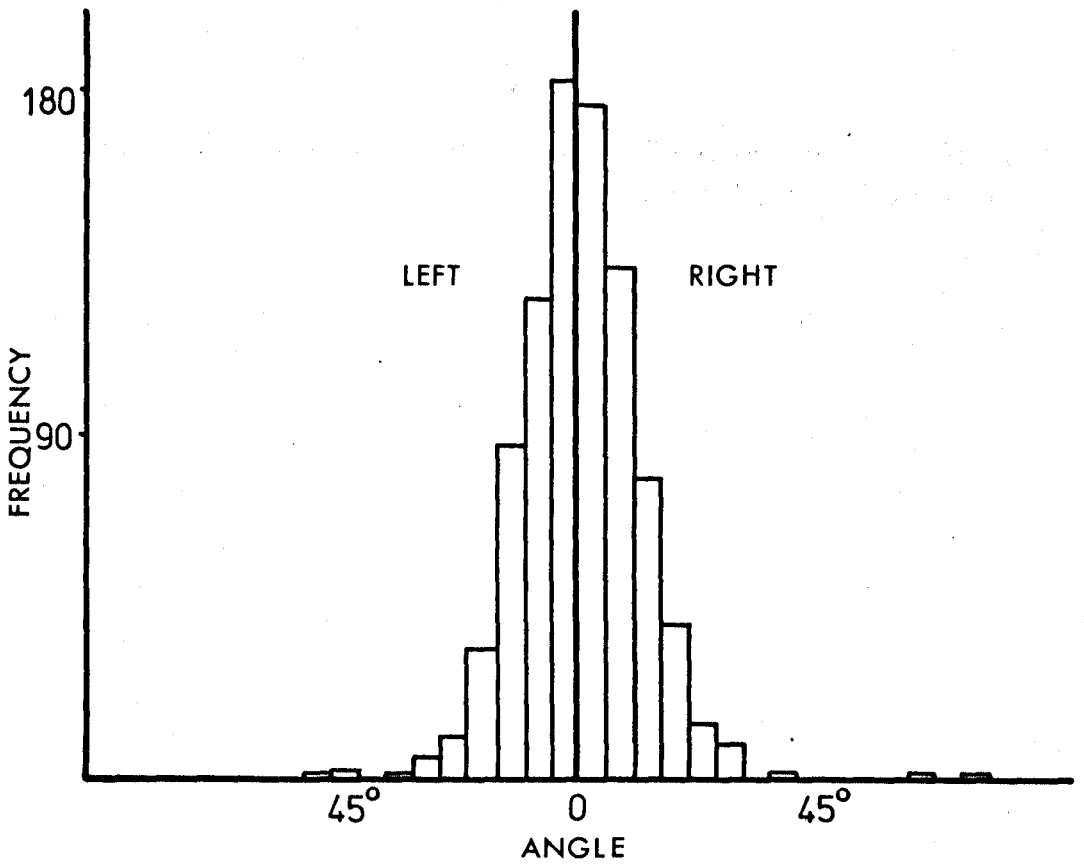
STEP	ANGLE OF DIRECTION
1	44.57
2	40.75
3	36.47
4	34.97
5	43.02
6	35.19
7	37.82
8	32.33
9	42.37
10	40.94
11	29.00
12	39.72
13	42.72
14	28.02
15	25.73
16	44.90
17	33.11
18	37.80
19	37.62
20	40.78
21	44.11
22	37.63
23	37.80
24	40.78
25	40.55
26	30.14
27	33.64
28	41.56
29	39.22
30	36.45
31	39.24
32	41.56
33	35.34
34	35.19
35	34.99
36	41.17
	degs

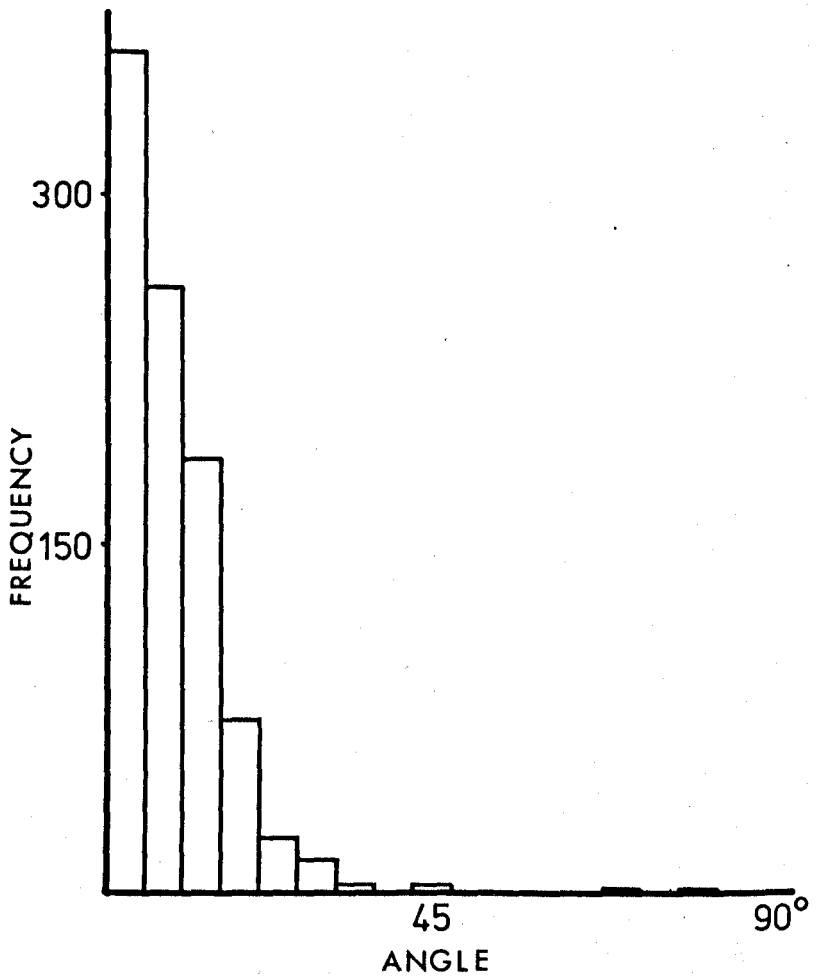
FIGURE 1.2.4

Frequency histogram of left and right angles turned by unstimulated miracidia

FIGURE 1.2.5

Frequency histogram of combined left and right angles of turn.


180
90
FREQUENCY
LEFT
RIGHT
45°
0
45°
ANGLE


300
150
FREQUENCY
45
90°
ANGLE

that the swimming of the miracidia is not biased to the left or to the right. The small standard error, indicating that the "spread" of the distribution is narrow, suggests in turn that the usual angle turned by the miracidia is also small. This result confirms that the miracidia usually deviate from a straight path by only small amounts. The distribution of the left and right angles combined is shown in Fig. 1.2.5 and was found to have a mean of 8.2° with a standard error of $\pm$ 0.25.

String length of tracks.

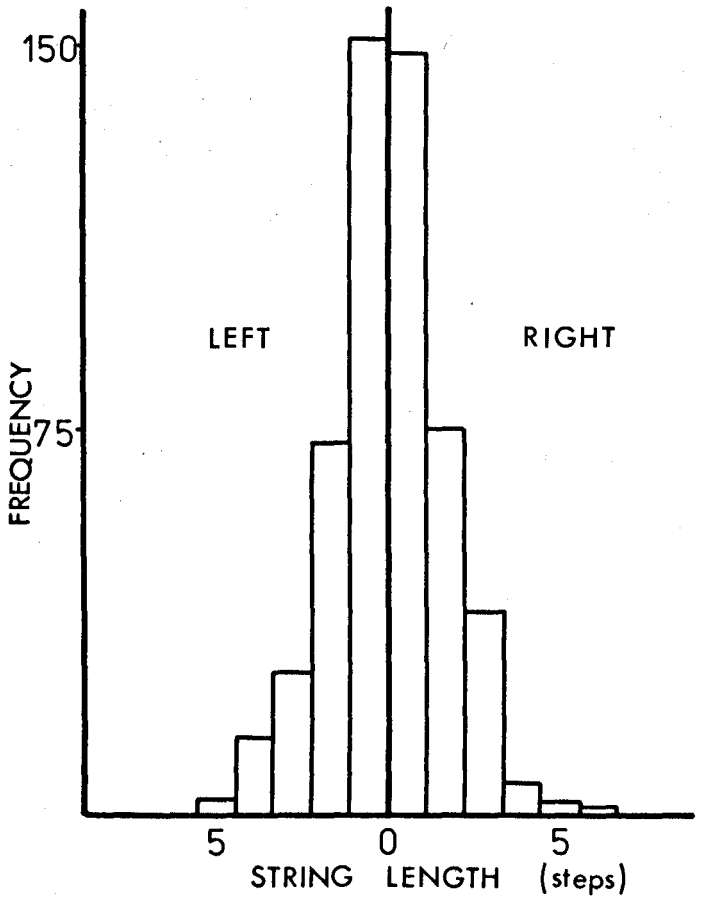
The frequency histogram of string length was produced by track analysis and is shown in Fig. 1.2.6 resolved into left and right strings. The distribution was found to be normally distributed about a mean of 0.01° with a standard error of $\pm$ 0.08 (n=540). This mean was found to be not significantly different from a mean of zero at the 10% level using the "d_o" test. This result again confirms that the miracidia turn to the left as often as they turn to the right. As, in general, a left turn follows a turn to the right, and vice versa, the miracidia will tend to swim in straight lines. The distribution of combined string lengths is shown in Fig. 1.2.7 and was found to have a mean of 1.7 and a standard error of $\pm$ 0.04.

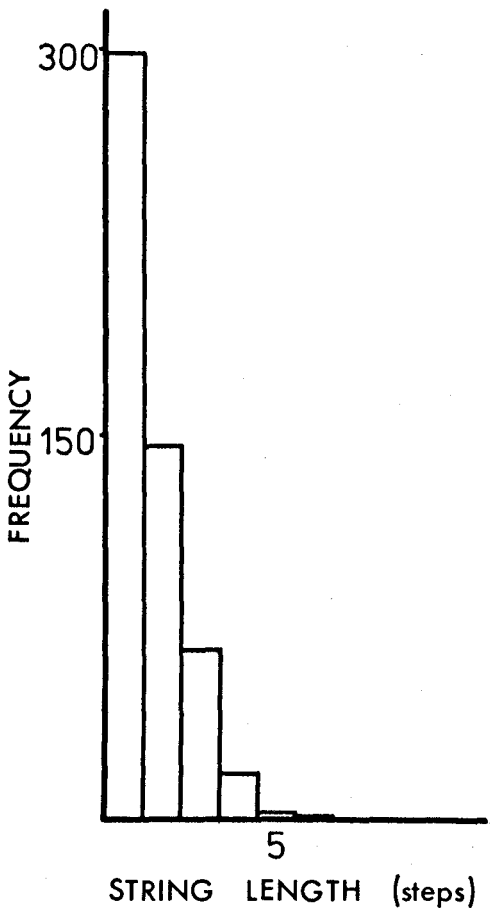
FIGURE 1.2.6

Frequency histogram of the left and right string lengths of unstimulated miracidia.

FIGURE 1.2.7

Frequency histogram of the combined left and right string lengths.


150
75
FREQUENCY
LEFT
RIGHT
5
0
5
STRING LENGTH (steps)


300
150
FREQUENCY
5
STRING LENGTH (steps)

1.2.2 RESPONSE OF MIRACIDIA TO CONTACT WITH THE WALLS OF THE TEST CELL.

A cine film of miracidia swimming into the glass wall of the test cell was taken in the normal way. Initial observation of the processed film suggested that although a few miracidia ($< 5\%$) showed marked responses to contact, the majority of the miracidia showed no response but just simply appeared to glance off. If this were the case, then the angle of incidence of a miracidium swimming towards the wall should equal the angle of reflection of the same miracidium swimming away. To investigate this, the paths of the first 20 miracidia to hit the wall were transcribed onto paper immediately before and after contact with the wall. The angles of incidence and reflection in relation to the wall of the cell were then measured using a protractor. The results are shown in Table 1.2.B. In general, the angle of reflection was within 30° of the angle of incidence. In only three cases was the difference between the two angles greater than 30°.

The small proportion of miracidia that showed a response upon contact with the wall was categorised by the methods of MacInnis (1965) and was found to fall into his category of 'contact with return'. The types of 'contact with return' observed were 'one loop', 'several loops' and the 'dipping response'. No attempt was made to quantify these responses as they were so infrequent and it was found difficult to place the miracidia into only one of the 'contact with return' categories.

The result that miracidia tend to glance off the walls of the cell provides an explanation for the observed

TABLE 1.2.B The angles of incidence and reflection of miracidia hitting a glass wall:

Miracidium number	Angle of incidence	Angle of reflection	Difference
1	51	24	-27
2	52	22	-30
3	70	68	- 2
4	63	87	24
5	66	67	1
6	80	105	25
7	52	26	-26
8	29	50	21
9	29	25	- 4
10	74	70	- 4
11	65	54	-11
12	12	7	- 5
13	74	117	43
14	55	40	-15
15	72	56	-16
16	15	35	20
17	66	45	-21
18	38	98	60
19	80	62	-18
20	39	88	49
	degs	degs	degs

preference of the miracidia to swim diagonally across the cell (see previous section). Upon re-examination of the cine film, it was found that, by chance, the field of view of the cine camera was close to one wall of the test cell. It is suggested that the frequency of the miracidia entering the field of view at an angle to the wall is greater than that of miracidia entering from other directions. In an infinitely large cell, the frequency histogram of direction of a large sample of miracidia should thus be rectangular.

1.2.3 SUMMARY OF SECTION 1.2

1. The speed of swimming of unstimulated miracidia which were less than two hours old was found to be 2.19 mm s^{-1}

2. The miracidia swam in relatively straight lines with occasional changes in direction. The straight path of the miracidia was made up of alternate small deviations to the right and left.

3. In general, miracidia hitting a glass wall simply glanced off at an angle approximately equal to the angle of incidence. A small proportion of miracidia showed 'contact with return' responses.

1.3 BEHAVIOUR CLOSE TO A SNAIL - EXPERIMENTS AND RESULTS.

1.3.1 CATEGORISATION OF MIRACIDIA SWIMMING CLOSE TO A SNAIL

Miracidia were prepared for filming as previously described and approximately 100 miracidia were pipetted into a test cell. The cell was placed horizontally on a glass plate underneath a vertically mounted cine camera. A snail (Biomphalaria glabrata), approximately 5 mm in diameter, was positioned horizontally against the cell so that its foot extended over the opening. The snail did not move to any great extent as it was held down by surface tension. Two minutes were allowed to pass before 100 feet of film were exposed as the miracidia approached the snail. The processed film was projected onto a screen and it was considered that the miracidia swimming within 5mm of the snail could be easily assigned to the following three categories:-

1) miracidia swimming as if unstimulated

2) miracidia showing a short period (< 2 seconds) of increased turning followed by 'unstimulated' swimming

3) miracidia showing a prolonged period of turning.

Proportion of miracidia in each category

The first 30 miracidia to swim within 5mm of the snail, starting from an arbitrary frame of the cine film, were observed and assigned to one of the three categories. It was found that 8 miracidia (26.7%) were in category 1, 11 miracidia (36.7%) were in category 2 and 11 miracidia were in category 3. Of all the miracidia only two actually contacted the snail and these were miracidia of category 3. All of the other miracidia eventually swam out of the area under observation.

Time spent by miracidia in the vicinity of the snail.

The same 30 miracidia used for categorisation were used to determine the time spent by each within the 5mm area close to the snail. The difference between the frame count when an individual entered and left the area was divided by the speed of filming in frames per second to give the time spent in the area in seconds. In addition to the 30 miracidia above, a further 30 miracidia swimming within 5mm of the glass wall of the test cell (in the absence of the snail) were also analysed in this way to provide a control. The results are shown in Table 1.3.A. Miracidia in category 3 spent a significantly longer time (at the 5% level) within 5mm of the snail than did unstimulated miracidia or miracidia in the other two categories. Miracidia in categories 1 and 2 spent a significantly shorter time (at the 5% level) than did either unstimulated miracidia or miracidia in category 3. There was no significant difference at the 5% level between the time spent by miracidia in categories 1 and 2 within the 5mm area.

TABLE 1.3.A

Time spent by miracidia in the vicinity of the snail.

		Control	Category 1	Category 2
	Mean (secs) sd n	P	P	P
Control	6.28 ±4.93 30			
Category 1	3.31 ±1.41 8	<.01		
Category 2	3.39 ±1.51 11	<.01	>.1	
Category 3	10.58 ±5.41 11	<.05	<.001	<.001

P is the probability that the difference between compared means would occur by chance.

1.3.2 TRACKS OF MIRACIDIA SWIMMING CLOSE TO A SNAIL

The 11 miracidia in category 3 (see previous section) were selected for tracking. Miracidia in the other categories were not used as their particular behaviour patterns were thought unlikely to increase the chance of an individual contacting the host. It was found that the miracidia in category 3 showing the increased turning, often swam on top of the track that had already been recorded onto paper. To allow complete tracks to be recorded the following procedure was adopted. Two reference points (eg. two specks of dust on the cell) were transcribed onto the paper and a fresh piece of paper placed on the screen. These reference points allowed the track sections to be accurately joined when the tracks were converted into coordinates. Sample track sections are shown in Fig. 1.3.1.

Speed of swimming.

The frequency histogram of the distance between points on the track was produced by track analysis and was found to be significantly skewed to the left at the 0.1% level with a significant degree of platykurtosis. The mean was at 0.15mm with a standard error of $\pm$ 0.001 (n=974). The distribution is shown in Fig. 1.3.2. The mean speed of swimming was thus found to be 1.67mm s^{-1} and appeared to be 16.5 percent slower than the speed of swimming of unstimulated miracidia. Although the difference between the speed of swimming in the absence and presence of a snail is considerable, much of this difference might be explained by an artefact of the recording system

FIGURE 1.3.1

Sample tracks of miracidia in category 3 swimming close to a snail.

The time interval between points on the track is 0.091 seconds.

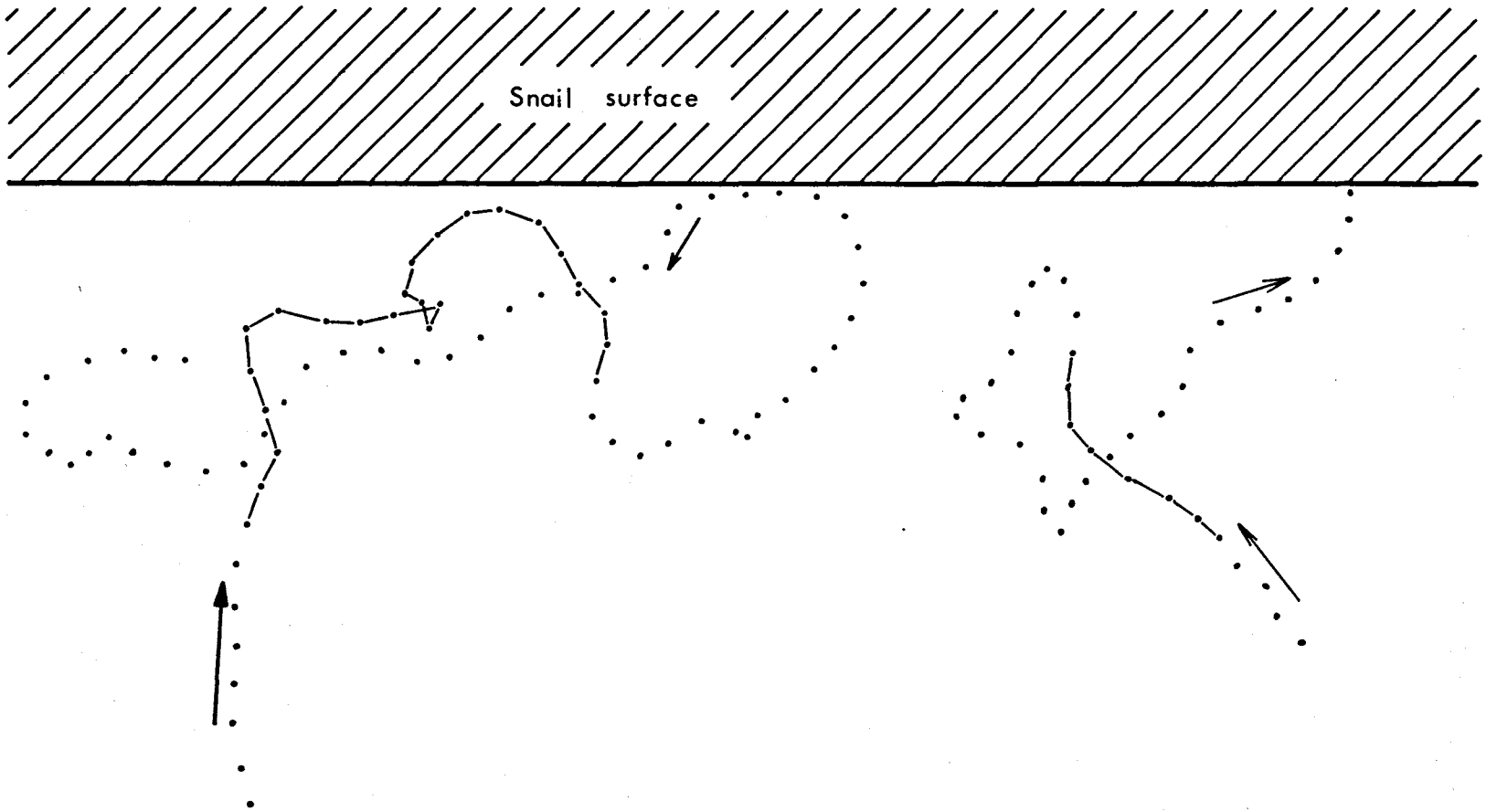
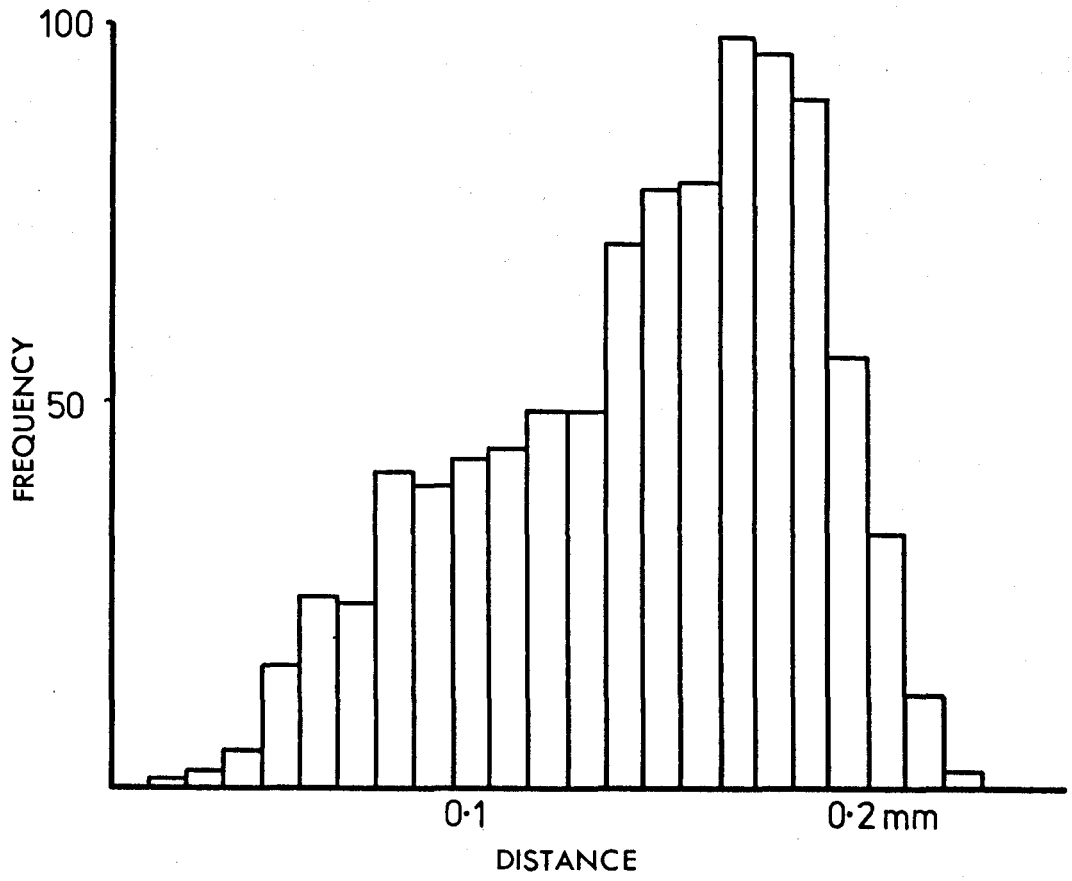

Snail surface

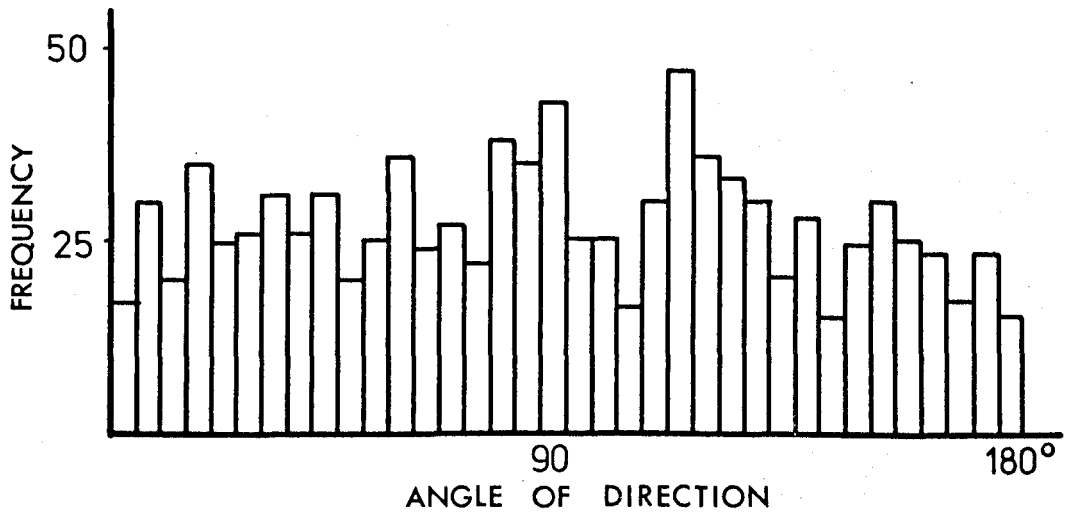
FIGURE 1.3.2

Frequency histogram of the distance between points on the tracks of miracidia swimming close to a snail.

FIGURE 1.3.3

Frequency histogram of the directionality of tracks of miracidia swimming close to a snail.


100
50
FREQUENCY
0·1
0·2 mm
DISTANCE


50
25
FREQUENCY
90
180°
ANGLE OF DIRECTION

(further discussed in the section on the angularity of tracks of miracidia close to a snail).

Direction of swimming close to a snail.

The frequency histogram of the directionality of tracks was produced by track analysis and is shown in Fig. 1.3.3. The analysis of a sample track is shown in Table 1.3.B. The direction in which the miracidia swim close to a snail seems to be generally random. This implies that an individual will swim away from the snail as often as it will swim towards it.

Angularity of the tracks of miracidia swimming close to a snail.

The frequency histogram of the angle turned was produced by track analysis and is shown in Fig. 1.3.4 resolved into left and right angles and in Fig. 1.3.5 as combined angles. The distribution of combined angles showed a mean of 30.93° and a standard error of $\pm$ 0.94 (n=962). The distribution of resolved angles was normal about a mean of 0.67° and with a standard error of $\pm$ 1.37. The mean of the resolved angle distribution was found to be not significantly different at the 10% level from the mean of the resolved angle distribution of unstimulated miracidia using the "d_1" test. The "spread" of these two distributions was significantly different at the 1% level using the variance ratio test.

These results imply that, although miracidia turn to the right to the same degree as they turn to the left when close to a snail, the actual angle turned by the miracidia is much greater than the angle turned by

TABLE 1.3.B Analysis for the angle of direction of miracidia swimming close to a snail.

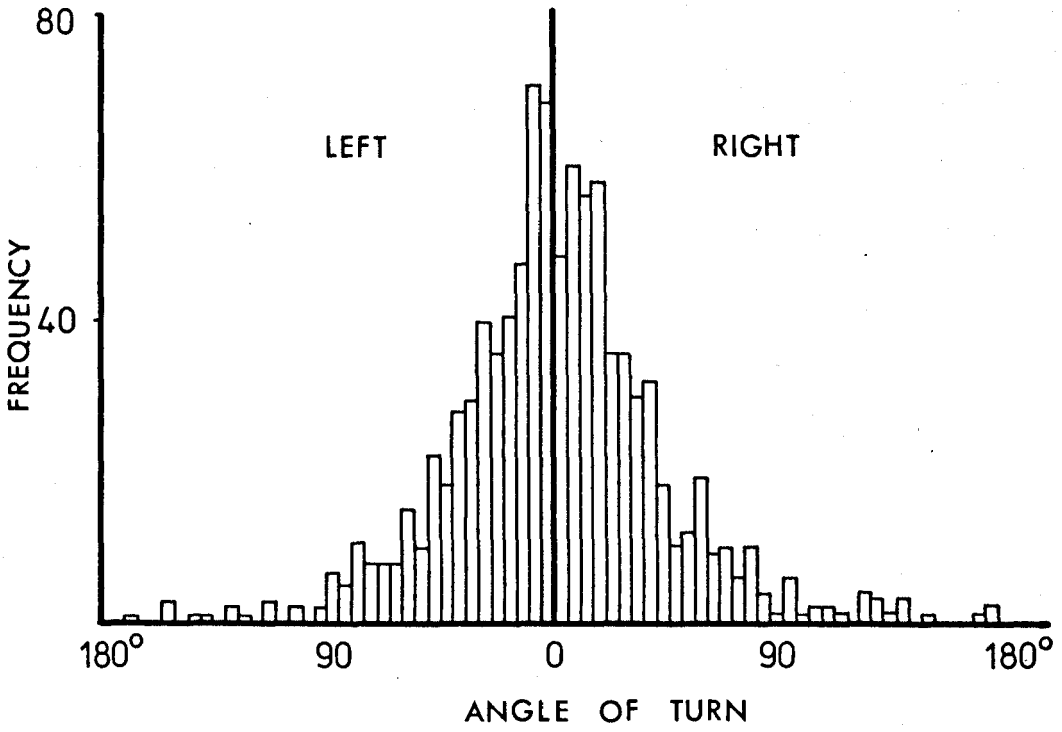
Step	Angle of direction	Step	Angle of direction
1	-149.26	37	-124.62
2	173.81	38	-84.71
3	147.03	39	-78.15
4	107.85	40	-121.66
5	116.59	41	-158.07
6	90.00	42	-144.90
7	108.65	43	-97.63
8	77.93	44	-64.05
9	22.06	45	12.18
10	162.04	46	87.56
11	157.57	47	111.08
12	-175.52	48	111.06
13	-135.24	49	73.86
14	-150.87	50	33.05
15	82.67	51	6.15
16	99.72	52	25.25
17	139.14	53	103.34
18	169.86	54	125.23
19	150.57	55	65.77
20	75.59	56	43.47
21	31.99	57	110.17
22	20.34	58	127.62
23	3.54	59	114.06
24	62.07	60	122.40
25	49.43	61	124.59
26	-10.14	62	109.46
27	-77.04	63	116.14
28	-166.34	64	100.89
29	114.05	65	71.49
30	83.40	66	90.00
31	45.69	67	112.79
32	30.71	68	120.97
33	-25.67	69	85.09
34	-64.29	70	25.28
35	-105.50	71	15.13
36	-122.03	72	43.55
	degs		degs

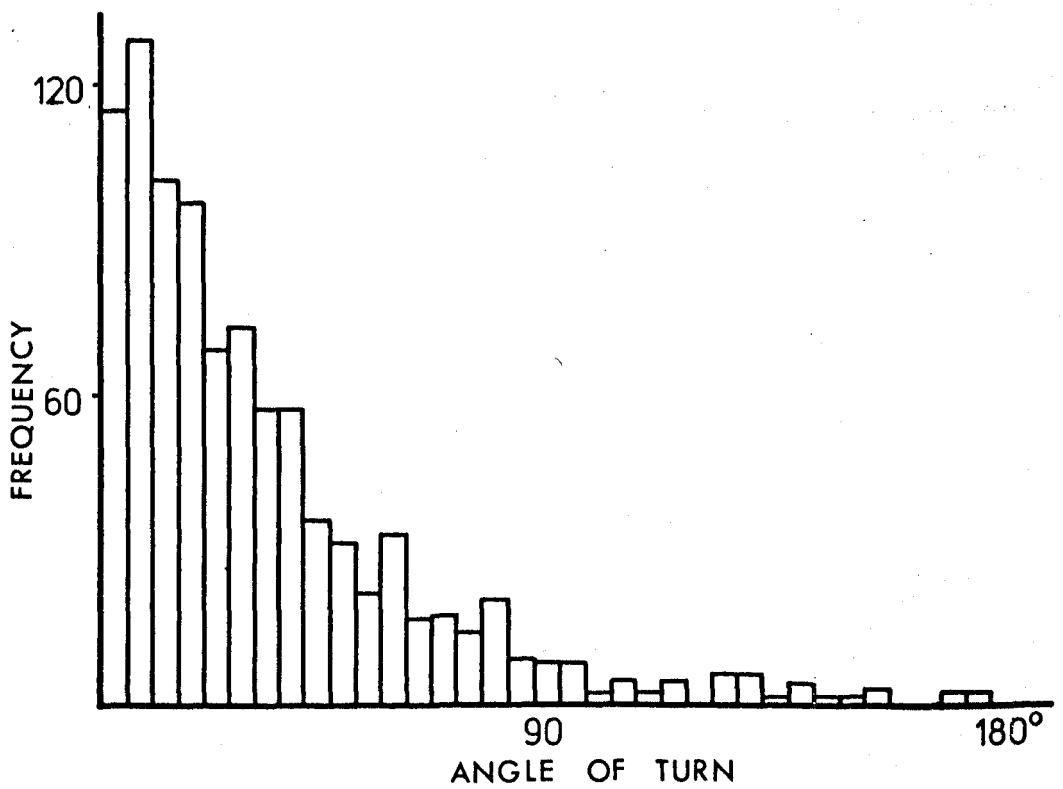
FIGURE 1.3.4

Frequency histogram of the angles turned on the tracks of miracidia swimming close to a snail resolved into left and right.

FIGURE 1.3.5

Frequency histogram of the angles turned on the tracks of miracidia swimming close to a snail.


80
40
FREQUENCY
LEFT
RIGHT
180°
90
0
90
180°
ANGLE OF TURN


120
60
FREQUENCY
90
180°
ANGLE OF TURN

unstimulated miracidia. This increase in the angle turned provides an explanation for the decrease in the speed of swimming which was observed close to the snail. If a tight circle is swum by a miracidium, the distances measured by track analysis will be the chords of the circle, so introducing an error. As the radius of the circle decreases, the underestimation of the distance (and so the speed) becomes greater. In addition, although the swimming of the miracidia is restricted to within 1mm away from or towards the camera, there will still be a small component of the swimming pattern in the third dimension which will increase as the angularity of the track increases. This will also lead to an underestimation of the swimming speed as a miracidium swimming directly towards or away from the camera will be recorded as if it were stationary. Indeed, a consideration of Fig. 1.3.2 suggests that the distribution might be bimodal, formed by the combination of the distribution of the distances moved by the miracidia swimming in relatively straight lines between periods of turning, and the distribution of the distances moved by the miracidia when turning.

Angularity of swimming at different distances from a snail.

The aim of assessing the angularity of miracidia swimming at different distances from a snail is to determine whether a true orientation response is taking place. If, for example, a direct klinokinesis occurred, then the angularity of the tracks would increase as the snail was approached.

The cine frame was divided into six, 0.5mm wide horizontal bands, starting from the side to which the snail was attached. Bands were used rather than circular orbits because the snail foot entirely covered the edge of the cell within the field of

view. Tracks of miracidia swimming within each of the bands were recorded onto paper and subjected to track analysis. The same miracidia were used for this analysis as were used elsewhere in this section.

The results are shown in Table 1.3.C. The mean angle turned is expressed in two ways. Firstly, the mean absolute angle was calculated (ie. disregarding whether the angle was to the right or to the left). Secondly, the mean of the angle turned resolved into left and right angles was calculated, left angles being negative. Significance tests were performed on the resolved means as combined left and right angles were found to be normally distributed.

The results show that, although there appears to be a general trend for the angle turned to increase as the snail was approached, there is no significant difference between the angles turned in the different bands.

String length of tracks close to a snail.

The frequency histograms of the string lengths of the tracks of miracidia swimming close to the snail were produced by track analysis. Fig. 1.3.6 shows the distribution of combined left and right strings. This distribution has a mean of 2.69 and a standard error of $\pm$ 0.11 (n=358). The frequency distribution of strings resolved into left and right is shown in Fig. 1.3.7. This distribution was found to be normal about a mean of -0.02 with a standard error of $\pm$ 0.18. This mean was found to be not significantly different at the 10% level from zero using the "d_o" test. The mean was also found to be not significantly different at the 10% level from the mean resolved string length of unstimulated miracidia using the "d_1" test but the "spread"

TABLE 1.3.C Angles turned by miracidia in regions close to a snail.

Distance from snail	n	Mean of absolute angle	sd	Mean of left and right	sd	P_o	P_1	Variance ratio	P_F
0.5	131	32.21	31.88	1.53	45.15	>.1			
1.0	283	31.12	27.47	0.24	42.44	>.1	>.1	1.27	>.05
1.5	336	30.92	30.65	2.36	43.64	>.1	>.1	1.05	>.05
2.0	95	27.82	25.15	-4.60	37.93	>.1	>.1	1.32	>.05
2.5	61	30.48	28.65	0.65	42.01	>.1	>.1	1.28	>.05
3.0	26	28.21	28.02	12.04	38.61	>.1	>.1	1.18	>.05

P_o the probability that the difference of the resolved mean from zero would occur by chance.

P_1 the probability that the difference between successive means would occur by chance.

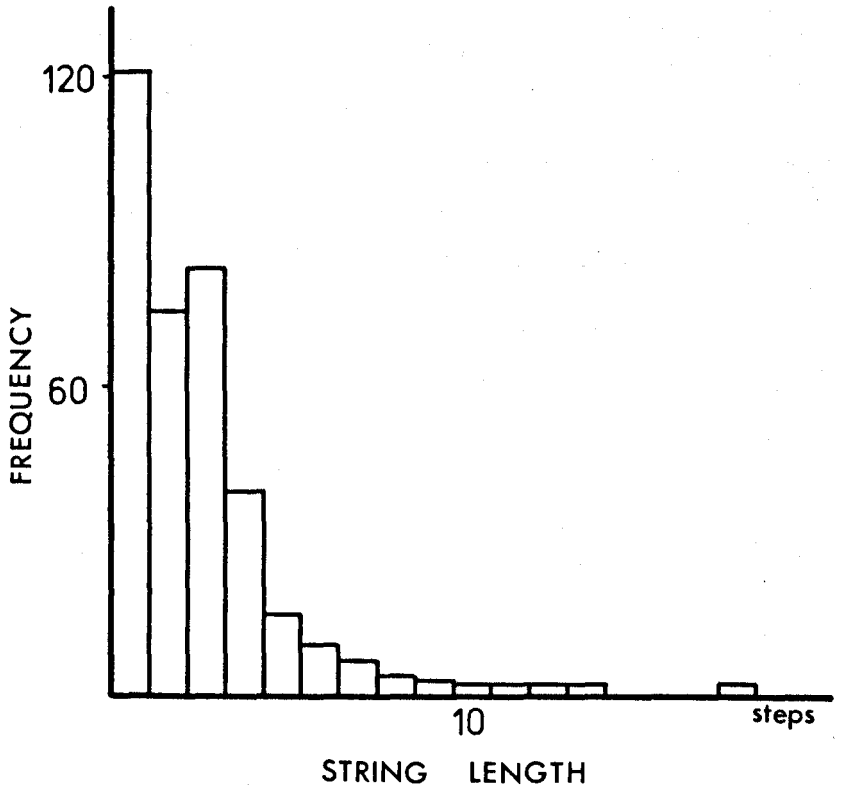
P_F the probability that the ratio of the variance about successive means would occur by chance.

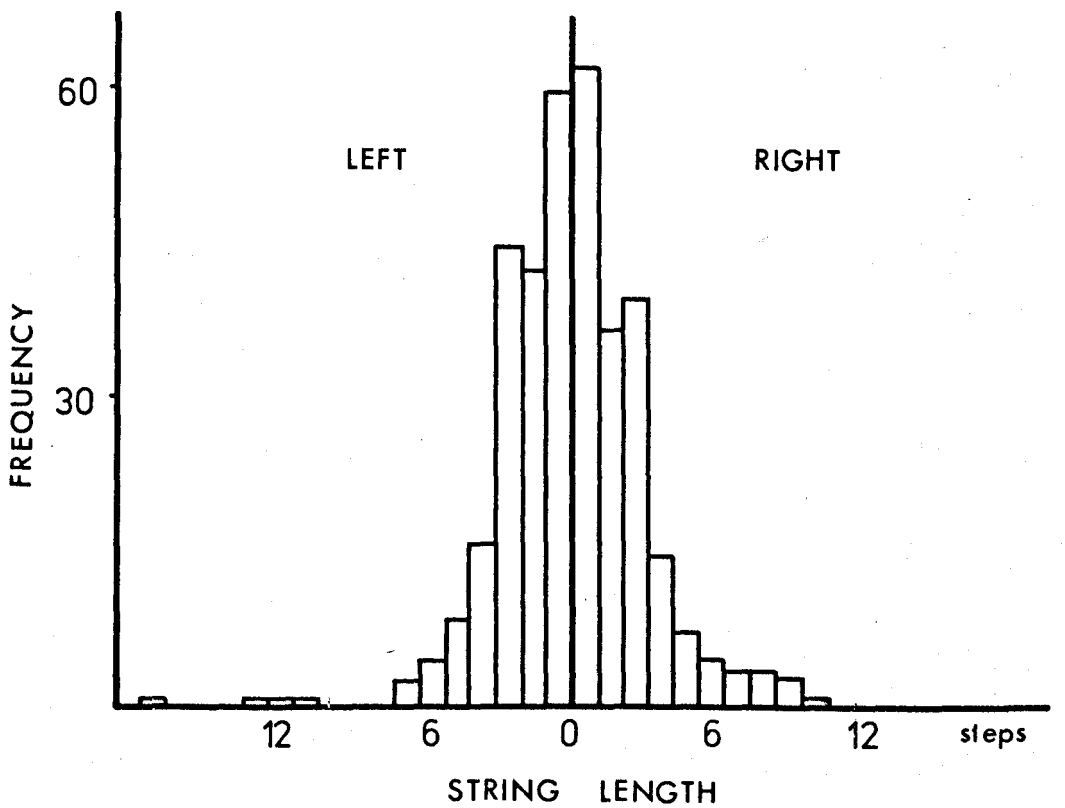
FIGURE 1.3.6

Frequency histogram of the string lengths of the angles turned by miracidia swimming close to a snail.

FIGURE 1.3.7

Frequency histogram of the string lengths of the angles turned by miracidia swimming close to a snail resolved into left and right strings.


120
60
FREQUENCY
10
steps
STRING LENGTH


60
30
FREQUENCY
LEFT
RIGHT
12
6
0
6
12
steps
STRING LENGTH

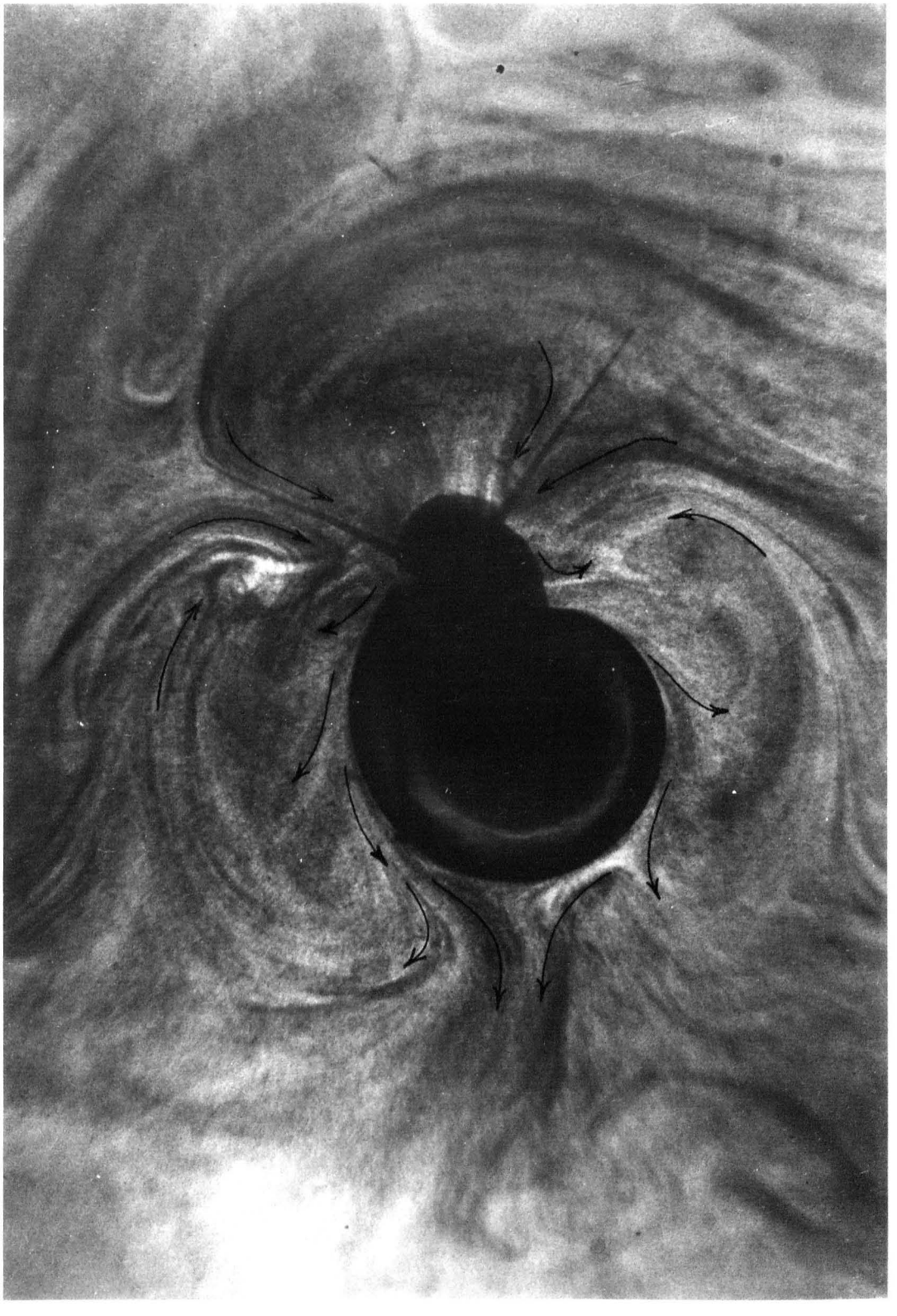
of these two distributions was found to be significantly different at the 1% level using the variance ratio test. This result implies that a turn to the left or the right is more often followed by a turn to the same side when the miracidia are close to the snail than when the miracidia are unstimulated. This in effect confirms the observation that miracidia close to a snail turn in tight circles.

1.3.3 WATER CURRENTS AROUND A SNAIL.

It is quite possible that the water currents generated by the cil iated epithelium of a snail might play a role in its location by a miracidium. The degree to which these currents disrupt the surrounding water is also important in determining whether gradients of stimulant chemicals would be set up.

To record the currents around a snail, a 5mm diameter snail was placed in a petri dish containing water to depth of approximately 7mm. The petri dish was placed under a vertically mounted cine camera and the area around the snail was filmed as a solution of colloidal graphite was pipetted, drop by drop, in front of the snail. A typical pattern of graphite particles is shown in Plate 4. In general, water is drawn down the tentacles, across the head, and down the body. Two vortices, one each side of the head behind the tentacles were set up. In addition, an area of relatively still water extended for several millimetres in front of the head.

It is obvious that a great amount of mixing takes place at the side of the head, so making the existence of chemical gradients in this area unlikely. In the region immediately in front of the head smooth chemical gradients might occur, but the sweeping of the tentacles from side to side and the progression of the snail itself make this rather unlikely. Rather, general areas of uniform chemical concentration are probably set up, those to the sides of the head being of higher concentration than those in front of and behind the snail.



1.3.4 SUMMARY OF SECTION 1.3

1. The miracidia within 5mm of a snail could easily be assigned into three categories. These were :-

a) miracidia showing no change in behaviour when compared with unstimulated miracidia.

b) miracidia showing a brief (<2 secs) increase in angularity.

c) miracidia showing a prolonged increase in angularity.

2. 26.7% of the miracidia were in category a), 36.7% in category b) and 36.7% were in category c). Only 18.2% of the miracidia in category c) succeeded in contacting the host during the period of observation. All other miracidia eventually left the proximity of the snail.

3. Miracidia in the third category tended to swim slower in the presence of the snail than did miracidia in the absence of the snail. Some of this difference is due to an artefact of the analysis.

4. Miracidia in the third category swim in a random direction close to a snail so that as many miracidia will swim away from the snail as will swim towards it.

5. The mean angle turned by the miracidia in the third category was significantly greater than that of miracidia swimming in the absence of the snail.

6. Miracidia in the third category show a greater tendancy to follow left turns with left turns and right turns with right turns than do unstimulated miracidia.

7. The mean angle turned in six 0.5mm bands close to the snail by miracidia in the third category did not significantly increase in the bands closest to the snail.

8. Analysis of the water currents around the snail suggests that chemical gradients traversing several millimetres are not present but instead, areas of relatively uniform concentration exist.

1.4 DISCUSSION

In this chapter, I have recorded and quantified the behaviour of the miracidia of Schistosoma mansoni in the presence and absence of their snail host. The speed of swimming of the unstimulated miracidia was found to be about 2 mm per second which agrees well with the reports of Chernin and Dunavan (1962) and Mason and Fripp (1976). The unstimulated miracidia swam in relatively straight lines with only occasional changes in direction. This type of swimming pattern typifies the ranging or scanning phase of the host-location mechanism which is often discussed in the literature.

Miracidia swimming into inert objects (eg. the glass walls of the test cell) tended to simply glance off and continue swimming as normal. A very small proportion of the miracidia did however show "contact with return" responses. This result is somewhat at odds with the report of Chernin and Dunavan (1962) that miracidia are likely to congregate at submerged margins, and these margins will be important in the infection process. This hypothesis was based on the observation that, in a petri dish, more miracidia were found around the edges of the dish than were found in the middle. This distribution of miracidia could be predicted from my results as a miracidium hitting the concave wall would glance off and proceed around the dish in a series of chords. However, there is another explanation for the results of Chernin and Dunavan as they state that the water used in their experimental vessels was taken from "aerated aquaria of the stock colony". This water would in effect be SCW so

that in their experiments the miracidia were probably stimulated even in the absence of a snail. The increase in the number of miracidia near the walls could thus be due to an increase in the number of "contacts with return" shown by the larvae (see MacInnis 1965). It is thus probable that submerged margins will only be important in the location of the host if snails are already present.

The speed of miracidia swimming within 5mm of a snail was found to be 1.7mm per second, which is significantly slower than the speed of unstimulated miracidia. Much of this difference can be explained by the underestimate of the distance travelled measured by track analysis due to the increased level of turning. This result, however, does not agree with the observations of Mason and Fripp (1976). They found an increase in the speed of miracidia swimming in SCW.

It is interesting to note that approximately 27 percent of the miracidia simply swam straight past a snail without showing stimulation and that a further 37 percent of the miracidia (category 2) showed a response that turned them away from the snail. These miracidia might represent the 50-60 percent of "defective" miracidia described by Chernin and Dunavan (1962). Two provisos, however, should be mentioned here. Firstly, the result that miracidia in category 1 spend a shorter time within 5mm of the snail than do the control miracidia was due to some of these miracidia (approximately half) only swimming across the very edge of the selected area. Whether these miracidia would have become stimulated if they had swum closer to the snail is a matter for conjecture. Secondly, it is possible that the response of miracidia in category 2 might increase in duration

as the miracidia age. Mason and Fripp (1976) have shown that the basal rate of change of direction (turning) varied as the larvae aged, reaching a peak about five hours after isolation. An investigation of the response of miracidia to the presence of a snail as the larvae age would be of value in the understanding of the dynamics of the infection process.

The most dramatic response of the miracidia to the presence of the snail was the increase in the rate of turning coupled with an increase in the number of turns of the same sign following each other. This behaviour pattern was shown by the miracidia in category 3. As the direction of the swimming tracks was essentially random, this response cannot be a taxis. Instead, the results I have presented in this chapter support the view that this response is a klinokinesis. However, the degree of turning does not seem to be affected by the distance away from the snail as would normally be expected if an organism was responding to a chemical gradient by a klinokinesis. Three possible explanations of this situation can be suggested. Firstly, the pooling of the tracks of many miracidia in the analysis might mask the directed response of an individual. Secondly, the miracidia may not be capable of responding to a change in the intensity of the stimulus except by an 'all or nothing' response. This latter situation is supported by the results of Mason and Fripp (1976) who found that the rate of change of direction of miracidia swimming in SCW was not altered if the SCW was diluted to 20 percent of the original. However, Wilson and Denison (1970 b) found that the angle turned by

the miracidium of Fasciola hepatica increased as the concentration of caprylic acid was increased. Lastly, chemical gradients may not be present at all, but only areas of uniform chemical concentration exist. The analysis of the water currents around the snail suggests indeed that any chemical gradients that might be set up would be totally disrupted by this mixing.

Mason (1977) has recently compared the area of chemical stimulation around a snail to the concept of an "active space" (Wilson 1970). An active space is formed by the dispersion of the stimulant chemical around the point of emission by currents or eddies so that a zone of stimulation is set up. This zone will be formed rapidly and will remain stable in conditions where more slowly forming gradients would be disrupted. Bossert and Wilson (1963) suggest that when the concentration of the stimulant in the "active space" reaches a threshold value, the organism will respond in such a way that the chance of contacting the source of the stimulation is increased. The similarity between the "active space" concept and the observed miracidium/snail interaction is obvious.

The response of miracidia to true chemical gradients could be studied using the assay system for chemostimulation described by Sponholtz and Short (1975), coupled with cinephotography and track analysis.

The increased turning around the snail has often been cited as a host-location mechanism in that the miracidia, spending an increased time in the vicinity of the snail, would increase its chances of randomly contacting the host.

Results, presented in this chapter, confirm that at least some of the miracidia spend a greater time in the vicinity of a snail. Testing the value of this response to the miracidium is very difficult and no attempts to do so have been reported. Experiments can be devised which would enable the experimenter to investigate the value of this response, but these would be generally difficult to perform or time intensive. For example, the number of contacts of miracidia with a host snail and with an inert model snail could be counted using a binocular microscope. If this experiment could practically be performed, it would tell us nothing about the mechanism used for the location of the host. I will show in Chapter 3 that simulation modelling on the computer is a very valuable tool, which as well as assessing the value of a selected response, can also provide information about the processes involved in the host-location mechanism.

CHAPTER 2.

THE ACTIVITY OF CERCARIAE

2.1 INTRODUCTION

The cercaria larva of Schistosoma mansoni becomes free-swimming when it is shed from its snail host. The number of cercariae that are shed from snails infected with schistosomes varies from location to location, from strain to strain, from snail to snail and even in an individual snail from day to day. Generally, it is agreed that the stimulus for the onset of shedding is light, and that the peak in the rate of shedding occurs a few hours after dawn or after the switch from dark to light in the laboratory (cf. Giovannola 1936, Barbosa et al 1954, Maldonado 1959, Jordan 1961, Asch 1972, Lawson 1977 etc). Lawson (1977), using the same culture of the/used in this study, reported that at 27°C the peak in the rate of shedding occurred between 5 and 6 hours after the lighting was switched on.

The cercaria of S.mansoni belongs to the group of cercariae bearing a well developed forked tail. Nuttman (1975), again using this particular culture of parasites, found that the external morphology of the inactive cercaria was similar to the dimensions reported by Khalil (1922). These were :-

mean body length	189μm
mean body width	73μm
mean tail stem length	250μm
mean tail stem width	40μm
mean furcae length	75μm

The cercaria is an intermittent swimmer. Periods of active swimming, which is usually upwards in direction, are followed by periods of passive downward sinking. Generally, a cercaria reaching the surface will stop swimming and start to sink downwards. A cercaria sinking onto the bottom

is often stimulated to swim upwards. These observations are supported by Haas (1976). The normal mode of swimming is tail first. Graefe et al (1967), using high speed cinephotography found that, while swimming, the body and tail appear to oscillate about two fixed points or nodes, one in the region of the ventral sucker and one near the distal end of the tail stem. The furcae were held at right angles to the tail axis during the propulsive beat and acted in a rowing action to produce the thrust. Nuttman (1975) supported the views of Graefe et al but further reported that during one complete propulsive beat, a slight asymmetry of the tail action was observed at the two points of maximum tail flexure. The tail stem twisted so that at one of these points, the furcae passed ventrally beneath the body and at the other, the furcae passed laterally above the body. He suggested that this asymmetry of beat made the main contribution to the slow spiral rotation about the longitudinal axis which is often observed when cercariae swim tail first.

Occasionally, particularly when stimulated, cercariae will reverse their direction of swimming and proceed head first. Graefe et al (1967) report that, while swimming forward, the furcae are not spread but lie close together and in line with the tail stem. In addition, the body lengthens by between 30 and 40 percent and as a result becomes narrower.

The speed of the tail-first swimming was shown by Valle et al (1974) to increase in a linear relationship with the increasing temperature. At 25°C, the speed of swimming was about 1.1mm per second. The mean swimming speed was

found to be 0.75mm per second at 24°C by Nuttman (1975) and 1.4mm per second at 27°C by Lawson (1977). Graefe et al (1967) quote only the maximum speed of swimming, which was 1.4mm per second, but the temperature at which the observations were made was not reported. The variations in the speed of swimming found by these workers are probably due to strain differences and to different experimental conditions (in particular the light intensity). Lawson (1977) measured the speed of swimming as the cercariae aged. She found that the speed of swimming steadily decreased from 10 hours after shedding onwards. Chapman (1971) describes a similar decline in the swimming speed of the cercaria of Himasthla secunda which started about 4 hours after shedding.

The vertical position of cercariae in a body of still water will depend on the relative distances moved upwards when swimming and downwards when sinking. The distance moved depends on the product of the duration and the speed of swimming or sinking. Changes in the activity or speed of the cercariae should thus alter their distribution in a body of water. Faust and Hoffman (1934), Rowan (1965) and the Third WHO Report on Bilharziasis (1965) give incidental references to an even distribution of cercariae. Faust and Hoffman mention that cercariae begin to congregate on the bottom in increasing numbers 18 to 24 hours after shedding. Krakower (1940) confirmed these observations and also noted that the cercariae reacted to stimuli in decreasing numbers as they aged. Stirewalt (1971) however found that the majority of cercariae are found near the surface, in a glass tube, for several hours after shedding. When a skin surface was positioned at the bottom of the tube the cercariae quickly congregated at the bottom. Pellegrino

and Valle (1974) found that in a depth of water of 5cm, 75 percent of the cercariae were found within 1cm of the surface. Lawson (1977) confirms that the majority of the cercariae were found in the top quarter of a small glass test cell 15 minutes after shedding. As the cercariae aged, the number found in the top of the cell gradually dropped. Conversely, the number of cercariae near or on the bottom steadily increased. In natural waters, the action of wind, turbulence, convection currents and general mixing would have a considerable disruptive effect on this distribution. In addition, cercariae of all ages would be present through the day as the snails shed cercariae over many hours.

According to reports in the literature, the survival of cercariae shows a degree of variation. Generally, significant mortality is reported to occur at about 24 hours after shedding (cf. Leiper 1915, Khalil 1924, Faust and Hoffman 1934, Krakower 1940 etc). The main influences on longevity appear to be temperature and the salt concentration in the water. Lawson (1977) reported that, at 27^{o}C and shortly after shedding, a small proportion of cercariae were already dead but that little additional mortality occurred until 10 hours after shedding. After this time, the death rate increased rapidly so that by 24 hours after shedding less than 3 percent of the cercariae were still alive. A distinction should be made between the cercariae that are alive and those that retain the ability to remain suspended in the water. As the cercariae age, an increasing proportion are found to be inactive on the bottom of the test vessel and are not engaged in active swimming (Lawson 1977).

The behaviour of schistosome cercariae is affected

by many stimuli, temperature, light, turbulence and chemicals being the most important. The source of stimulation may be of host origin or simple diurnal or seasonal fluctuations in temperature and light intensity. Fluctuations in the seasonal or diurnal temperature are likely to change the behaviour of cercariae by a direct effect on the metabolism of the organism rather than by being detected by sensory receptors (cf. Valle et al 1974). The effect of an increased overall level of light intensity has been reported for several species of cercaria other than the schistosomes (Dönges 1963, Haas 1969, Chapman 1971). Two of the species showed a greater swimming activity at increasing light intensities and one (Chapman 1971) showed the reverse. Assuming that the swimming and sinking speed remained constant in each case, the increased swimming activity should result in an upward migration of cercariae whereas decreased swimming activity would bring cercariae towards the bottom.

In terms of a host location mechanism, it is the response of the cercariae to host generated stimuli which is likely to be the most important. A man wading through water will cast shadows, cause turbulence and chemicals will dissolve from the skin surface. In addition, temperature differentials may exist between the skin surface and the surrounding water. The response of cercariae to brief shadows or drops in light intensity has been reported for a number of schistosome species (Bracket 1940, Neuhaus 1952, Nuttman 1975, Lawson 1977) and in other digenean species by Miller and McCoy (1930), Miller and Mahaffy (1930), Dönges (1963,1964) and Haas (1969,1971). An initiation of swimming following a shadow stimulus was found in most cases.

Haas (1969) has showed that the shadow response of Diplostomum spathaceum cercariae is very short lived. Lawson (1977) found that cercariae of S.mansoni inactive on the bottom of a test cell became active after a shadow stimulus. The observed migration of cercariae upwards in the cell reached a peak about 5 minutes after the stimulus was applied. Cercariae of all ages responded in this way although the response was less marked with the older cercariae.

Miller and Mahaffy (1930) and Haas (1969), observing schistosome and Diplostomum spathaceum cercariae respectively, found that mechanical stimulation by directly touching the cercariae, resulted in an initiation of swimming. It was assumed in both cases that the cercariae would respond in a similar way to turbulence and water currents.

The reactions of cercariae to host chemicals has usually been concerned with penetration activity and seldom has the free-swimming behaviour been studied (cf. Bolwig 1955, Stirewalt 1970,1971, Wagner 1959, Clegg 1969, MacInnis 1969, Austin et al 1972, Shiff et al 1972 etc). Stirewalt (1971) showed that the distribution of schistosome cercariae was altered by the presence of host skin at the bottom of a tube. Hubbard and MacInnis (1969) found that S.mansoni cercariae were attracted to a small well cut in agar gel and filled with aspartic or butyric acids. Shiff et al (1972) reported that cercariae of S.mansoni and S.haematobium became "hyperactive" in the presence of a chemical stimulus and Bolwig (1955) suggested that the cercariae of S.haematobium and S.bovis respond to host tissues in a klinokinetic orientation.

Haas (1976), observing S.mansoni cercariae in close

proximity to a host substrate described several different swimming reactions. These included a reversal reaction, where normal tail-first swimming became head first, and a reaction which led to fixation or attachment. He found that the best situation for fixation was a natural host substrate at a higher temperature than the surrounding water. Neither temperature nor the substrate alone had such a great effect on fixation. Nuttman (1975) however, measured the surface temperature of a human forearm when it was immersed in water at 28°C. One minute after immersion, a differential of only 0.5°C existed, but this differential was then maintained. He also found that in a temperature gradient of 10°C, consistently more cercariae were found at the warmer end. However he concluded that, although temperature differentials may be important in penetration and do affect the behaviour of cercariae, they are unlikely to be important in host location.

Recent work by Becker and Lutz (1976) has indicated that the sodium or magnesium ion concentration of the water has an effect on the activity of cercariae. In a balanced salt solution approximately 60 percent of the cercariae were active whereas only about 20 percent were active in distilled water controls. This increased activity was not diminished if the sodium ions in the salt solution were replaced by magnesium ions. The replacement of the sodium ions by potassium ions however, reduced the percentage activity to the control levels. The role of this change of behaviour in a host location mechanism is unclear but might be related to some of the activities engaged by humans in water (eg. washing and work involving simple chemical compounds).

In this chapter the behaviour patterns of cercariae as they age and in response to stimuli are recorded and analysed. A comprehensive study of all cercarial responses is not intended. Instead, the behavioural responses which are potentially important in the location of the host have been selected. The stimuli chosen to be studied were shadows, a stimulus simulating water currents (turbulence) and host chemicals. A temperature stimulus was not included after the consideration of the results of Nuttman (1975), who found that only very small temperature differentials existed between the skin surface and the water one minute after immersion. Thus it is very unlikely that this stimulus could be remotely sensed and so would not play a major role in the location of the host. The verification of this supposition could be the subject of future work.

The approach used in this chapter allows the tool of computer simulation modelling (see Chapter 3) to be used to assess the value of a selected response as a host-location mechanism.

2.2 UNSTIMULATED BEHAVIOUR - EXPERIMENTS AND RESULTS.

The unstimulated behaviour of the cercariae of S.mansoni has been well described by Lawson (1977). However, a similar study has been undertaken here to provide a record of the basal activity against which the responses to stimuli can be compared. In the following experiments, both the lighting conditions and the size of the test cell are different from those used by Lawson. It is assumed, possibly without justification, that different batches of cercariae prepared using identical methods, will on average behave in the same way. All experiments were carried out in a constant temperature room set at 25°C.

2.2.1 AGE RELATED DISTRIBUTION OF CERCARIAE.

Number of cercariae remaining suspended in the test cell in relation to their age.

Cercariae were pipetted into the test cell as previously described and photographs of the entire cell were taken on a Linhoff plate camera at hourly intervals as the cercariae aged from 1 to 10 hours after shedding. In addition, photographs were also taken at 11.5 and 13 hours after shedding. The cut film photographs were processed and printed and the total number of cercariae visible was counted. A typical photograph of the cell is shown in Plate 5. These cercariae represent those of the total population that are alive and off the bottom of the cell (ie. those that are suspended in the water either actively swimming or passively sinking). The results are shown in Fig. 2.2.1 expressed as a percentage of the maximum number of cercariae counted in the cell. The means and standard

PLATE 5

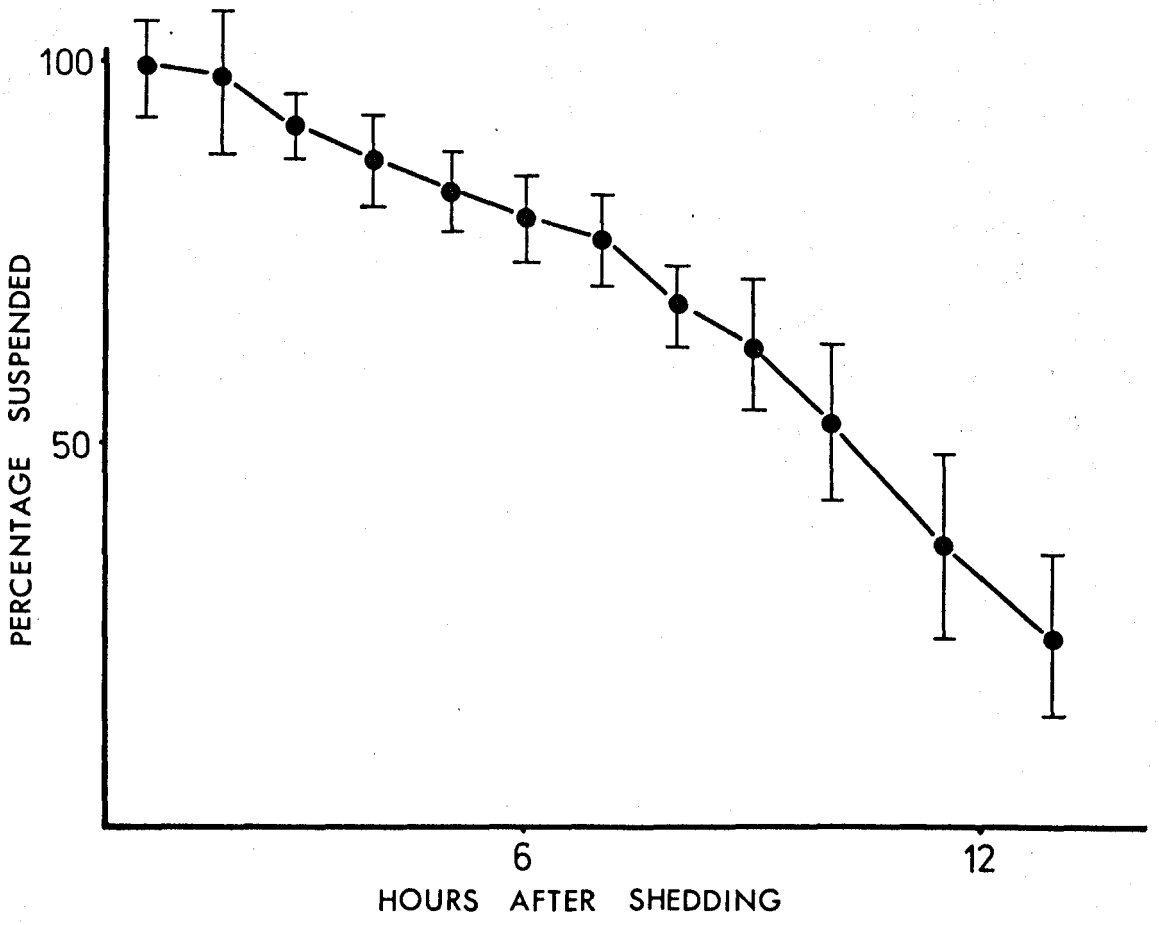
A typical photograph of the entire test cell containing cercariae taken on the plate camera.

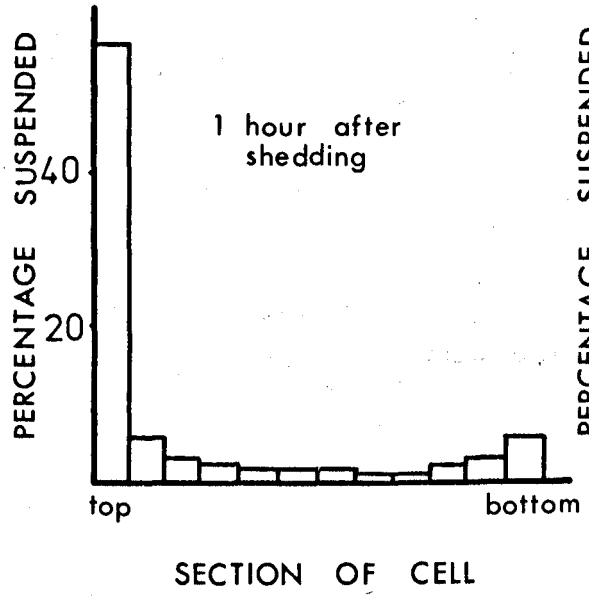
FIGURE 2.2.1

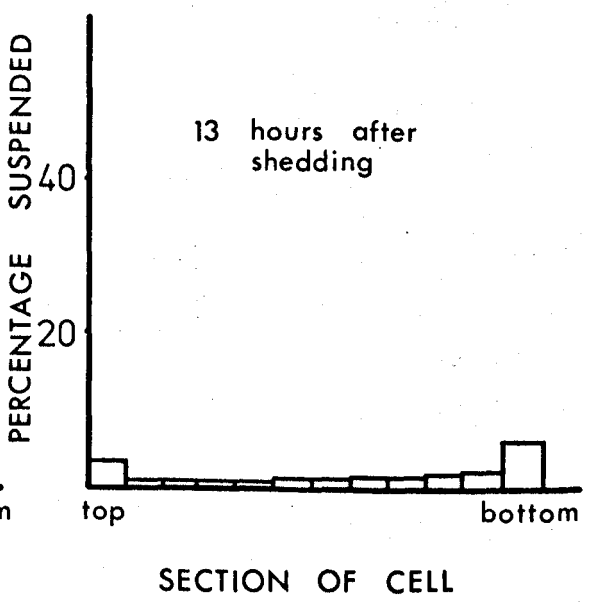
Percentage of cercariae suspended in the cell as they aged.
Results expressed as the mean of 13 observations calculated using the arcsine transformation and converted back to percentages. The limits are the standard deviation of the transformed data.

FIGURE 2.2.2

Distribution of cercariae in the cell as they aged.
The results are expressed as the mean of 13 observations calculated using the arcsine transformation and converted back to percentages. The complete set of results are tabulated in Appendix 2.


100
50
PERCENTAGE SUSPENDED
6
12
HOURS AFTER SHEDDING


40
20
PERCENTAGE SUSPENDED
1 hour after shedding
top
bottom
SECTION OF CELL


40
20
PERCENTAGE SUSPENDED
13 hours after shedding
top
bottom
SECTION OF CELL

deviations were calculated using the arcsine transformation (see Appendix 1).

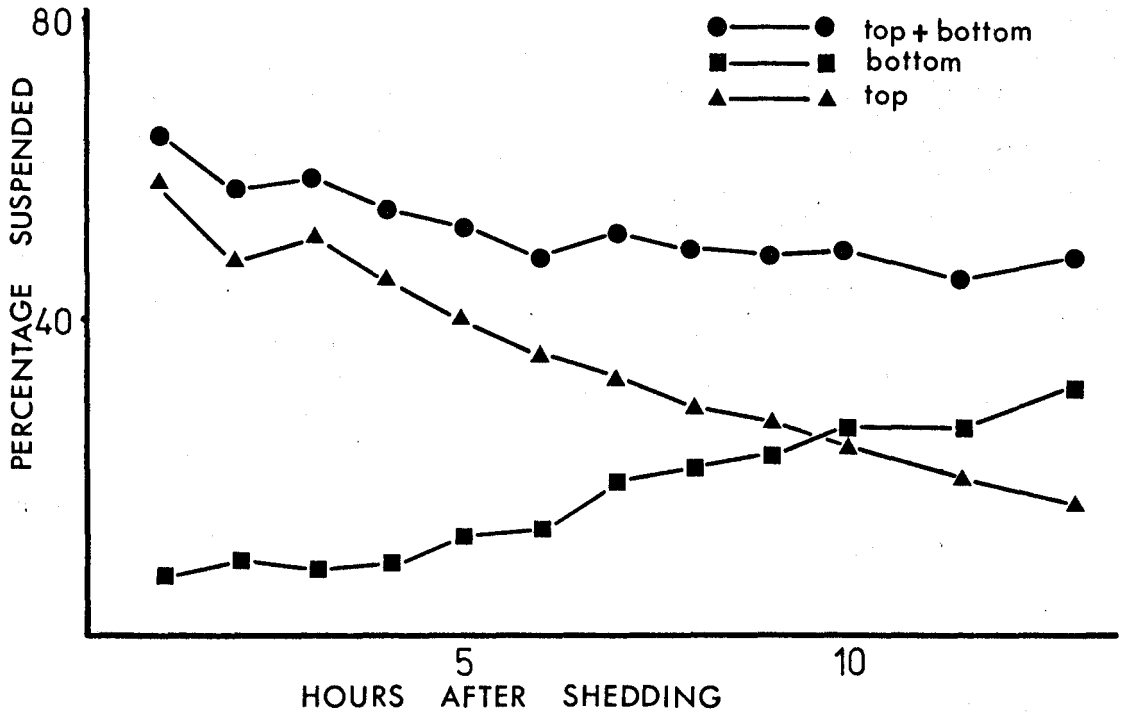
As the cercariae aged, an increasing proportion lost their ability to remain suspended in the water. It follows that there was an increase in the number of cercariae that were either inactive or dead on the bottom of the cell.

Distribution of cercariae in the test cell as they aged.

To determine the distribution of cercariae, the photographs taken in the previous experiment were divided into 12 equal horizontal sections and the number of cercariae in each section was counted. One hour after shedding, the majority of the cercariae were found towards the top of the test cell (see Fig. 2.2.2). Thirteen hours after shedding, the majority of the cercariae still suspended in the water were found towards the bottom of the cell. A study of the data in Appendix 2 shows that in addition to the gradual decrease in the numbers of cercariae remaining suspended in the cell as they aged, there was also a gradual reduction in the ability of the cercariae to remain near the surface. The number of cercariae suspended in the top and bottom twelfths of the cell at different times after shedding is shown in Fig. 2.2.3 expressed as the percentage of the total number of cercariae suspended at each time. It can be seen that between 65 and 45 percent of the cercariae suspended in the cell were found in the top and bottom twelfths of the cell throughout the course of the experiment. The gradual tran-sition of cercariae from the top of the cell to the bottom can clearly be seen, equal numbers of cercariae occurring at the top and bottom at about 10 hours after shedding.

FIGURE 2.2.3

Percentage of cercariae suspended in the top and bottom twelfths of the test cell.
The number of cercariae in the selected section is expressed as a percentage of the cercaria suspended in the cell at each cercarial age.
The results are shown as the mean of 13 observations calculated using the arcsine transformation and converted back to percentages.


80
40
PERCENTAGE SUSPENDED
top + bottom
bottom
top
5
10
HOURS AFTER SHEDDING

2.2.2 AGE RELATED ACTIVITY PATTERN OF CERCARIAE.

Two parameters were chosen to describe the activity of the cercariae. Firstly, the lengths of swim and sink periods were recorded and measured using cinephotography and an event recorder. Secondly, the percentage activity of the population (ie. the number of cercariae swimming expressed as a percentage of the total number of cercariae) was measured again using cinephotography and an event recorder.

Duration of sink and swim periods.

For the purposes of this experiment, the cercariae were considered as two discrete populations, cercariae within 1cm of the top of the cell and cercariae within 1cm of the bottom. As was shown in section 2.2.1, this division of the population includes the majority of the suspended cercariae. In the first set of experiments, the cercariae and test cell were prepared as previously described and 20 feet of cine film exposed one hour after shedding and then every two hours until 11 hours after shedding. No recording was made if less than five cercariae were present in the field of view. This procedure was repeated for cercariae both at the top and bottom of the cell. The processed films were loaded into a Specto Motion Analysis projector and projected onto a screen. At the start of each 20 feet section of film, cercariae were chosen randomly and beginning with the first swim period of an individual, the frame counter was zeroed and the film was advanced until the cercaria stopped swimming. The count was noted and the film again advanced until the cercaria now stopped sinking. The frame count was again noted. In this way, the duration of

alternate swim and sink periods was measured (in frames). When possible, five complete swim and sink periods for ten different individuals were recorded. From these data and the speed of filming, the duration of the swim and sink periods could be calculated in seconds.

In the second set of experiments, the cercariae were directly observed through a binocular microscope and the duration of the swim and sink periods was recorded on an event recorder. Ten individuals at the top and ten individuals at the bottom of the cell were selected and followed for at least five complete swim and sink periods. At the bottom of the cell, the measurement of the sinking period included any time spent by the cercariae on the bottom of the cell.

The results are shown in Fig. 2.2.4 for cercariae at the top of the cell and in Fig. 2.2.5 for cercariae at the bottom, the overlays being the results from the cine film analysis. There appears to be little difference in the mean duration of the swim periods at both the top and the bottom of the cell as the cercariae aged, although the swim periods at the bottom were slightly longer. The sink periods at the top of the cell showed an increase from about 5 seconds (1 to 3 hours after shedding) to about 12 seconds (7 hours after shedding), the latter being a duration similar to that shown by cercariae at the bottom of the cell.

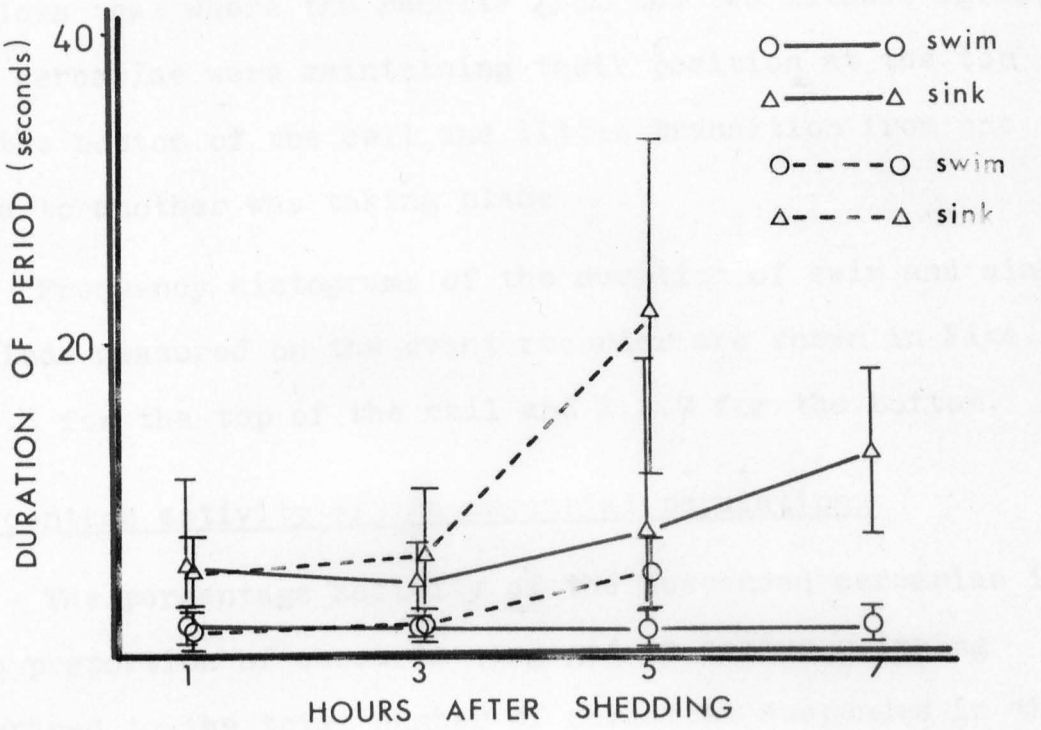
The differences between the results from each method of measurement may partially be due to the different methods used to select the cercariae (see Techniques For Recording the Movements of Microscopic Organisms). It

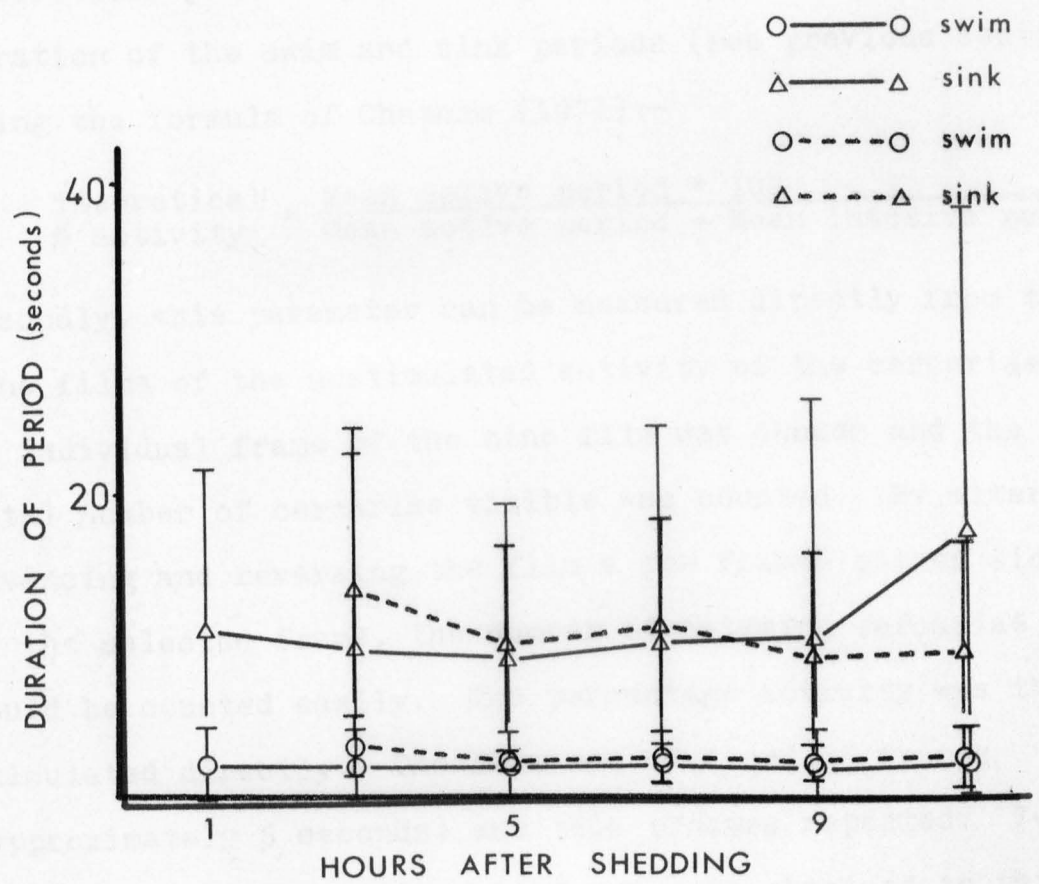
FIGURE 2.2.4

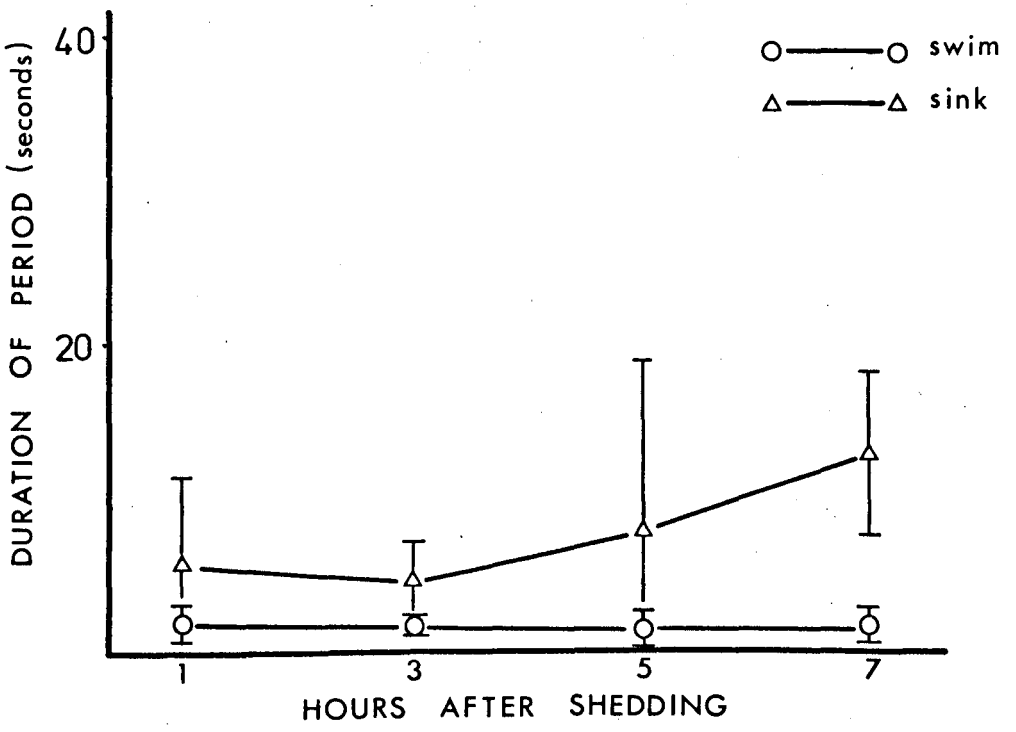
The duration of swim and sink periods at the top of the cell as the cercariae aged.
The lower graph shows the duration of the periods as measured by the event recorder.
The upper graph shows the duration of the periods as measured from cine film.

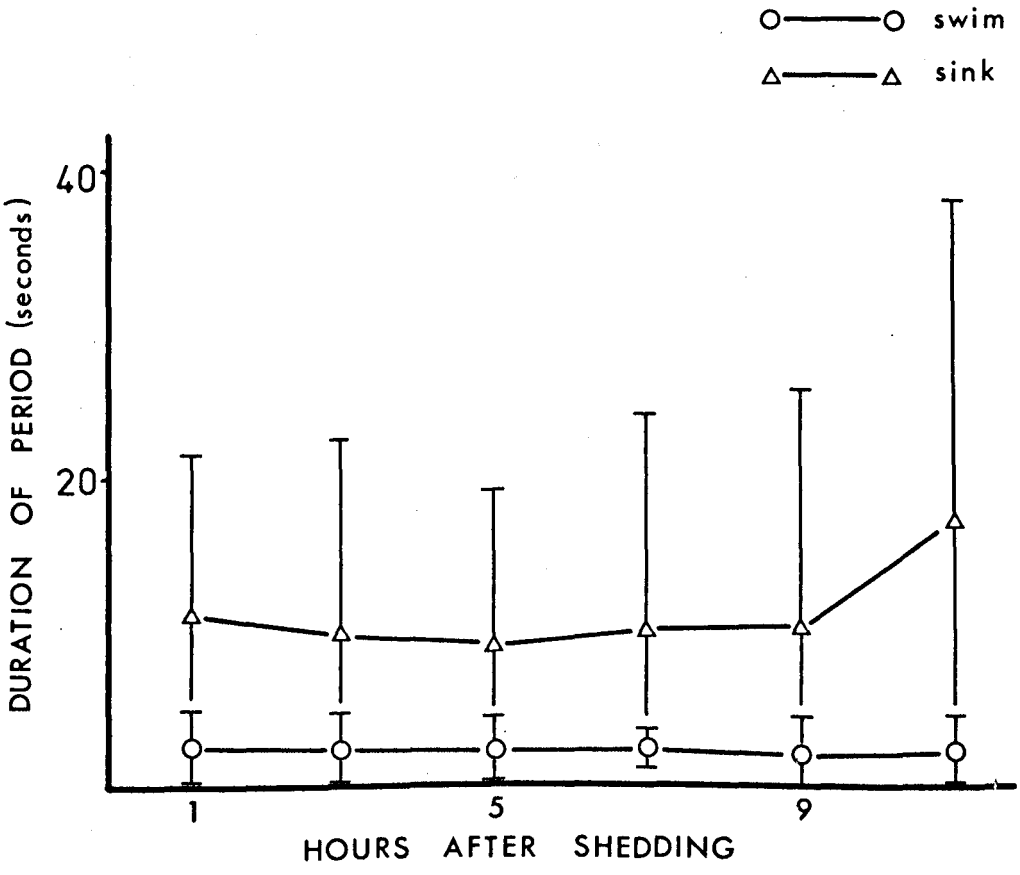
FIGURE 2.2.5

The duration of swim and sink periods at the bottom of the cell as the cercariae aged.
The lower graph shows the duration of periods as measured by the event recorder.
The upper graph shows the duration of periods as measured from cine film.


40
20
DURATION OF PERIOD (seconds)
swim
sink
swim
sink
1
3
5
HOURS AFTER SHEDDING


swim
sink
swim
sink
40
20
DURATION OF PERIOD (seconds)
1
5
9
HOURS AFTER SHEDDING


40
20
DURATION OF PERIOD (seconds)
swim
sink
1
3
5
7
HOURS AFTER SHEDDING


swim
sink
40
20
DURATION OF PERIOD (seconds)
1
5
9
HOURS AFTER SHEDDING

follows that where the results from the two methods agree, the cercariae were maintaining their position at the top or the bottom of the cell and little transition from one area to another was taking place.

Frequency histograms of the duration of swim and sink periods measured on the event recorder are shown in Figs. 2.2.6 for the top of the cell and 2.2.7 for the bottom.

Percentage activity of the cercarial population.

The percentage activity of the suspended cercariae is the proportion of cercariae engaged in active swimming compared to the total number of cercariae suspended in the water (those actively swimming plus those passively sinking). This parameter was measured in two ways. Firstly, the theoretical percentage activity can be derived from the duration of the swim and sink periods (see previous section) using the formula of Chapman (1971):-

$$\text{Theoretical \% activity} = \frac{\text{Mean active period} * 100}{\text{Mean active period} + \text{Mean inactive period}}$$

Secondly, this parameter can be measured directly from the cine films of the unstimulated activity of the cercariae. An individual frame of the cine film was chosen and the total number of cercariae visible was counted. By alternately advancing and reversing the film a few frames either side of the selected frame, the number of swimming cercariae could be counted easily. The percentage activity was then calculated directly. The film was advanced 55 frames (approximately 5 seconds) and this process repeated. Ten observations for each cercarial age were obtained in this way.

FIGURE 2.2.6

Frequency histograms of the duration of swim and sink periods of cercariae at the top of the cell as they aged.

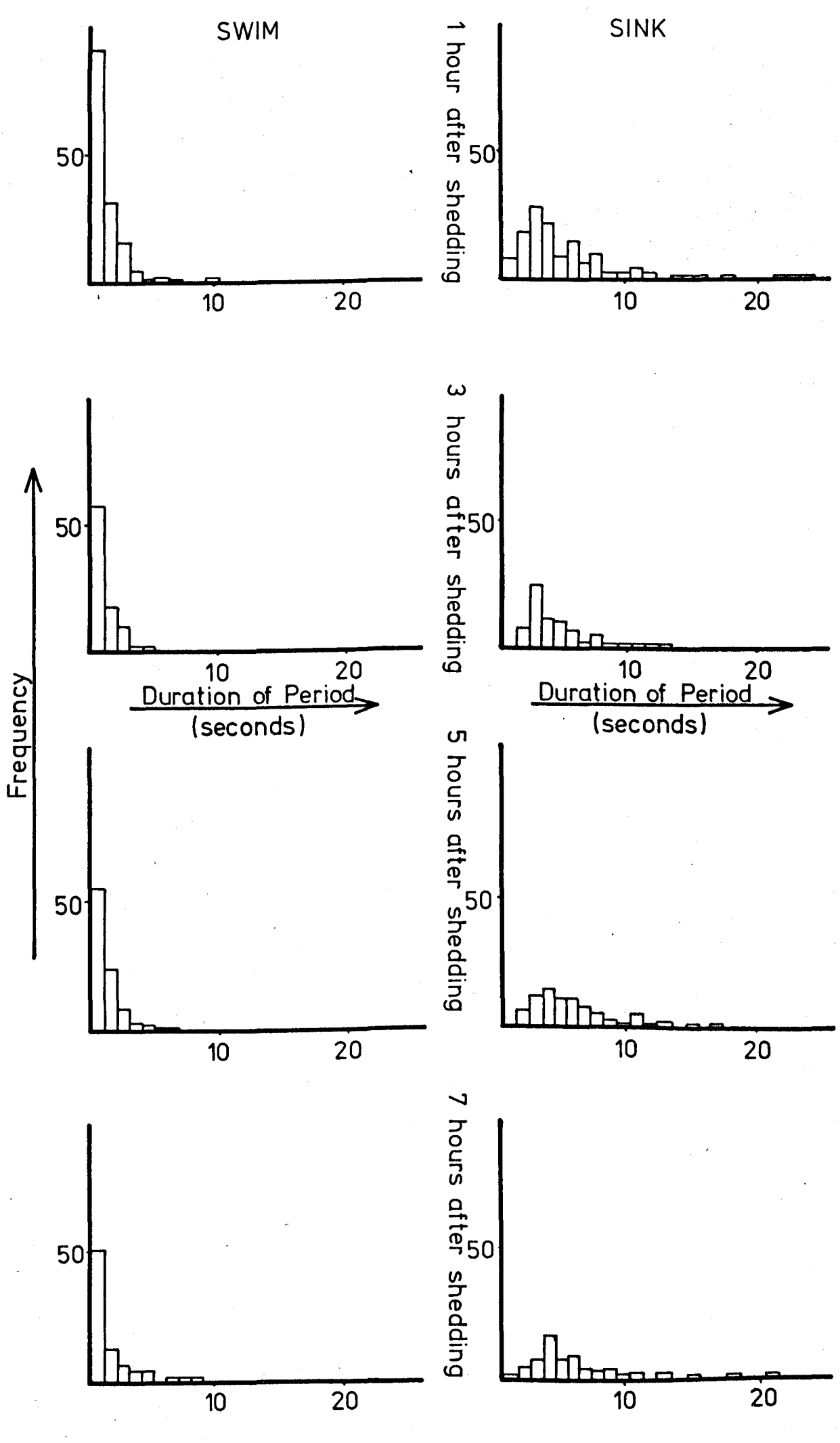
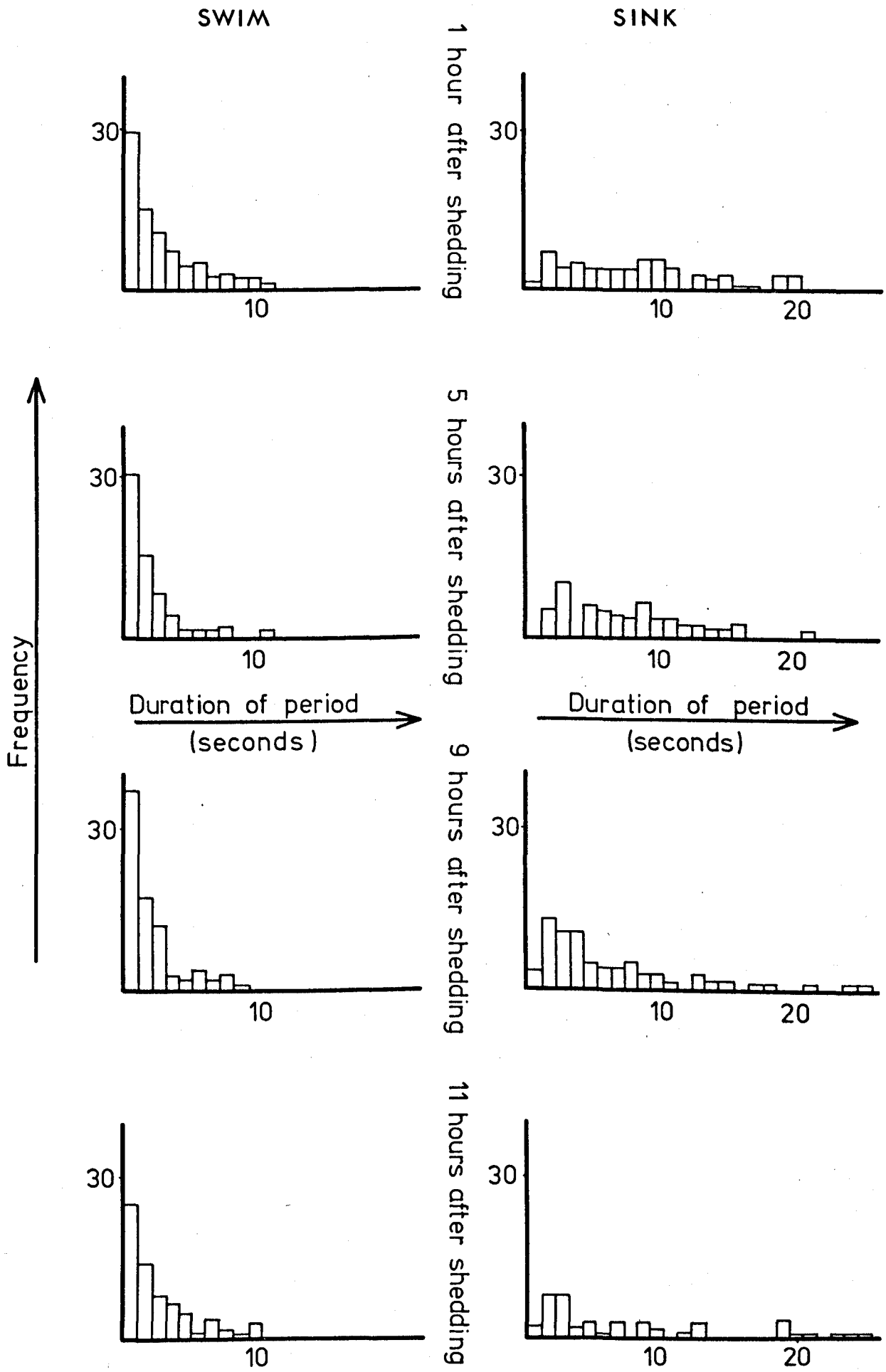

SWIM
SINK
1 hour after shedding
3 hours after shedding
5 hours after shedding
7 hours after shedding
50
10
20
Frequency
Duration of Period
(seconds)

FIGURE 2.2.7

Frequency histograms of the duration of swim and sink periods of cercariae as they aged at the bottom of the cell.


SWIM
SINK
1 hour after shedding
5 hours after shedding
9 hours after shedding
11 hours after shedding
Frequency
Duration of period
(seconds)
30
10
20

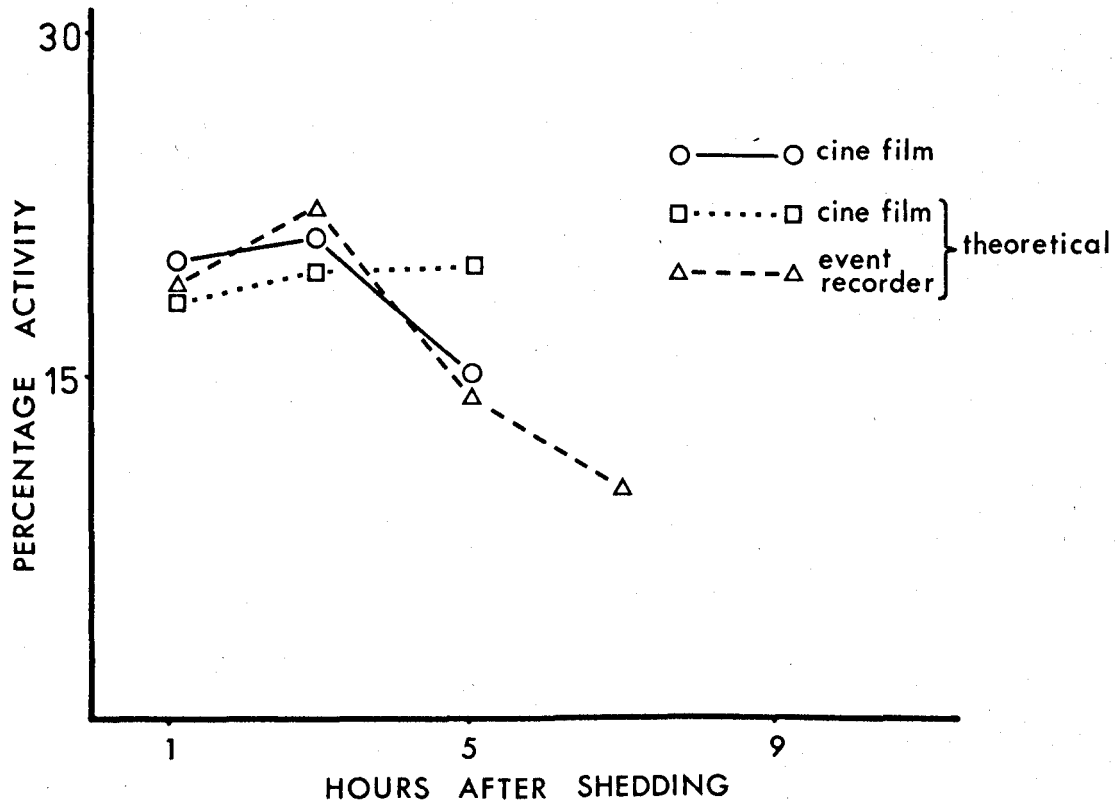
The results from each of these methods for determining the percentage activity are shown in Fig. 2.2.8 for cercariae at the top of the cell and in Fig. 2.2.9 for cercariae at the bottom. The theoretical percentage activity calculated from cine film remains relatively constant as the cercariae aged. This result was expected due to the biased selection of the cercariae. The theoretical percentage activity from the event recorder and the direct measurement from the cine film agree well for cercariae at the top of the cell. At the bottom of the cell, the direct measurement gave a lower percentage activity than the event recorder for the first five hours after the cercariae were shed. This might be due partially to the small number of cercariae which were present in the cine film frame for the first few hours. In general, a drop in the level of activity, starting 3 hours after shedding at the top of the cell and 9 hours after shedding at the bottom of the cell was indicated. This decrease in the activity is due to an increase in the time that the cercariae spent in sinking (see previous section).

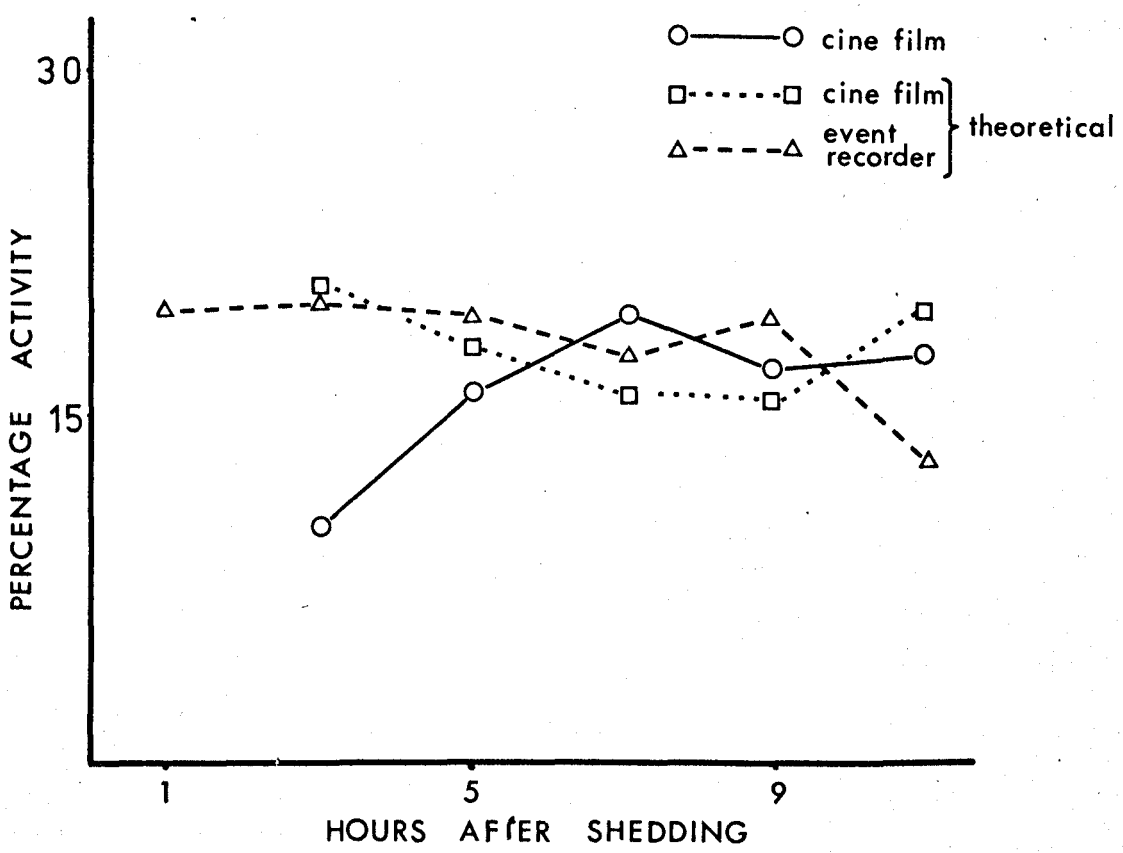
FIGURE 2.2.8

Percentage activity of cercariae as they aged at the top of the cell.

FIGURE 2.2.9

Percentage activity of cercariae as they aged at the bottom of the cell.


30
15
PERCENTAGE ACTIVITY
cine film
cine film
event recorder
theoretical
1
5
9
HOURS AFTER SHEDDING


30
15
PERCENTAGE ACTIVITY
cine film
cine film
event recorder
theoretical
1
5
9
HOURS AFTER SHEDDING

2.2.3 TRACKS OF AGE RELATED SWIMMING PATTERNS.

The films produced in a previous experiment (see Duration of swim and sink periods) were used to obtain the tracks of the behaviour of individual cercariae. At each cercarial age, for cercariae at the top and the bottom of the cell, ten individuals were picked at random and five successive swim and sink periods were recorded onto paper from each. The swimming positions were transcribed at two frame intervals and the sinking positions at ten frame intervals. Sample tracks are shown in Fig. 2.2.10 for cercariae at the top of the cell and Fig. 2.2.11 for cercariae at the bottom. These tracks were then subjected to the track analysis previously described.

Speed of swimming and sinking.

Frequency histograms for the speed of swimming and sinking were produced by track analysis and were found to be normally distributed about their means for cercariae at the top and bottom of the cell. The differences between successive mean speeds were tested using the "d_1" test (see Appendix 1) and all the results are presented in Table 2.2.A. Cercariae at the top of the cell swam at about 0.7mm per second and there appears to be a small but significant decrease in the speed between 3 and 5 hours after shedding. The sinking speed at the top of the cell remained constant at 0.12mm per second. The swimming speed at the bottom of the cell was much more variable and there appears to be a significant increase in speed compared to cercariae at the top. There was also a general trend for the cercariae at the bottom to swim slower as they aged. The sinking speed

FIGURE 2.2.10

Tracks of cercariae swimming and sinking at the top of the cell.

The time interval between points on all cercarial swimming tracks is 0.185 seconds.
The time interval between points on all cercarial sinking tracks is 0.926 seconds.

FIGURE 2.2.11

Tracks of cercariae swimming and sinking at the bottom of the cell.

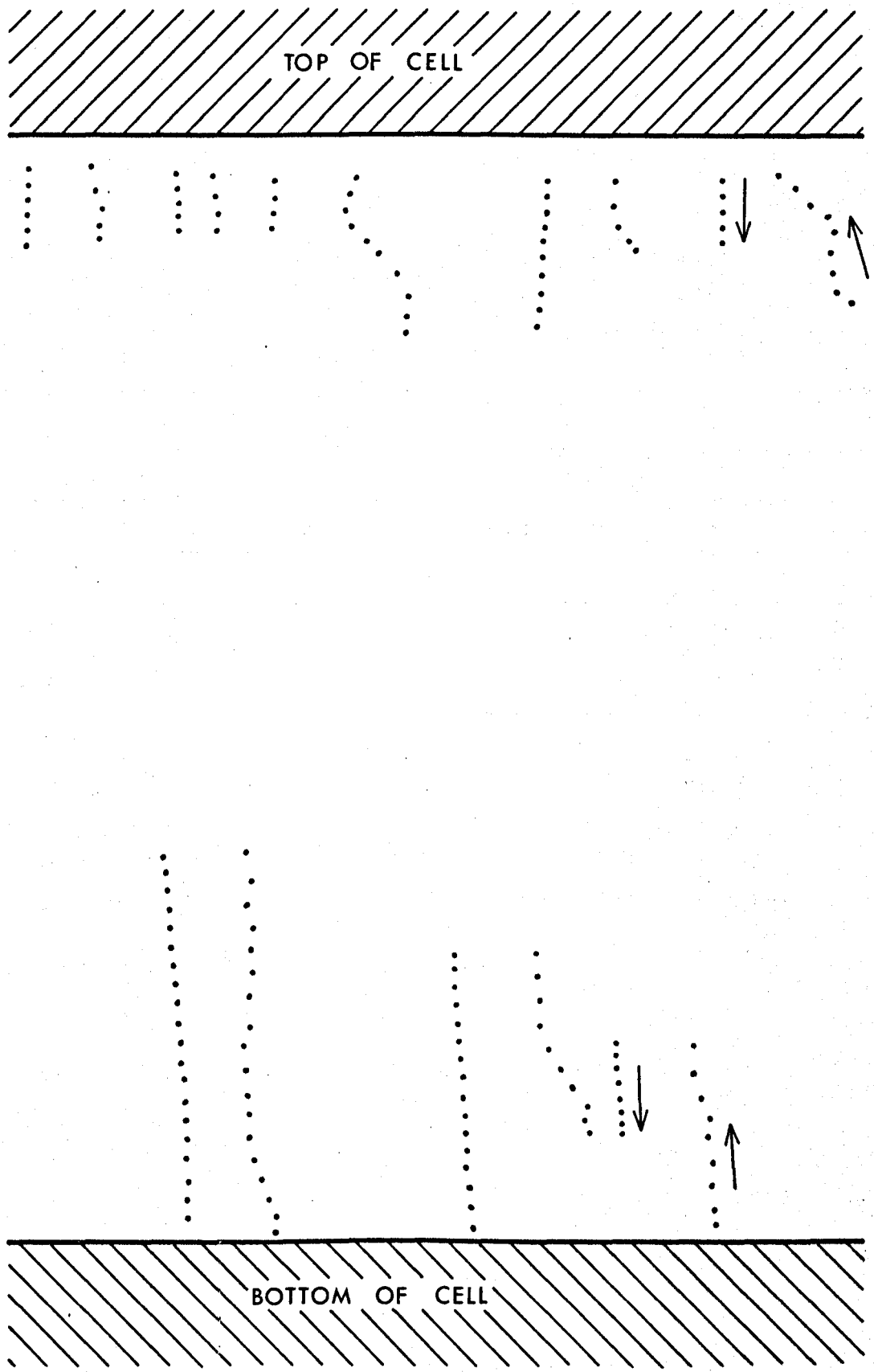

TOP OF CELL
BOTTOM OF CELL

TABLE 2.2.A

The speed of swimming and sinking cercariae as they aged at the top and bottom of the test cell.

		SWIM					SINK				
	Hour	n	mean	sd	P_1	P_F	n	mean	sd	P_1	P_F
Top	1	274	0.73	0.14			253	0.12	0.02		
	3	331	0.73	0.14	>.1	>.1	299	0.12	0.02	>.1	<.001
	5	220	0.68	0.17	<.001	<.01	122	0.12	0.02	>.1	<.05
Bottom	3	114	0.92	0.17			52	0.15	0.02		
	5	392	0.76	0.17	<.001	>.1	278	0.14	0.02	<.001	>.1
	7	357	0.92	0.21	<.001	<.001	293	0.14	0.02	>.1	>.1
	9	351	0.76	0.19	<.001	>.05	296	0.14	0.02	>.1	>.1
	11	357	0.59	0.17	<.001	<.05 >.01	147	0.14	0.03	>.1	<.01
			mm/s					mm/s			

P_1 - the probability that the difference between successive means would occur by chance. ("d_1" test)

P_F - the probability that the ratio of the variances about successive means would occur by chance.

at the bottom remained constant at 0.14mm per second after an initial slight but significant reduction between 3 and 5 hours after shedding. Cercariae at the bottom would also appear to sink significantly faster than cercariae at the top of the cell.

Direction of swimming.

Frequency histograms for the directionality of tracks (0^{o} being directly upwards, 180^{o} directly downwards) were produced by track analysis. The histogram for cercariae swimming at the top of the cell one hour after shedding is shown in Fig. 2.2.12. If the directionality is resolved into its left and right components, assigning a negative value to the left orientated directions, the resulting distribution is normal about a mean close to zero. The means and standard errors along with tests for significant differences between means are presented in Table 2.2.B. These results suggest that although unstimulated cercariae swim predominately upwards, this swimming is not directly towards the surface but shows considerable deviations from a staight path. Cercariae swimming upwards at the bottom of the cell showed a slight swimming bias towards the left and there was a general degree of variation from sample to sample.

Direction of sinking.

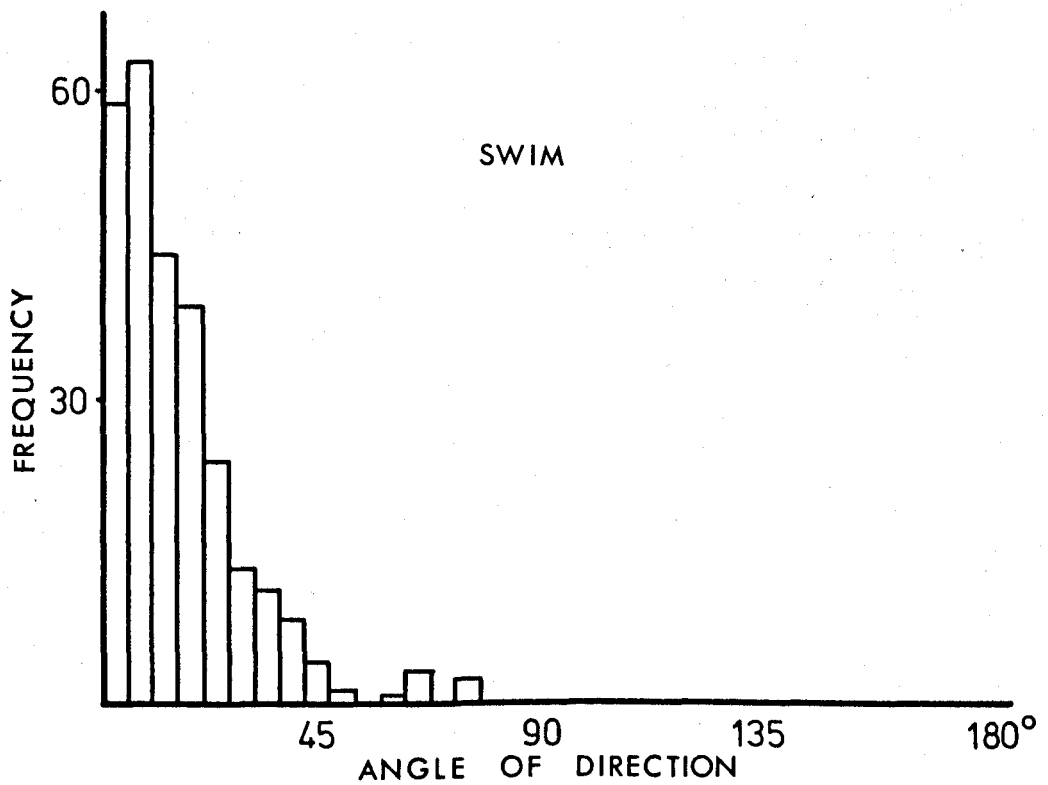
Frequency histograms for the directionality of sinking tracks (0^{o} being directly upwards, 180^{o} directly downwards) were produced by track analysis and the distribution for cercariae sinking at the top of the cell 1 hour after shedding is shown in Fig. 2.2.13. The values for the directionality were again not normally distributed. Normal

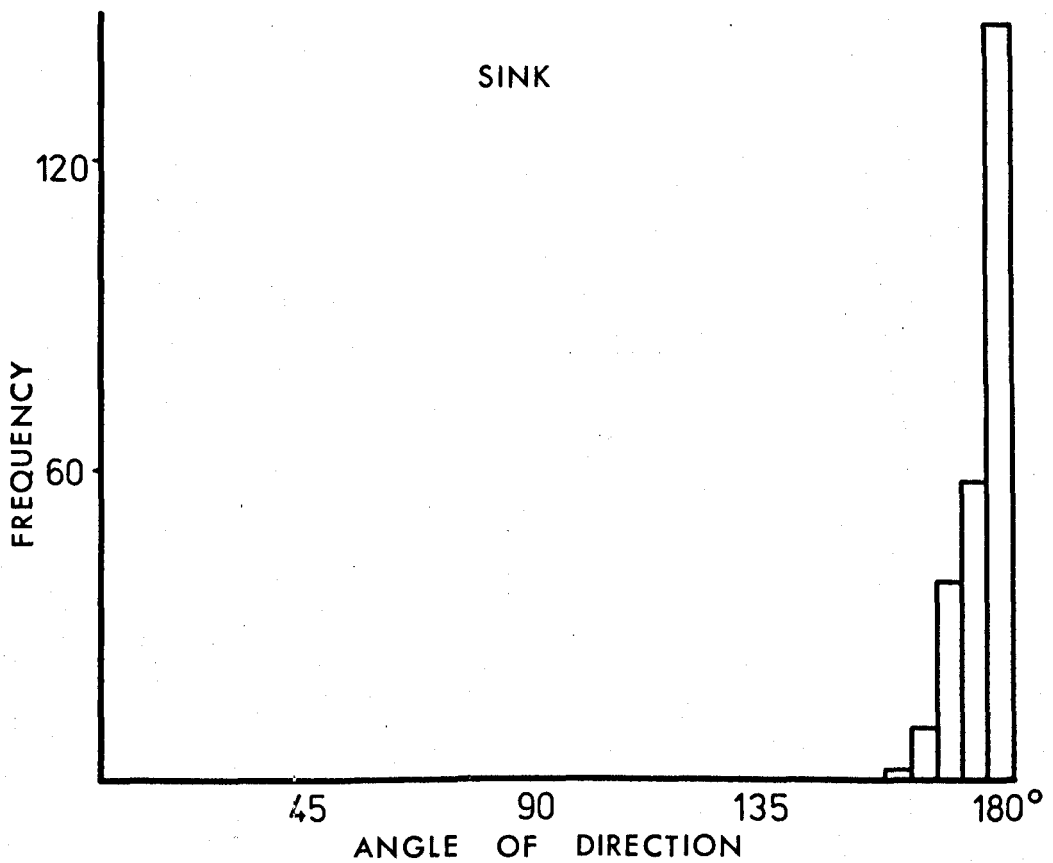
FIGURE 2.2.12

Frequency histogram of the direction of cercariae swimming at the top of the cell one hour after shedding.

FIGURE 2.2.13

Frequency histogram of the direction of cercariae sinking at the top of the test cell one hour after shedding.


SWIM
60
30
FREQUENCY
45
90
135
180°
ANGLE OF DIRECTION


SINK
120
60
FREQUENCY
45
90
135
180°
ANGLE OF DIRECTION

distributions for statistical comparison were produced by subtracting 180° from the left and right components of the directionality and then taking the absolute value of the right orientated directions. The resulting distribution was normal about a mean close to zero. The means and standard errors along with tests for significant differences are shown in Table 2.2.C. The sinking tracks were generally orientated downwards within 5° of the normal. Tests for significance showed that, where significant differences did occur, the difference between means was again less than 5°.

Angularity of tracks.

Frequency histograms for the angularity of tracks were produced by track analysis and the distribution of the angles turned by cercariae swimming at the top of the cell one hour after shedding is shown in Fig. 2.2.14 and for cercariae sinking in Fig. 2.2.15. Similar distributions for cercariae swimming and sinking three hours after shedding at the bottom of the cell are shown in Figs. 2.2.16 and 2.2.17. The angles were not normally distributed and so were resolved into left and right components (left being negative) to allow comparison of means. The results are shown in Table 2.2.D for swimming cercariae and in Table 2.2.E for sinking cercariae.

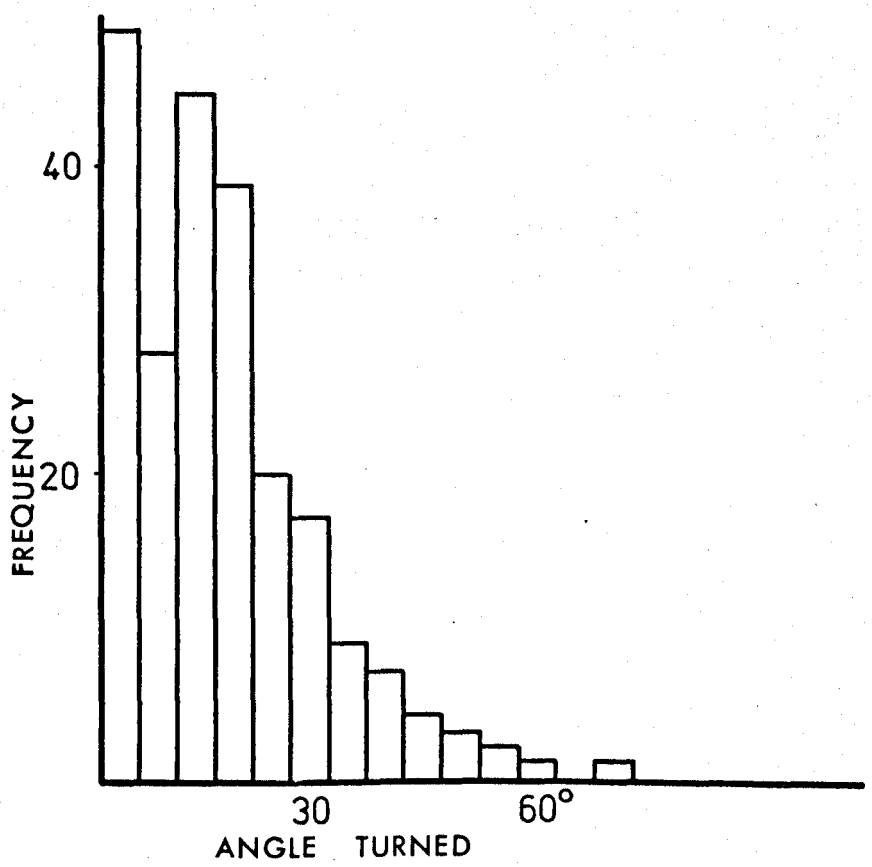
The angularity of all the tracks remained relatively constant as the cercariae aged and there was no bias towards the right or the left. As would be expected, swimming cercariae showed a larger angular component in their tracks than did the passively sinking cercariae.

FIGURE 2.2.14

Frequency histogram of the angles turned by cercariae swimming at the top of the test cell one hour after shedding.

FIGURE 2.2.15

Frequency histogram of the angles turned by cercariae sinking at the top of the test cell one hour after shedding.


40
20
FREQUENCY
30
60°
ANGLE TURNED

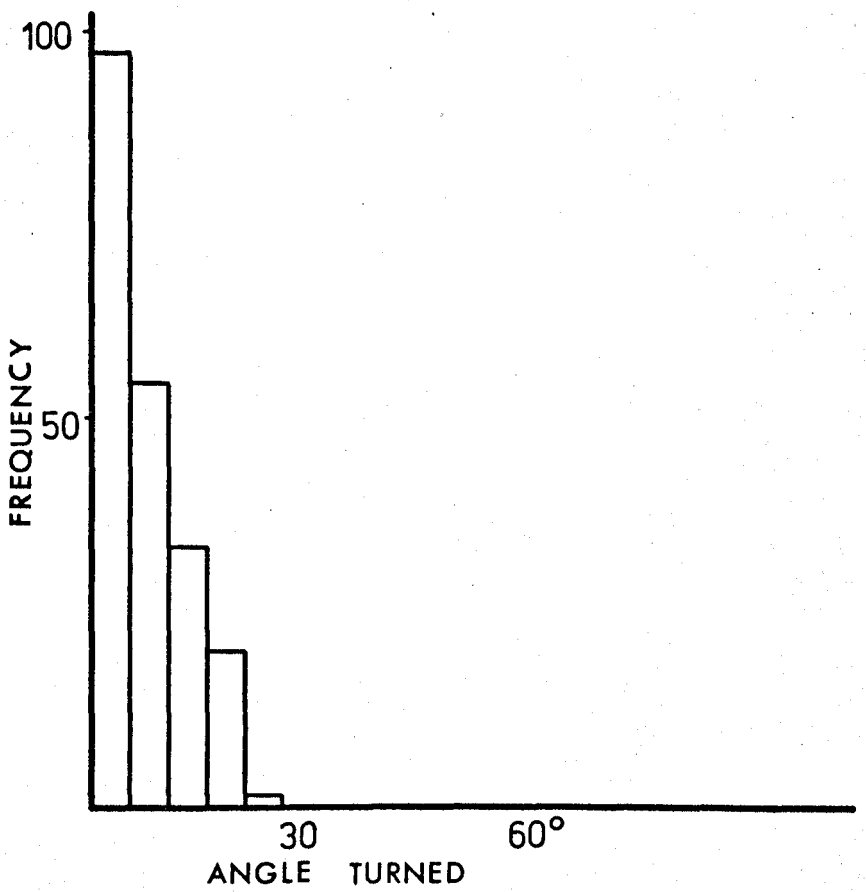
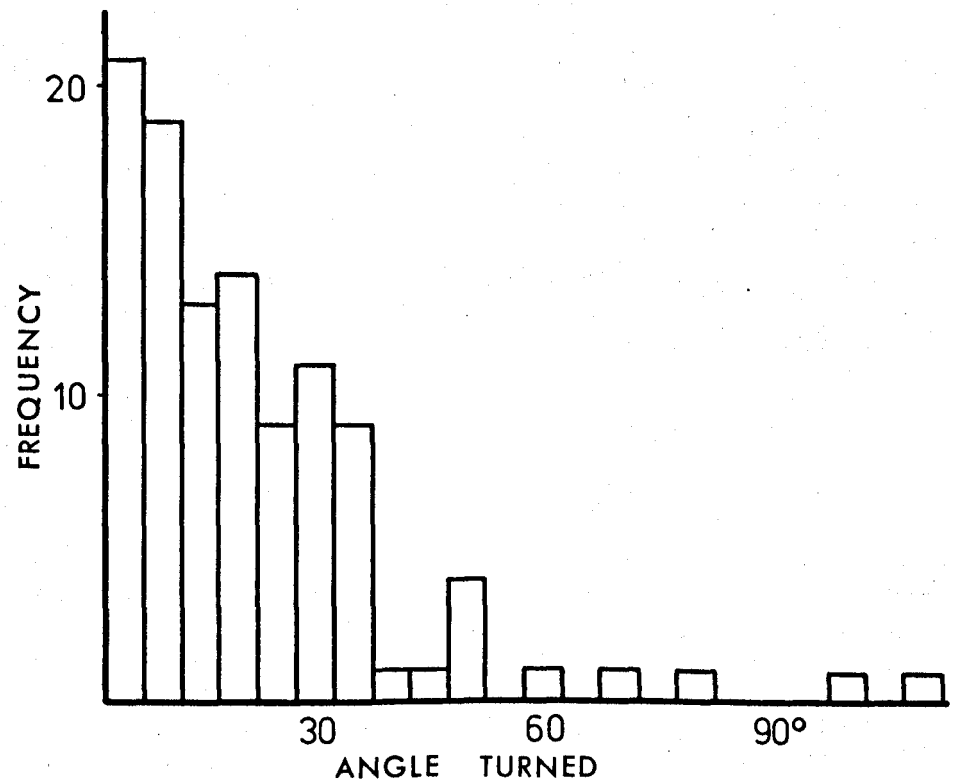

100
50
FREQUENCY
30
60°
ANGLE TURNED

FIGURE 2.2.16

Frequency histogram of the angles turned by cercariae swimming at the bottom of the test cell three hours after shedding.

FIGURE 2.2.17

Frequency histogram of the angles turned by cercariae sinking at the bottom of the test cell three hours after shedding.


20
10
FREQUENCY
30
60
90°
ANGLE TURNED

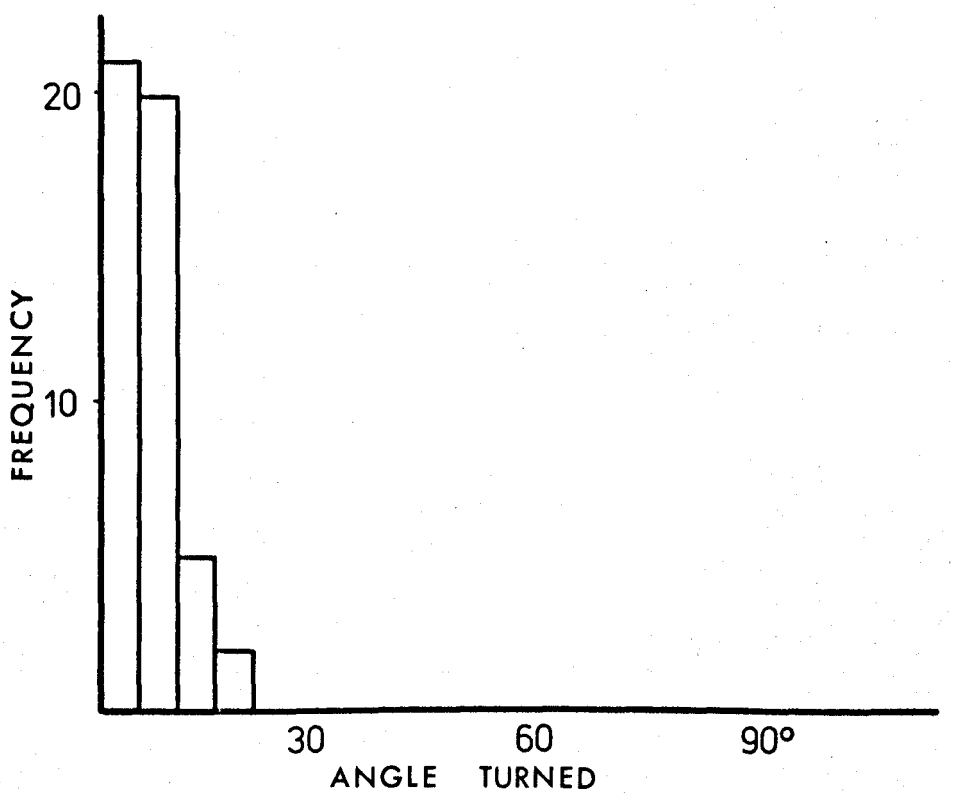

20
10
FREQUENCY
30
60
90°
ANGLE TURNED

TABLE 2.2.D

The angularity of cercariae swimming at the top and bottom of the cell as they aged.

	hour	n	mean angle	se	mean of left and right	se	P_0	P_1	P_F
top	1	225	15.88	0.82	-0.48	1.34	>.1		
	3	286	18.33	0.95	-0.45	1.44	>.1	>.1	<.01
	5	211	16.41	0.99	0.95	1.50	>.1	>.1	>.05
bottom	3	108	20.14	2.11	1.09	2.87	>.1		
	5	354	17.13	0.81	-0.25	1.22	>.1	>.1	<.001
	7	329	13.29	0.58	0.61	0.93	>.1	>.1	<.001
	9	317	18.65	0.81	0.34	1.33	>.1	>.1	<.001
	11	330	17.61	0.81	0.35	1.27	>.1	>.1	>.1
			degs		degs				

TABLE 2.2.E

The angularity of cercariae sinking at the top and bottom of the cell as they aged.

	hour	n	mean angle	se	mean of left and right	se	P_0	P_1	P_F
top	1	208	6.73	0.37	0.20	0.60	>.1		
	3	253	6.73	0.36	0.35	0.56	>.1	>.1	>.1
	5	103	6.09	0.46	-0.62	0.76	>.1	>.1	>.05
bottom	3	48	5.55	0.66	0.02	1.04	>.1		
	5	250	5.58	0.33	-0.20	0.48	>.1	>.1	>.1
	7	265	6.01	0.34	0.15	0.50	>.1	>.1	>.1
	9	265	5.07	0.36	0.06	0.49	>.1	>.1	>.1
	11	130	6.62	0.49	0.11	0.76	>.1	>.1	>.05
			degs			degs			

For both tables:-

P_0 - see Table 2.2.C

P_1 and P_F - see Table 2.2.A

String length of swimming and sinking cercariae.

Frequency histograms for the string length (number of successive angles of the same sign) were obtained by track analysis. The distributions for cercariae swimming and sinking one hour after shedding at the top of the cell are shown in Fig. 2.2.18 and for cercariae swimming and sinking at the bottom of the cell three hours after shedding in Fig. 2.2.19. The distributions were again resolved into string lengths of left angles and string lengths of right angles to allow the use of statistical tests for significance. The results are shown in Table 2.2.F for swimming cercariae and in Table 2.2.G for sinking cercariae.

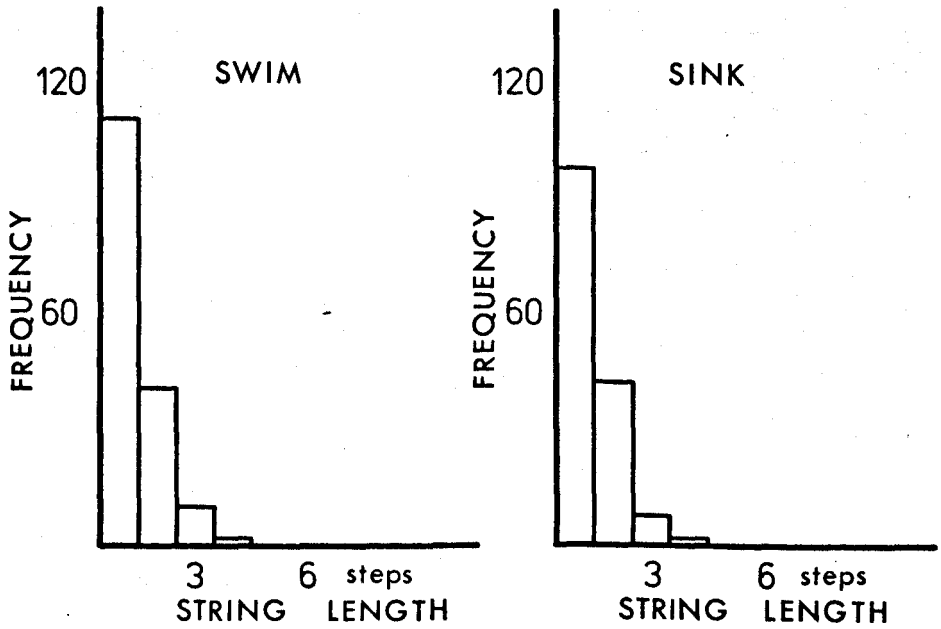
The results indicate that generally, right and left angles alternated and that the degree of alternation varied little as the cercariae aged.

FIGURE 2.2.18

Frequency histograms of the string lengths of cercariae swimming and sinking at the top of the cell one hour after shedding.

FIGURE 2.2.19

Frequency histograms of the string lengths of cercariae swimming and sinking at the bottom of the cell three hours after shedding.


SWIM
120
60
FREQUENCY
3
6 steps
STRING LENGTH
SINK
120
60
FREQUENCY
3
6 steps
STRING LENGTH

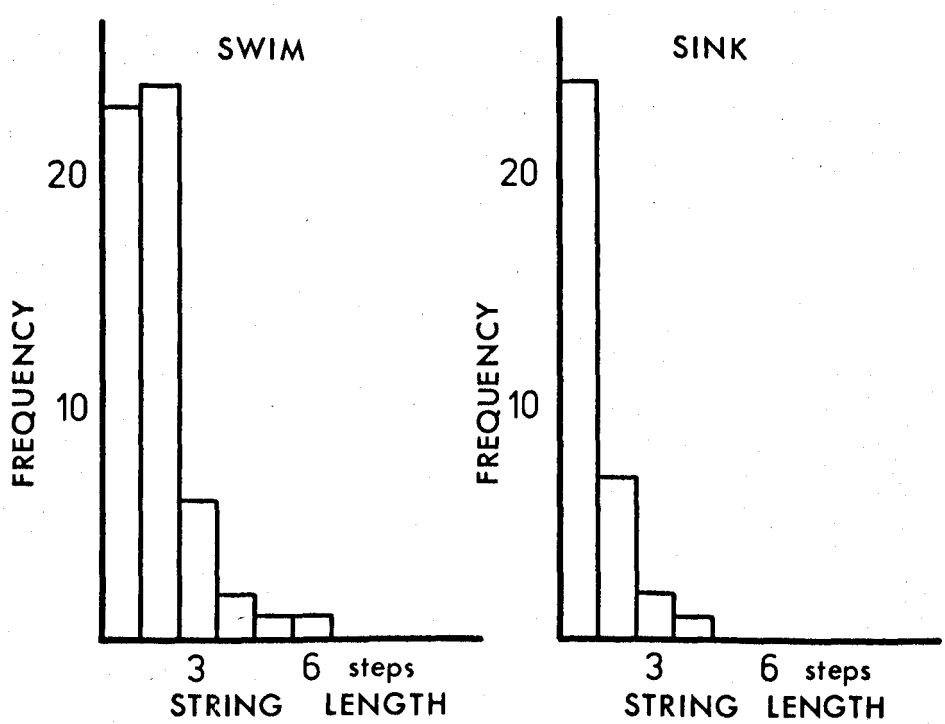

SWIM
20
10
FREQUENCY
3
6 steps
STRING LENGTH
SINK
20
10
FREQUENCY
3
6 steps
STRING LENGTH

TABLE 2.2.F

The string lengths shown by cercariae swimming at the top and bottom of the cell as they aged.

	hour	n	mean string length	sd	mean of left and right	sd	P_0	P_1	P_F
top	1	162	1.39	0.62	-0.04	1.53	>.1		
	3	187	1.53	0.71	0.00	1.64	>.1	>.1	>.05
	5	145	1.46	0.82	0.02	1.69	>.1	>.1	>.05
bottom	3	57	1.89	1.06	0.18	2.19	>.1		
	5	198	1.79	0.98	-0.01	2.11	>.1	>.1	>.05
	7	218	1.51	0.74	0.12	1.62	>.1	>.1	<.05
	9	197	1.61	0.98	0.05	1.82	>.1	>.1	>.05
	11	204	1.62	1.00	0.01	1.14	>.1	>.1	>.05

TABLE 2.2.G

The string lengths shown by cercariae sinking at the top and bottom of the cell as they aged.

	hour	n	mean string length	sd	mean of left and right	sd	P_0	P_1	P_F
top	1	149	1.39	0.61	0.15	1.46	>.1		
	3	181	1.40	0.69	0.14	1.61	>.1	>.1	>.05
	5	70	1.47	0.74	0.10	1.67	>.1	>.1	>.05
bottom	3	34	1.41	0.76	0.35	1.57	>.1		
	5	175	1.43	0.79	0.22	1.59	>.05	>.1	>.05
	7	169	1.57	0.91	0.37	1.82	>.01	>.1	>.05
	9	176	1.51	0.80	0.24	1.72	>.05	>.1	>.05
	11	83	1.57	1.00	0.31	1.82	>.1	>.1	>.05

For both tables:-

P_0 - the probability that the difference of the resolved mean from zero would occur by chance. ("d_o" test).

P_1 - the probability that the difference between successive means would occur by chance. ("d_1" test).

P_0 - the probability that the ratio of the variances about successive means would occur by chance.

2.2.4 SUMMARY OF SECTION 2.2

1. The number of cercariae that remained suspended in a vessel decreased as the cercariae aged.

2. Shortly after shedding, the majority of the cercariae were found towards the top of the test cell. As the cercariae aged, there was a gradual transition of larvae from the top to the bottom.

3. This transition would appear to be due to an increase in the duration of the sinking periods and not due to a decrease in the time spent swimming.

4. Cercariae swam predominantly upwards with considerable deviations from a straight course. Sinking was directly downwards.

5. The speed of swimming tended to decrease as the cercariae aged. The sinking speed remained relatively constant.

6. The angularity of the swimming and sinking tracks remained relatively constant.

7. In general, cercariae followed one or two turns to the left with one or two turns to the right when swimming upwards, and *vice versa*. This result was unaffected by the age of the cercariae.

2.3 RESPONSE TO SHADOW STIMULUS - EXPERIMENTS AND RESULTS

Cercariae of many species have often been observed to respond in a positive way to a shadow stimulus or a drop in light intensity. Lawson (1977) has shown that inactive cercariae on the bottom of a small test vessel were activated by a shadow stimulus and that many cercariae of all ages would respond in this way. It might be suggested that the shadow response would become increasingly more important in a host-location mechanism as the cercariae age, as I have demonstrated that the number of cercariae on or near the bottom of a large test cell increases with time.

In this section, I have used the analyses developed in section 2.2 to quantify the response of cercariae to a simple shadow stimulus as they age. The shadow stimulus was produced by switching off the light above the test cell for between 3 and 4 seconds.

2.3.1 AGE RELATED CHANGE IN DISTRIBUTION IN RESPONSE TO A SHADOW STIMULUS.

Number of cercariae suspended in the cell after the stimulus.

Cercariae were pipetted into the test cell as previously described and photographs of the entire cell were taken on a Linhoff plate camera, immediately before and at 0.5, 1, 5 and 10 minutes after the stimulus was applied. This procedure was replicated four times for cercariae one hour after shedding and then at two hourly intervals until eleven hours after shedding. The photographs were processed and printed and the total number of cercariae visible was counted. An increase was observed in the number of cercariae of all ages suspended in the cell after the shadow stimulus.

Fig. 2.3.1 shows the number of additional cercariae suspended in the cell one minute after the application of the stimulus. The number of cercariae inactive or dead on the bottom of the cell is also shown estimated from the data presented in section 2.2.1. The percentage increase in numbers at each of the selected intervals can be found in Appendix 3.

Distribution of cercariae after the application of the stimulus.

The distribution of cercariae in the photographs taken in the previous experiment was calculated by dividing the photographs into 12 equal horizontal sections and by counting the number of cercariae visible in each section. Selected distributions are shown in Fig. 2.3.2 and the complete data are presented in Appendix 3. In general, a migration of cercariae upwards in the cell was observed. The peak in the number of cercariae at the top of the cell occurred at an increasing interval of time after the stimulus as the cercariae aged. One hour after shedding, this peak occurred only 0.5 minutes after the stimulus was applied. Eleven hours after shedding, the peak was not observed until 5 minutes after the application of the stimulus.

FIGURE 2.3.1

The number of additional cercariae suspended in the test cell one minute after the application of a shadow stimulus.

Δ----Δ percentage increase in the number of cercariae suspended in the cell after the application of the stimulus. Mean and standard error of four observations.

O——O percentage of the cercariae estimated to be on the bottom of the test cell. Mean of thirteen observations.

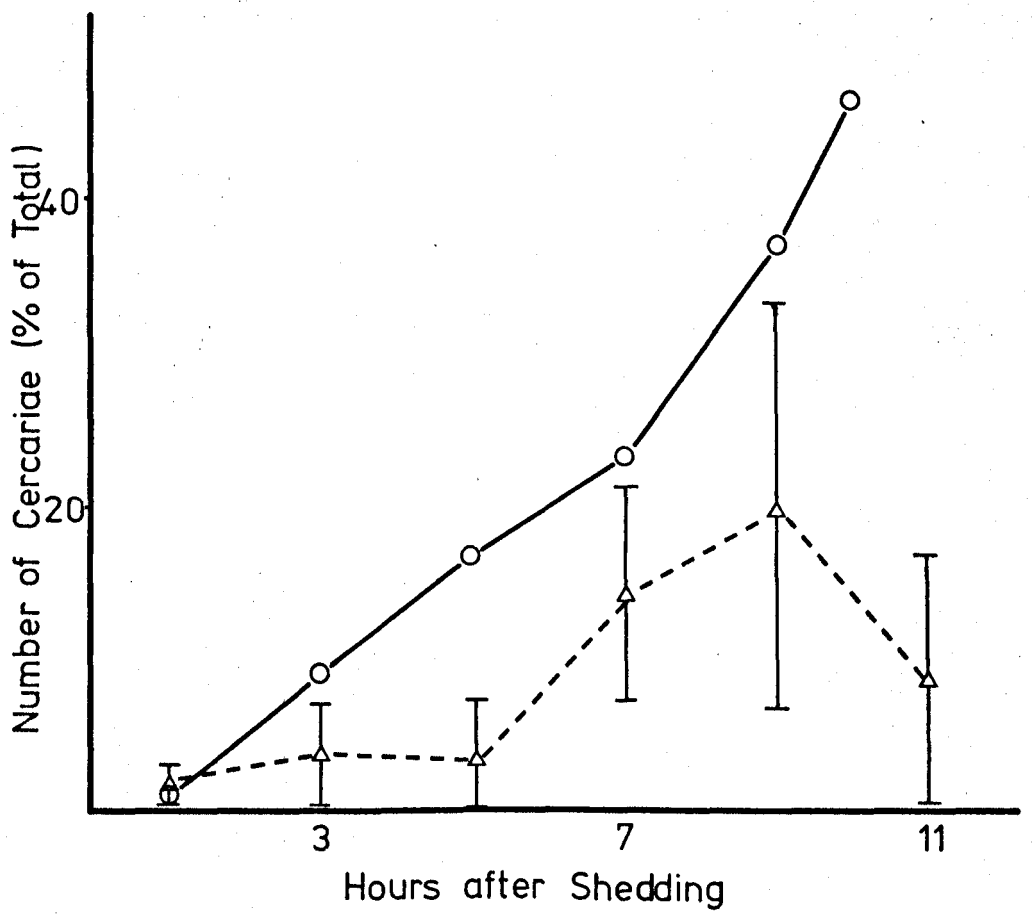
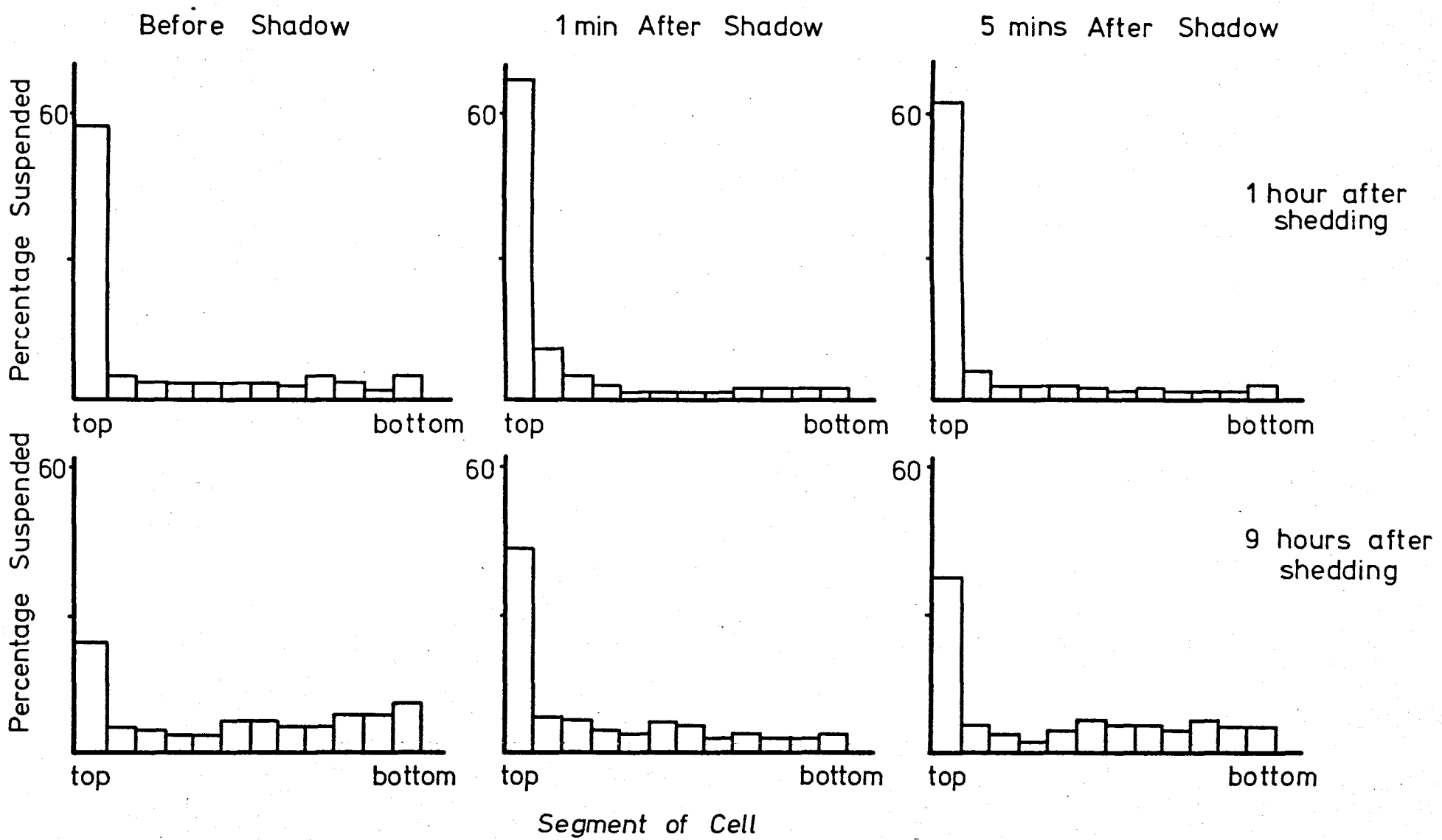

Number of Cercariae (% of Total)
40
20
3
7
11
Hours after Shedding

FIGURE 2.3.2

Selected histograms of the distribution of cercariae in the test cell at intervals after the application of a shadow stimulus. The distributions of cercariae one hour and nine hours after shedding are shown.


Before Shadow
1 min After Shadow
5 mins After Shadow
Percentage Suspended
60
top
bottom
1 hour after shedding
9 hours after shedding
Segment of Cell

2.3.2 AGE RELATED ACTIVITY PATTERN OF CERCARIAE IN RESPONSE TO A SHADOW STIMULUS.

The previously described procedures using cinephotography were used to record the swimming pattern of cercariae in response to the shadow stimulus.

Duration of sink and swim periods after the application of the shadow stimulus.

The length of the first swim and the first sink period after the top light was switched back on was measured from the cine film by counting the number of frames between the beginning and end of each period. Only cercariae that showed a positive response were considered. The duration of a period in frames was converted to the duration in seconds by dividing by the speed of filming. The results are shown in Table 2.3.A. As the distribution of the duration of the periods was non-normal, significance tests were not performed but it can be seen that, in general, the first swim period after the application of the stimulus was longer than before the stimulus and the first sink period was shorter than before.

Percentage activity of the cercarial population after the shadow stimulus.

The cine films used for the analysis of swim and sink periods were analysed for the percentage activity before, during and after the shadow stimulus at one second intervals, by counting both the total number of cercariae and those engaged in active swimming. The results are shown in Table 2.3.B and selected results for the top and bottom of the cell are shown graphically in Figs. 2.3.3 and 2.3.4. A dramatic increase in the percentage activity occurred throughout and after the application of the stimulus. This

TABLE 2.3.A

Duration of the first swim and the first sink periods immediately after the application of a shadow stimulus.

	hour		after shadow			unstimulated		
			n	mean	sd	n	mean	sd
top	1	swim	10	7.47	5.35	50	1.17	0.51
		sink	8	3.24	1.86	50	5.00	2.42
	3	swim	8	5.65	6.67	50	1.49	1.07
		sink	6	3.82	3.90	50	6.28	5.01
	5	swim	8	3.59	1.95	8	3.34	2.03
		sink	8	2.62	3.12	6	13.06	10.18
bottom	5	swim	9	4.26	1.45	40	2.01	1.04
		sink	9	6.31	7.65	35	9.24	7.62
	7	swim	10	9.23	9.36	41	2.13	2.24
		sink	10	11.41	15.31	33	10.55	8.06
	9	swim	10	7.90	2.51	40	1.73	1.07
		sink	10	7.26	9.85	39	8.56	7.59
	11	swim	8	8.17	7.72	42	2.07	1.88
		sink	7	5.22	6.42	37	8.62	8.87
				secs			secs	

TABLE 2.3.B

Percentage activity of cercariae after the application of a shadow stimulus for cercariae of different ages. The shadow stimulus lasted for between three and four seconds.

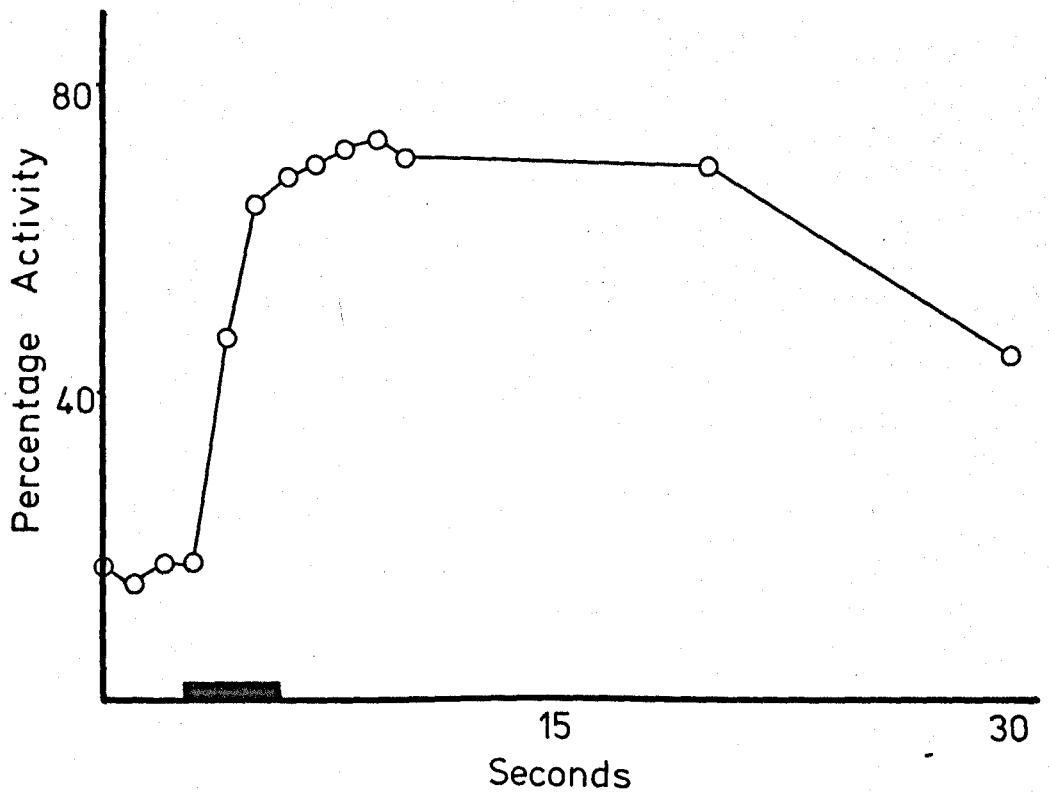
		seconds after the application of the stimulus													
	hour	-3	-2	-1	1	2	3	4	5	6	7	8	17	27	n
top	1	17.5	15.0	17.5	17.5	47.5	65.0	68.3	70.0	72.5	73.2	71.4	60.9	44.7	40-47
	3	17.1	15.0	15.0	15.0	51.2	56.1	62.5	72.5	63.6	70.0	67.5	53.6	44.2	40-44
	5	15.0	10.0	10.5	15.0	33.3	61.9	80.0	65.0	60.0	55.5	57.8	57.8	43.5	18-23
	7	7.1	14.2	14.2	14.2	50.0	64.3	56.3	50.0	56.3	50.0	53.3	53.3	40.0	14-16
bottom	3	7.1	10.7	10.7	34.4	64.5	51.7	50.0	45.2	36.6	16.1	11.7	16.6	20.0	28-34
	5	8.7	9.9	7.8	10.0	67.8	57.6	50.0	50.0	42.5	36.5	27.1	17.9	13.9	84-96
	7	10.5	10.9	9.8	8.7	50.0	59.8	58.7	46.7	54.3	42.4	35.9	23.9	15.2	86-92
	9	11.1	11.1	8.9	9.6	40.0	51.1	58.9	63.3	61.1	55.6	44.4	25.6	16.7	83-90
	11	13.3	13.3	11.1	11.6	34.4	45.6	48.9	43.3	47.8	47.8	45.6	33.3	16.7	86-91
		percentage activity													

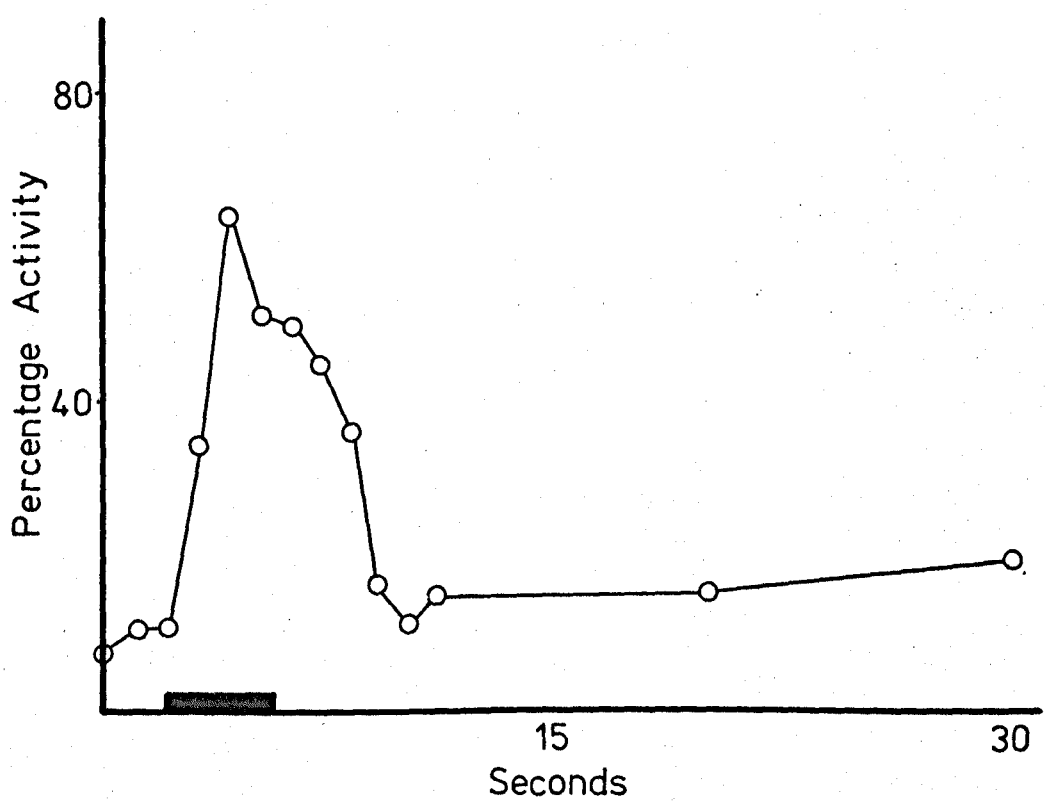
FIGURE 2.3.3

The percentage activity of cercariae at the top of the test cell one hour after shedding and after the application of a shadow stimulus.

FIGURE 2.3.4

The percentage activity of cercariae at the bottom of the test cell three hours after shedding and after the application of a shadow stimulus.


80
40
Percentage Activity
15
30
Seconds


80
40
Percentage Activity
15
30
Seconds

increased level of activity appeared to be maintained at the top of the cell, but rapidly died away at the bottom, the activity returning to basal levels within six seconds after the application of the stimulus. This difference in the duration of the response is primarily due to an artefact of the analysis. At the bottom of the cell, active cercariae will swim out of the cine film frame and so will be lost from the analysis. At the top of the cell, active cercariae will swim into the cine film frame and so will be included in the analysis.

Cerariae of all ages responded in this way (see Table 2.3.B) although the peak in activity decreased as the cercariae at the bottom of the cell aged.

2.3.3 TRACKS OF AGE RELATED SWIMMING ACTIVITY DIRECTLY AFTER A SHADOW STIMULUS.

The tracks of the first swim period after the application of the shadow stimulus were produced from the films exposed in the previous section and were subjected to track analysis along with control unstimulated tracks. Sample tracks for cercariae at the top of the cell one hour after shedding are shown in Fig. 2.3.5 and for cercariae at the bottom, three hours after shedding in Fig. 2.3.6.

Speed of swimming after the shadow stimulus.

Frequency histograms of the speed of swimming were found to be normally distributed about their means. Differences between means were tested for significance using the "d_1" test. The results are presented in Table 2.3.C. There was no significant difference between the speed of the cercariae directly before and directly after the shadow stimulus. The fall in the swimming speed after the stimulus as the cercariae aged parallels the drop in speed shown by unstimulated cercariae (see section 2.2.3).

Direction of swimming.

Frequency histograms for the direction of swimming after the application of a shadow stimulus were produced by track analysis. The histogram of the direction of cercariae swimming at the bottom of the cell, three hours after shedding is shown in Fig. 2.3.7. At the bottom, the direction of swimming was upward and the distributions were normalised by resolving into left and right angles of direction to allow comparison. The results are shown in Table 2.3.D. Although significant differences from unstimulated cercariae

FIGURE 2.3.5

Sample tracks of cercariae swimming at the top of the test cell one hour after shedding and directly after the application of a shadow stimulus.

FIGURE 2.3.6

Sample tracks of cercariae swimming at the bottom of the test cell three hours after shedding and directly after the application of a shadow stimulus.

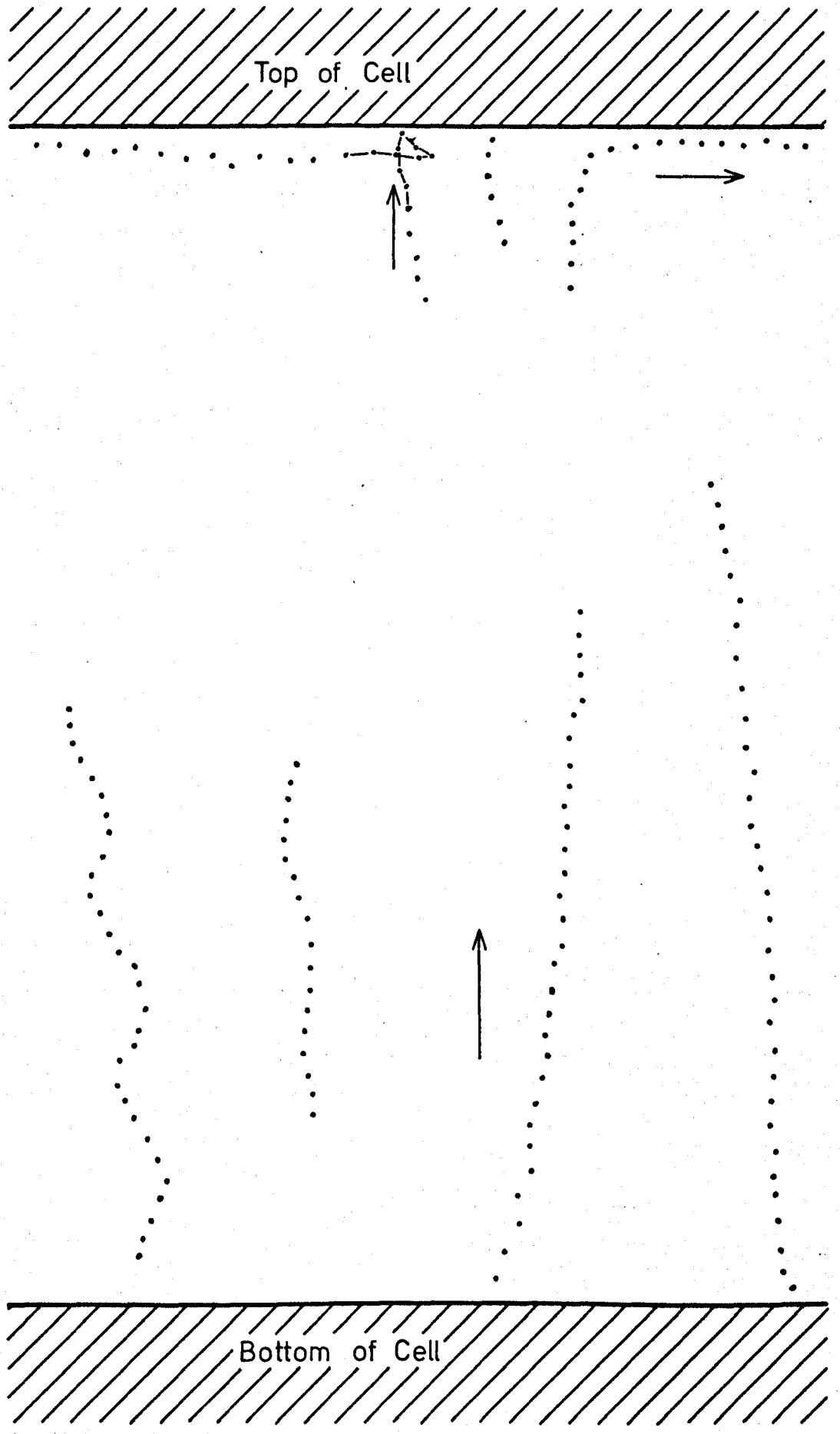

Top of Cell
Bottom of Cell

TABLE 2.3.C

The speed of cercariae of different ages directly after the application of a shadow stimulus.

	hour	n	mean	sd	P_1	P_F
top	unstim hour 1	81	0.91	0.18		
	1	368	0.89	0.19	>.1	>.05
	3	192	0.81	0.18		
bottom	unstim hour 3	109	0.98	0.21		
	3	121	1.01	0.22	>.1	>.05
	5	227	1.00	0.15		
	7	281	0.77	0.17		
	9	325	0.89	0.18		
	11	222	0.83	0.15		
			mm/s			

For both tables:-

P_0, P_1 and P_F see Table 2.2.G.

TABLE 2.3.D

The direction of cercariae of different ages at the bottom of the test cell and directly after the application of a shadow stimulus.

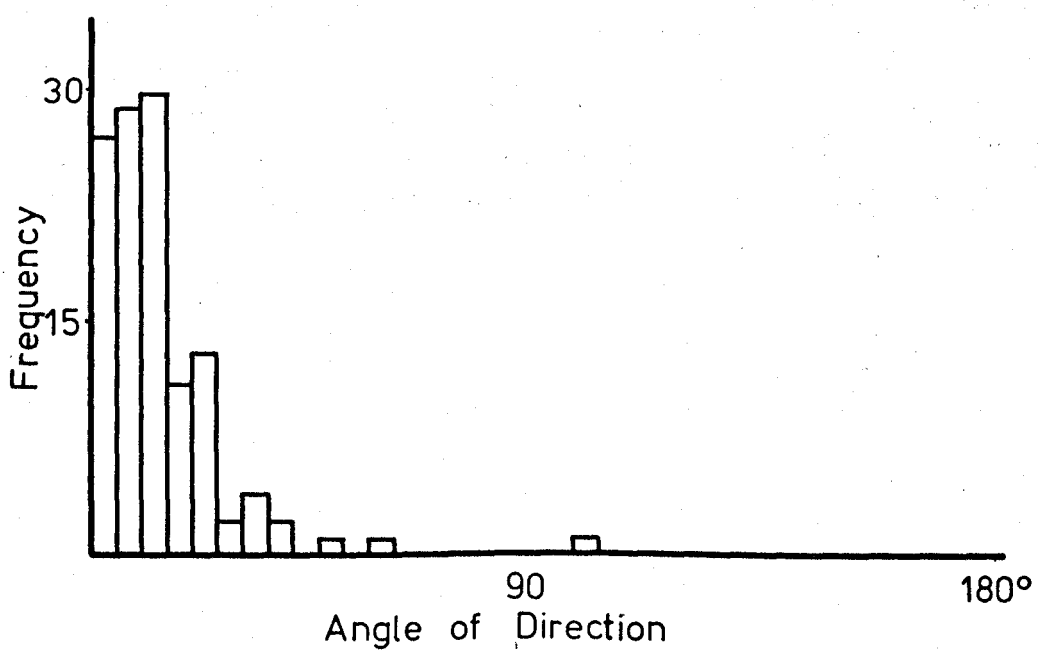
hour	n	mean	se	mean of left and right	se	P_0	P_1	P_F
unstim hour 3	109	10.08	0.80	0.10	1.26	>.1		
3	121	13.17	1.15	-5.38	1.59	<.001	<.01 >.001	<.05
5	227	14.52	0.88	6.23	1.24	<.001		
7	281	18.52	1.62	-8.09	1.90	<.001		
9	325	14.09	0.67	1.95	1.03	>.1		
11	222	13.83	0.84	-0.89	1.25	>.1		
		degs		degs				

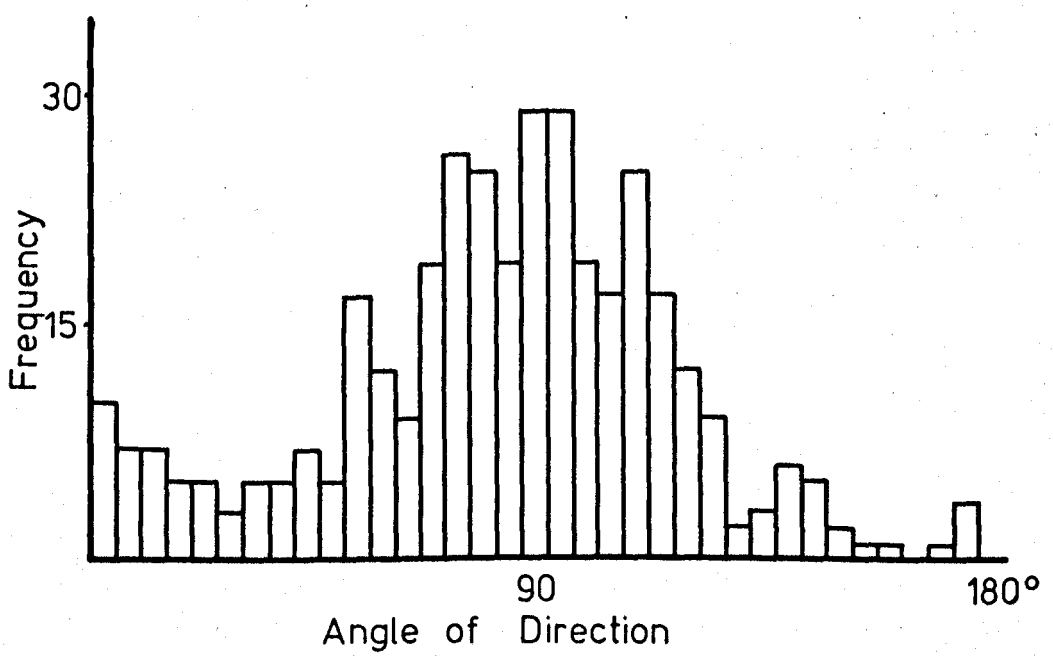
FIGURE 2.3.7

The frequency histogram of the angles of direction shown by cercariae swimming at the bottom of the test cell three hours after shedding and directly after the application of a shadow stimulus.

FIGURE 2.3.8

The frequency histogram of the angles of direction shown by cercariae swimming at the top of the test cell one hour after shedding and directly after the application of a shadow stimulus.


30
15
Frequency
90
180°
Angle of Direction


30
15
Frequency
90
180°
Angle of Direction

appear to occur, the direction of swimming was still basically upwards.

At the top of the cell, the swimming was not only directed upwards but also parallel to and away from the surface. The frequency histogram of the direction at the top of the cell is shown in Fig. 2.3.8.

Angularity of swimming tracks.

Frequency histograms of the angle turned between points on the track are shown in Fig. 2.3.9 for cercariae at the top of the cell one hour after shedding and in Fig. 2.3.10 for cercariae at the bottom three hours after shedding. All distributions were normalised by resolving into their left and right components. The results are shown in Table 2.3.E. It would appear that there was a significant increase in the "spread" of the normalised angle distributions at the top of the cell after the application of the stimulus. This implies that the usual angle turned is also greater, as is indicated by the mean of the non-normal angle distributions. There was little difference in the angles turned before and after the stimulus was applied at the bottom of the cell.

String lengths of swimming cercariae.

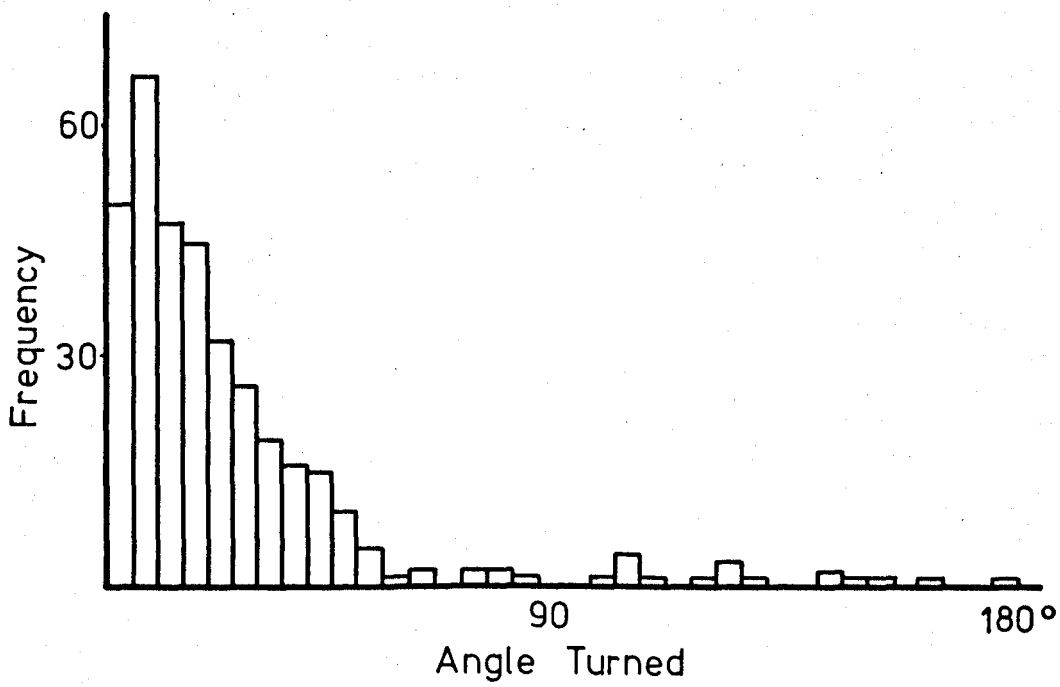
Frequency histograms of the string length are shown in Fig. 2.3.11 for cercariae at the top of the cell one hour after shedding and in Fig. 2.3.12 for cercariae at the bottom three hours after shedding. The distributions were normalised by resolving into their left and right components. The results are shown in Table 2.3.F. There was no significant difference in the alternation of left and right turns after the application of the shadow stimulus.

FIGURE 2.3.9

The frequency histogram of the angles turned by cercariae swimming at the top of the cell one hour after shedding and directly after the application of a shadow stimulus.

FIGURE 2.3.10

The frequency histogram of the angles turned by cercariae swimming at the bottom of the test cell three hours after shedding and directly after the application of a shadow stimulus.


60
30
Frequency
90
180°
Angle Turned

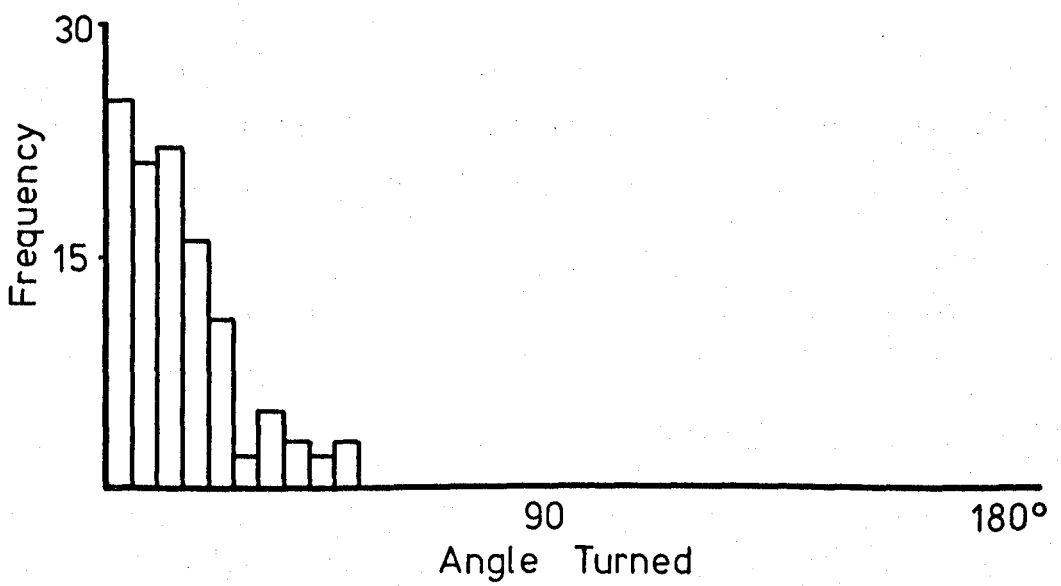

30
15
Frequency
90
180°
Angle Turned

TABLE 2.3.E

The angularity of cercariae swimming directly after the application of a shadow stimulus.

	hour	n	mean	se	mean of left and right	se	P_0	P_1	P_F
top	unstim hour 1	70	17.24	1.25	0.44	2.56	>.1		
	1	358	24.42	1.47	-1.66	1.95	>.1	>.1	<.05
	3	181	23.19	1.62	4.32	2.35	<.05 >.01		
bottom	unstim hour 3	99	13.61	1.00	1.32	1.71	>.1		
	3	110	13.90	1.08	-0.28	1.72	>.1	>.1	>.05
	5	216	13.21	0.69	0.20	1.34	>.1		
	7	271	17.21	0.90	-0.49	1.38	>.1		
	9	315	14.32	0.65	-0.18	1.04	>.1		
	11	212	14.35	0.87	0.09	1.31	>.1		
			degs		degs				

TABLE 2.3.F

The string lengths of the tracks of cercariae swimming directly after the application of a shadow stimulus.

	hour	n	mean	sd	mean of left and right	sd	P_0	P_1	P_F
top	unstim hour 1	48	1.42	0.83	-0.08	1.80	>.1		
	1	198	1.81	0.98	0.10	2.11	>.1	>.1	>.05
	3	105	1.70	1.13	0.13	2.05	>.1		
bottom	unstim hour 3	71	1.39	0.76	0.11	1.60	>.1		
	3	74	1.49	0.69	-0.05	1.63	>.1	>.1	>.05
	5	140	1.54	0.71	0.03	1.66	>.1		
	7	153	1.77	0.87	-0.05	1.98	>.1		
	9	196	1.61	0.84	0.05	1.82	>.1		
	11	139	1.53	0.83	-0.04	1.77	>.1		

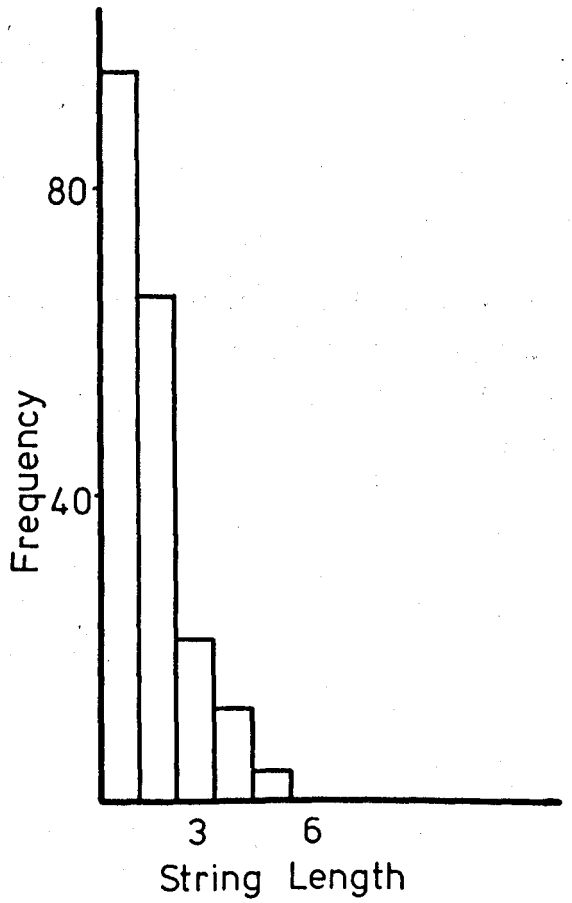
For P_0, P_1 and P_F see Tables 2.2.A and 2.2.C

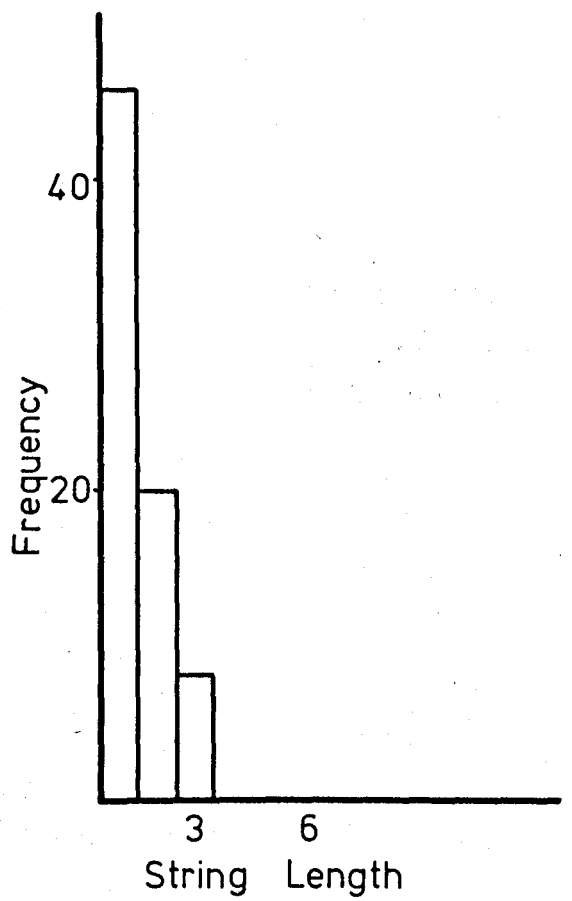
FIGURE 2.3.11

The frequency histogram of the string lengths of the angles turned by cercariae swimming at the top of the test cell one hour after shedding and directly after the application of a shadow stimulus.

FIGURE 2.3.12

The frequency histogram of the string lengths of the angles turned by cercariae swimmming at the bottom of the test cell three hours after shedding and directly after the application of a shadow stimulus.


80
40
Frequency
3
6
String Length


40
20
Frequency
3
6
String Length

2.3.4 SUMMARY OF SECTION 2.3

1. The number of cercariae suspended in the cell increased after the application of a shadow stimulus. This response was observed in cercariae of all ages.

2. The number of cercariae in the top section of the cell increased after the stimulus was applied. One hour after shedding, the peak in numbers at the top was found 0.5 minutes after the application of the stimulus. Eleven hours after shedding, this peak was reached 5 minutes after the stimulus.

3. The migration of cercariae upwards in the cell was coupled with a dramatic increase in activity and a lengthened period of swimming.

4. The stimulus did not greatly affect the speed of swimming cercariae, either at the top or bottom of the cell.

5. The direction of swimming was not greatly affected by the stimulus for cercariae at the bottom of the cell. Many of the cercariae at the top of the cell however, no longer stopped swimming upon contact with the surface, but continued to swim parallel to and away from the top of the cell.

2.4 RESPONSE TO RAPID MIXING - EXPERIMENTS AND RESULTS.

A man wading through water will cause several types of mechanical stimulation which might be sensed by cercariae. Firstly, he will create currents or turbulence in the water. Secondly, direct stimulation might result from physical contact with the man himself or from the turbulence forcing the cercariae to collide with objects in the water. Thirdly, vibrations might be set up in the water. Of these stimuli, turbulence and perhaps direct contact are the most important as far as a host-location mechanism is concerned. Only two reports of the reactions of cercariae to either direct contact stimuli or turbulence could be found (Miller and Mahaffy 1930, Haas 1969). In both cases, only direct stimulation of an individual cercaria was used and it was assumed that cercariae would respond in a similar way to turbulence or water currents.

I have found that pipetting cercariae rapidly into a test cell will provide a combined stimulus of both turbulence and direct contact of cercariae with the walls of the cell. It should be noted that this procedure will also probably involve rapid changes in light intensity. Due to the large internal surface area of the cell, the currents induced rapidly die away. However, photographs of the cell cannot be taken until about 15 seconds after the cell is filled. It is this rapid mixing stimulus that was chosen as a good representation of the turbulence and direct stimulation that might be caused by the host. Other methods used in this section are the same as those developed in section 2.2 for similar experiments. Cercariae were used one hour after shedding for all the experiments in this section.

Time did not permit a study of this response as the cercariae aged. Such a study could be the subject of future work.

2.4.1 CHANGE IN THE DISTRIBUTION OF CERCARIAE AFTER RAPID MIXING

Photographs of the entire cell were taken at 15, 30, 45, 75, 195 and 315 seconds after the cell was filled with cercariae. The distribution of cercariae was determined from these photographs and the results are shown in Fig. 2.4.1. Assuming a random distribution of cercariae initially, there was a rapid migration of cercariae upwards in the cell. The distribution of cercariae had returned to the distribution displayed by cercariae in section 2.2.1 about 5 minutes after the cell was filled.

2.4.2 ACTIVITY PATTERN OF CERCARIAE AFTER RAPID MIXING.

Duration of swim and sink periods

The duration of the swim and sink periods of ten individuals was measured using the event recorder. The swimming period showed a dramatic increase in duration directly after the cell was filled. The length of the swimming period then slowly returned to basal levels. The duration of the response varied from individual to individual but each showed the huge increase in the swimming period. The successive swim periods of a typical individual are shown in Fig. 2.4.2. (see Appendix 4 for complete results).

The sinking periods of the cercariae showed no difference from unstimulated cercariae and remained relatively constant throughout the experiment.

Percentage activity of cercariae after rapid mixing.

The behaviour of cercariae at the top of the cell directly

FIGURE 2.4.1

The distribution of cercariae in the test cell after rapid mixing.

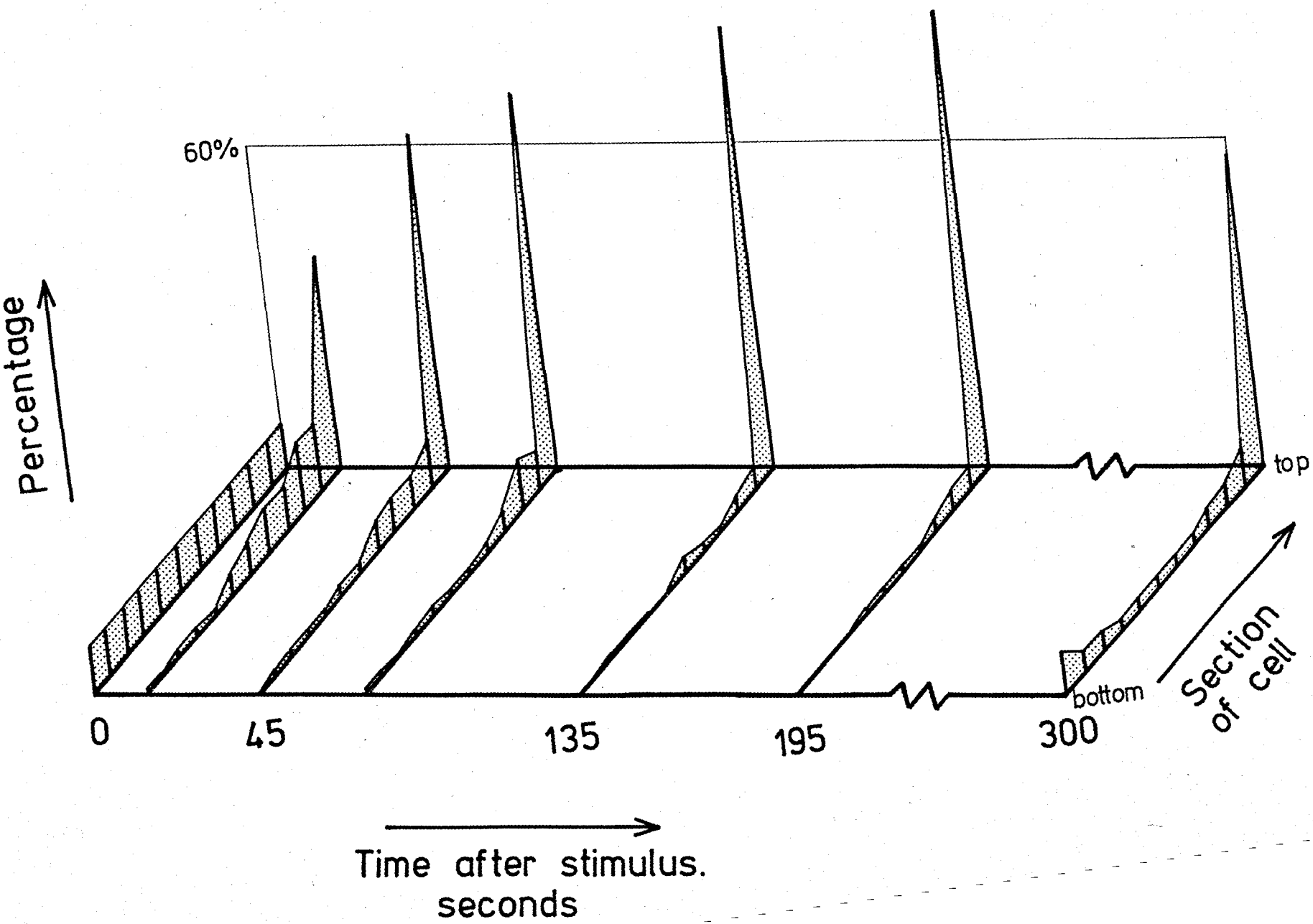
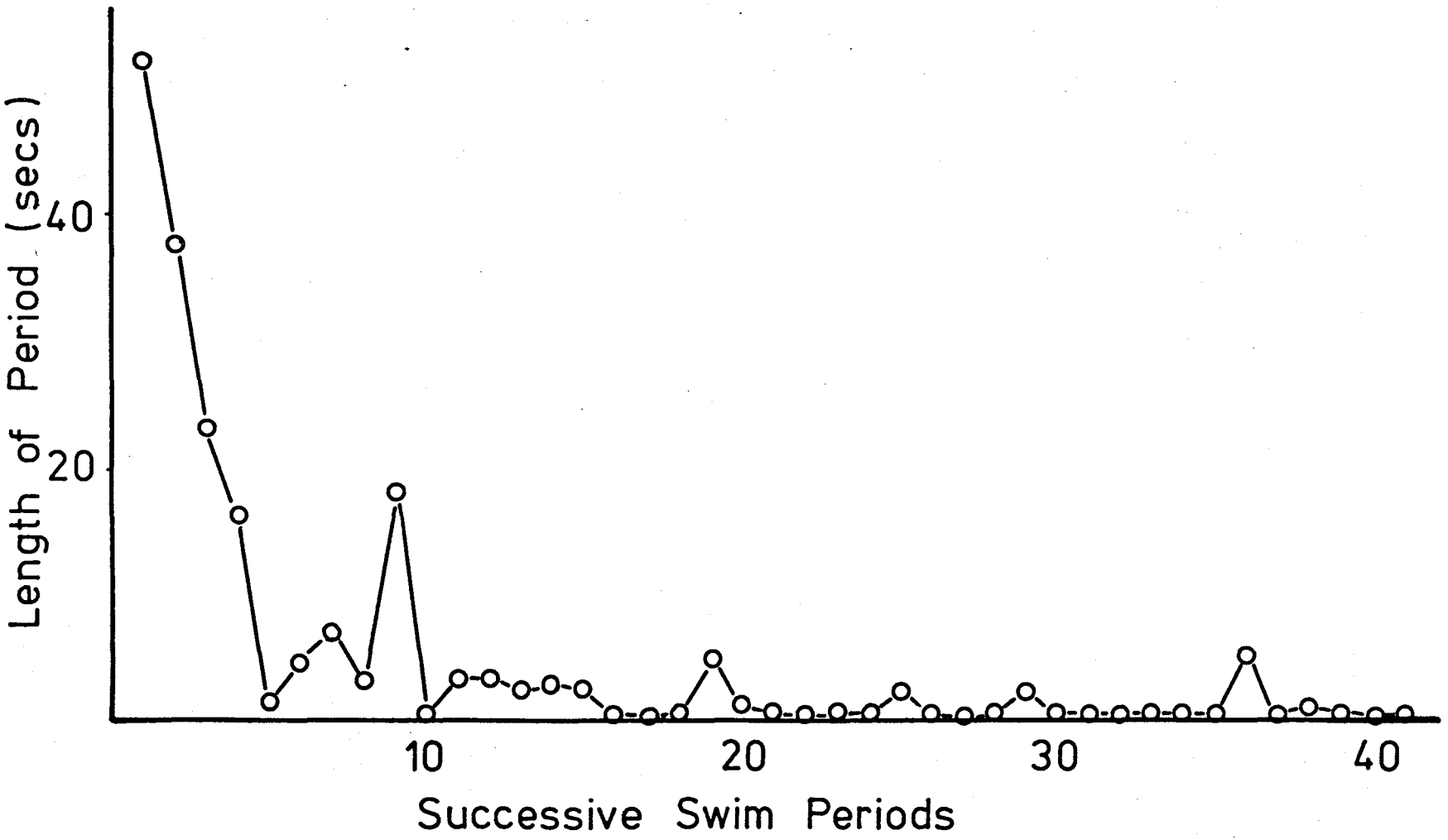

60%
Percentage
top
bottom
Section of cell
0
45
135
195
300
Time after stimulus.
seconds

FIGURE 2.4.2

The successive swimming periods of a typical cercaria directly after being pipetted into the test cell.


Length of Period (secs)
40
20
10
20
30
40
Successive Swim Periods

after filling, was recorded on cine film. The percentage activity was determined directly from this film at 5 second intervals. The results are shown in Fig. 2.4.3. The activity of the cercariae is a factor of three greater after rapid mixing than when the cercariae are unstimulated. This increased level of activity slowly returned to basal levels.

2.4.3 TRACKS OF SWIMMING ACTIVITY AFTER RAPID MIXING.

The tracks of the first swim period after the cell was filled with cercariae were produced from the films exposed in the previous section and were subjected to track analysis along with control, unstimulated tracks. Sample tracks of cercariae swimming after the application of the rapid mixing stimulus are shown in Fig. 2.4.4.

Speed of swimming after rapid mixing.

The frequency histogram of the speed of swimming was found to be normally distributed about a mean of 0.81mm per second with a standard error of $\pm$ 0.13 (n=333). This speed of swimming was not significantly different at the 5% level from the mean speed of cercariae swimming 10 minutes after filling the cell.

Direction of swimming after rapid mixing.

The frequency histogram for the direction of swimming is shown in Fig. 2.4.5. The swimming is directed predominantly upwards as is typical of unstimulated cercariae.

Angularity of swimming after rapid mixing.

The frequency histogram of the angle turned after rapid mixing is shown in Fig. 2.4.6. This distribution was

FIGURE 2.4.3

The percentage activity of cercariae in the test cell directly after rapid mixing.

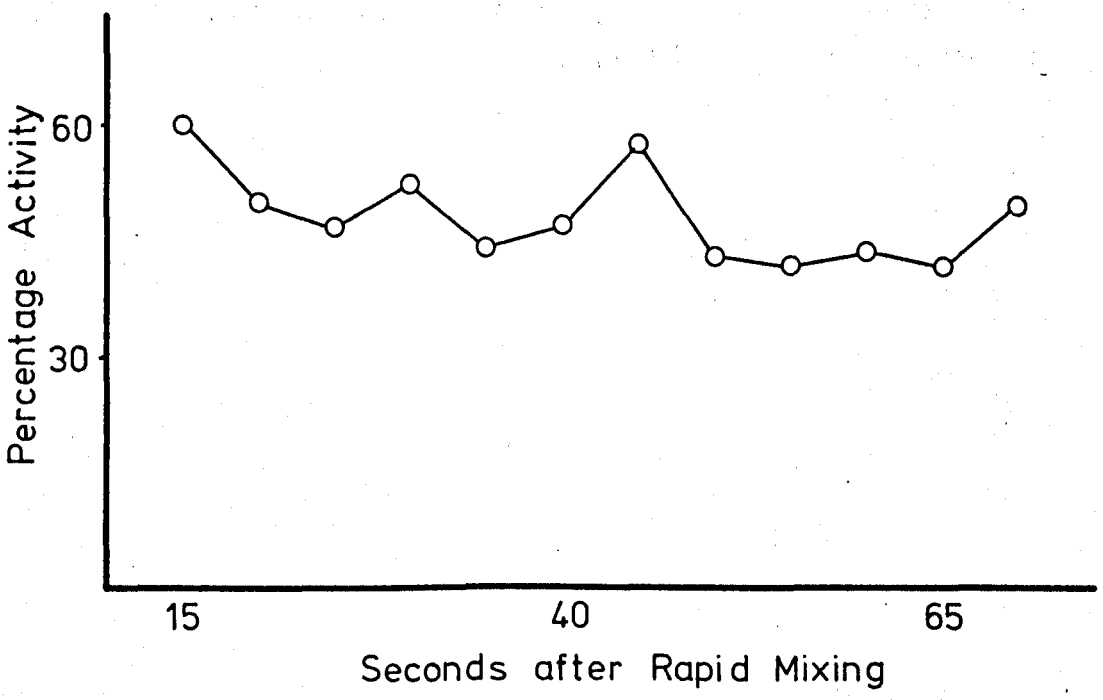

Percentage Activity
60
30
15
40
65
Seconds after Rapid Mixing

FIGURE 2.4.4

Sample tracks of cercariae swimming in the test cell directly after rapid mixing.

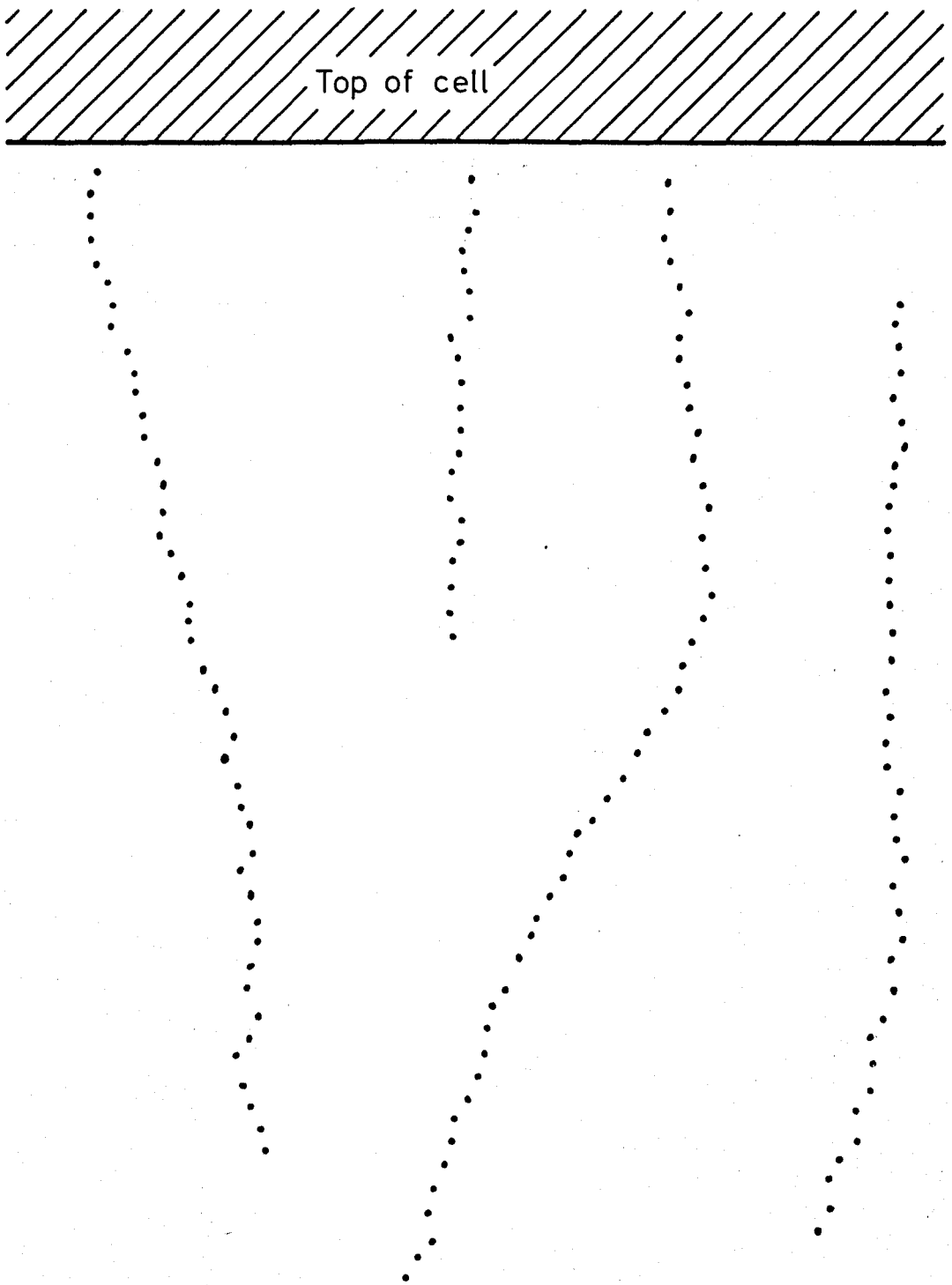
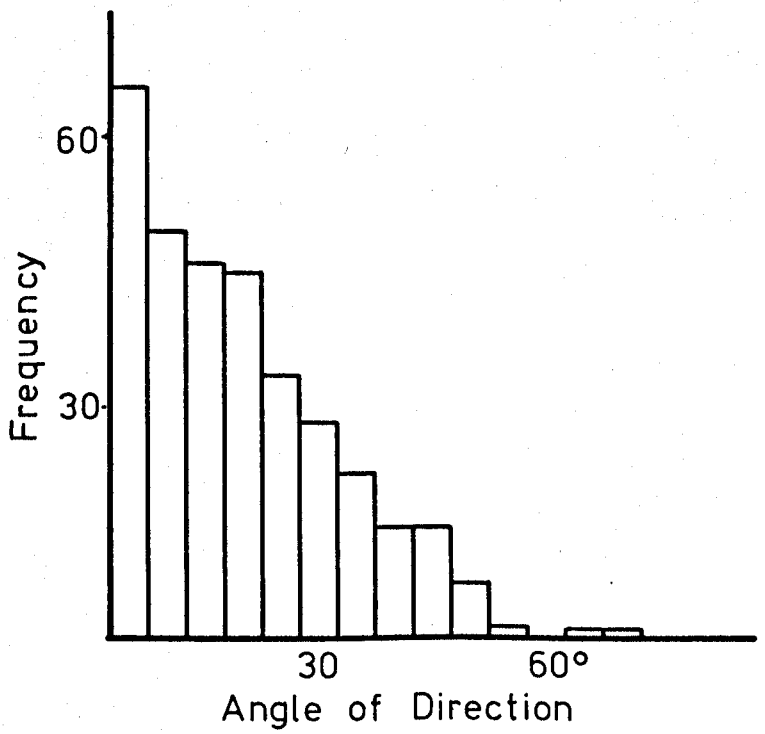

Top of cell

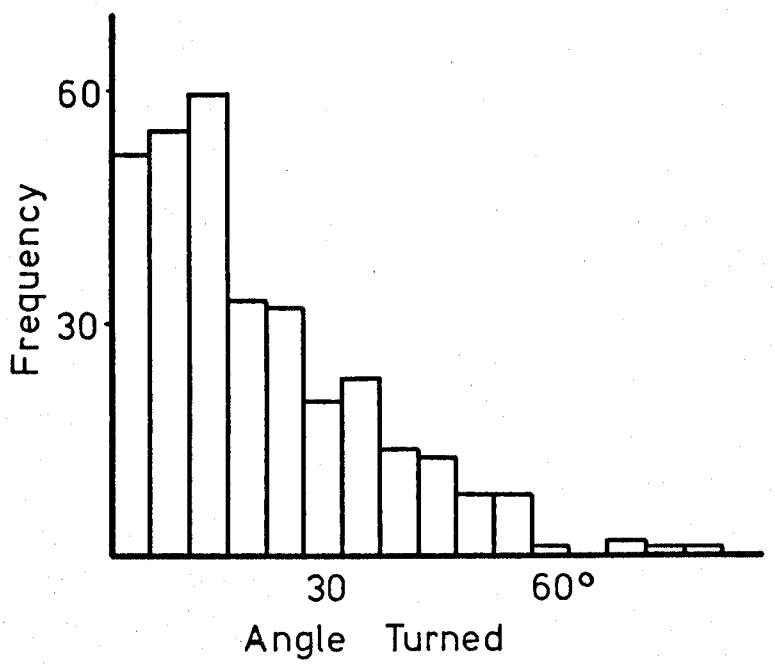
FIGURE 2.4.5

The frequency histogram of the direction of cercariae swimming in the test cell directly after rapid mixing.

FIGURE 2.4.6

The frequency histogram of the angles turned by cercariae swimming in the test cell directly after rapid mixing.


60
30
Frequency
30
60°
Angle of Direction


60
30
Frequency
30
60°
Angle Turned

normalised by resolving into left and right angles. The results are shown in Table 2.4.A. The pattern of angles turned after rapid mixing is typical of unstimulated cercariae swimming at the top of the cell.

String lengths of cercariae swimming after rapid mixing.

The frequency histogram of string lengths is shown in Fig. 2.4.7. This distribution was normalised by resolving into left and right string lengths. The results are shown in Table 2.4.B. There was no significant difference observed at the 5% level between cercariae swimming directly after and 10 minutes after rapid mixing.

TABLE 2.4.A

The angularity of cercariae swimming directly after rapid mixing.

n	mean	se	mean of left and right	se	P_0
323	18.76	0.83	-0.38	1.30	>.1
	degs		degs		

TABLE 2.4.B

n	mean	se	mean of left and right	se	P_0
202	1.56	0.05	-0.06	0.13	>.1

The string lengths of cercariae swimming in the test cell directly after rapid mixing.

For both tables:-

P_0 - the probability that the difference of the resolved mean from zero would occur by chance. ("d_o" test).

FIGURE 2.4.7

The frequency histogram of the string lengths of the angles turned by cercariae swimming directly after rapid mixing.


100
50
Frequency
3
6
String Length

2.4.4 SUMMARY OF SECTION 2.4

1. There was a rapid migration of 1 hour old cercariae upwards in the test cell in response to rapid mixing. Three minutes after the application of the stimulus, over 80% of the cercariae could be found within the top half centimetre of the cell. The distribution had returned to normal levels within 5 minutes of filling the cell.

2. There was a great increase in the duration of the swimming period directly after the cell was filled. The length of the swimming periods then slowly returned to basal levels. The duration of the sinking periods was unaffected by the stimulus.

3. The percentage of the cercariae swimming was also increased by the stimulus. The level of activity was a factor of three greater than for unstimulated cercariae.

4. The speed, directionality, angularity and string lengths of the swimming tracks were unaffected by the rapid mixing stimulus.

2.5 RESPONSE TO SKIN SURFACE CHEMICALS - EXPERIMENTS AND RESULTS

Most of the reports on the response of cercariae to skin surface chemicals have been concerned with the penetration activity. Usually when the swimming response has been observed, it has either simply been reported or types of orientation behaviour have been ascribed to the response without a detailed analysis of the behaviour patterns being undertaken (Bolwig 1955, Shiff *et al* 1972). Haas (1976) has made a detailed study of the swimming and fixation reactions of *S.mansoni* cercariae close to host substrates and he described changes in behaviour which included brief spells of head-first swimming and a reaction which directly led to fixation or attachment.

There is some controversy about the actual nature of the chemical that stimulates penetration behaviour (MacInnis 1969, Austin *et al* 1971, Stirewalt 1971, Shiff *et al* 1972, Nuttman 1975, etc). Most of these workers however, suggest that skin surface lipids of some type, are probably the stimulant. It would be reasonable to assume that the chemical involved in the penetration response is the same chemical that would be remotely sensed by the free-swimming cercariae. This assumption obviously cannot be verified until the precise nature of the stimulant has been discovered.

In this section, I have not attempted to identify the stimulant chemical, but I have simply used crude extracts of human skin surface chemicals and live host tissue as general chemical stimuli. The human skin surface chemical extract I have called Human Conditioned Water (HCW). HCW was prepared by swabbing a human forearm with cotton wool

soaked in water, squeezing out the cotton wool and filtering. The cercariae showed no response to a control filtrate produced in the same way but without swabbing the forearm. To study the response of cercariae to HCW, the test cell was half filled with the extract and a concentrated suspension of cercariae rapidly pipetted in, so ensuring a good mix.

The live tissue used for a second set of experiments was the tail of an anaesthetised mouse. A special fused glass cell was produced by Optiglass Ltd., which enabled the mouse tail to be pushed vertically into it at one side. The mouse was held in a plastic restrainer shown in Plate 6. The behaviour of the cercariae swimming close to the tail could thus be observed through a binocular microscope or recorded onto cine film.

Time did not permit a study of the reactions to host skin surface chemicals while the cercariae aged. Such a study could be the subject of future work.

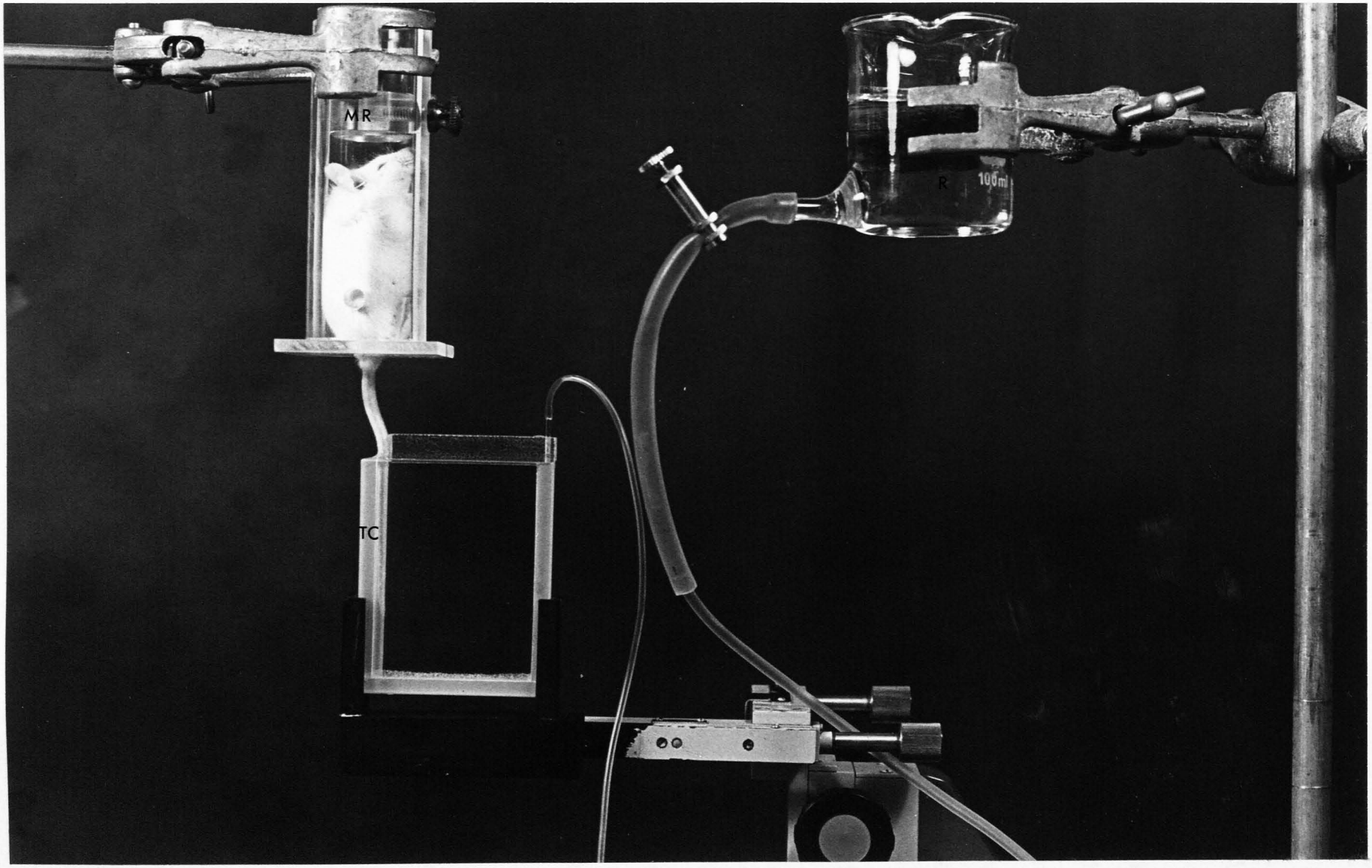
PLATE 6

The arrangement of the test cell for the photographic recording of cercariae swimming close to a mouse tail.

MR Mouse restrainer and anaesthetised mouse

TC Test cell

R Reservoir and siphon


MR
TC
R
100ml

2.5.1 ACTIVITY PATTERN OF CERCARIAE IN RESPONSE TO SKIN SURFACE CHEMICALS.

Duration of swim and sink periods

The duration of swim and sink periods was measured with an event recorder for ten individuals immediately after filling the cell containing a mouse tail and also 10 minutes later. In addition, the duration of the first swim and sink period after filling a cell containing HCW was recorded on cine film and the tracks of ten individuals analysed. The results are shown in Table 2.5.A. It can be seen that the duration of the swim period was dramatically increased by the presence of skin surface chemicals and that the stimulus of rapid mixing enhanced the response.

Percentage activity of cercariae after filling the cell containing HCW.

The percentage activity was measured directly from a cine film taken directly after the cell containing HCW was filled with cercariae. The results are shown in Fig. 2.5.1a. Initially, all the cercariae in the field of view at the top of the cell were found to be swimming. This high level of activity slowly subsided, although even 5 minutes after filling the cell, 35.6% of the cercariae were still actively swimming.

TABLE 2.5.A

The duration of swim and sink periods of cercariae responding to skin surface chemicals.

	mean swim	se	mean sink	se
First period after filling the cell containing HCW	n=10 12.3	30.27	n= 8 2.60	1.06
First period after filling the cell containing a mouse tail	n=10 34.6	8.35	9.78	4.40
Periods ten mins after filling the cell containing a mouse tail	n=86 5.8	0.76	5.28	0.82
	secs		secs	

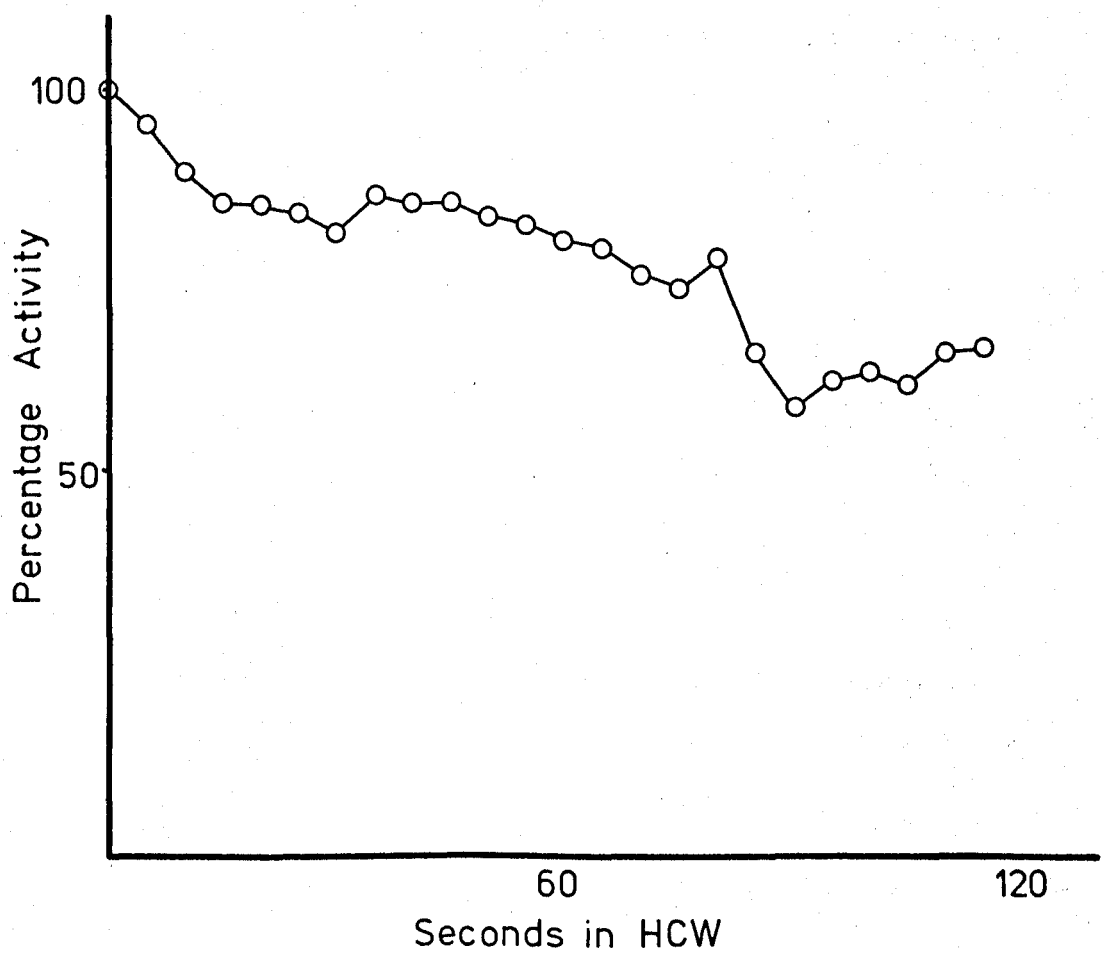
TABLE 2.5.B

The speed of swimming of cercariae responding to skin surface chemicals.

Stimulus	n	mean	sd
Mouse tail	382	1.00	0.20
HCW	526	1.14	0.23
HCW after 10 minutes	286	1.10	0.17
Unstimulated	81	0.91	0.18
		mm/s	

FIGURE 2.5.1a

The percentage activity of cercariae swimming in a test cell containing HCW directly after the larvae were pipetted into the cell.


100
50
Percentage Activity
60
120
Seconds in HCW

2.5.2. TRACKS OF SWIMMING ACTIVITY.

Films were taken of cercariae swimming within one centimetre of a mouse tail positioned vertically in the cell. The distance of one centimetre was chosen simply because it was only within this distance that a change in the swimming pattern of the cercariae could easily be observed. Additional films were taken of cercariae swimming in HCW, directly after the cell was filled and also 10 minutes later. Sample tracks of cercariae swimming in HCW are shown in Fig. 2.5.1b. These films were subjected to the track analysis described in a previous section.

Speed of swimming.

The frequency histograms of the speed of swimming were found to be normally distributed about their means. The results are shown in Table 2.5.B. There appears to be a slight but significant increase in the speed of swimming shown by cercariae responding to skin surface chemicals.

Direction of swimming.

The frequency histogram of the direction of cercariae swimming close to a mouse tail is shown in Fig. 2.5.2. Fig. 2.5.3 shows the directionality of cercariae swimming in HCW immediately after the cell was filled and Fig. 2.5.4 shows the directionality of another sample of the same cercarial population 10 minutes later.

Cercariae close to a mouse tail and those in HCW with rapid mixing swam in all directions. Ten minutes later in HCW, the cercariae were swimming more normally (ie. upwards), although a few were still swimming in other directions. Many of the cercariae showed reversals in the mode of swimming, ie.

FIGURE 2.5.1 b

Sample tracks of cercariae swimming in HCW directly after the cell was filled.

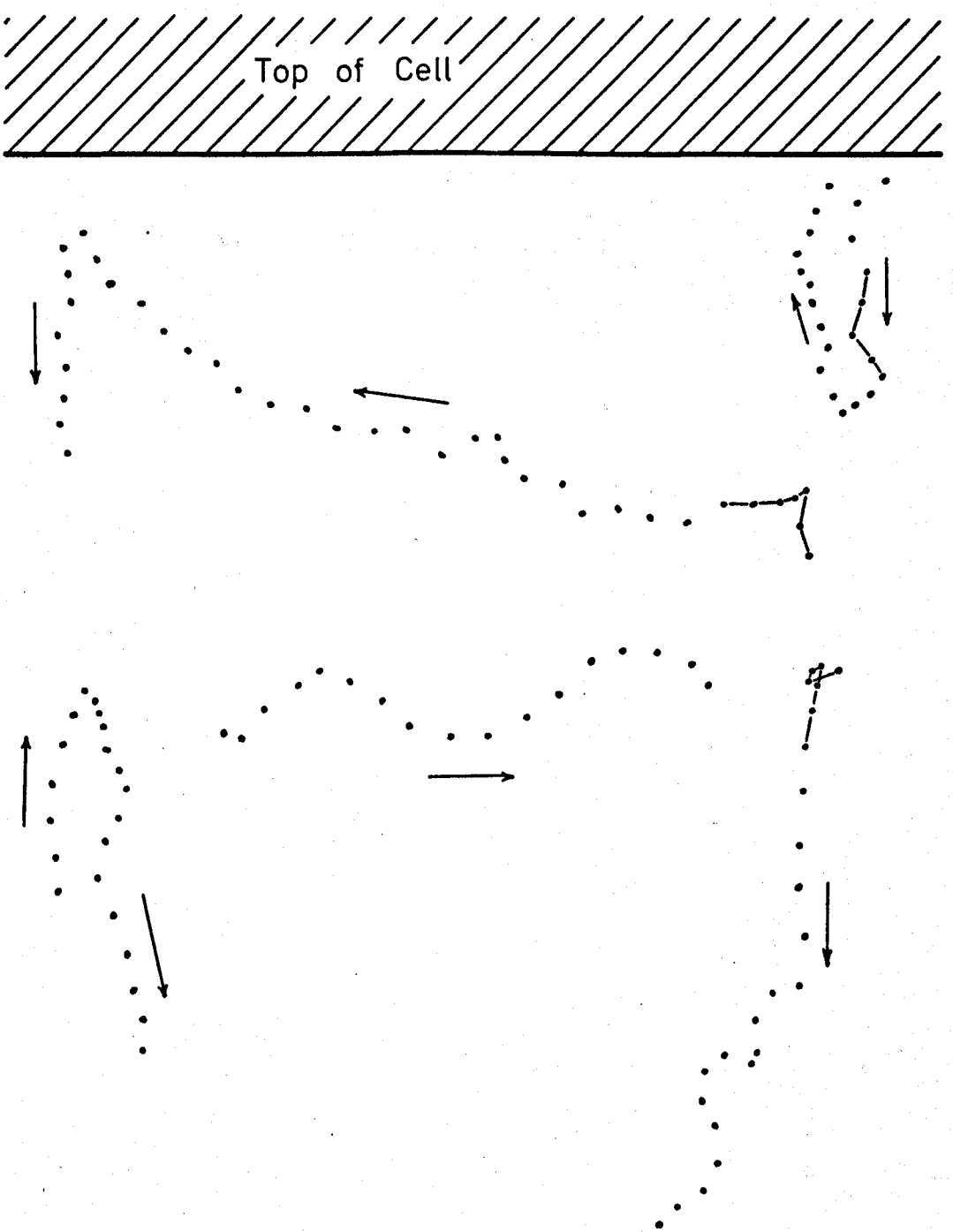
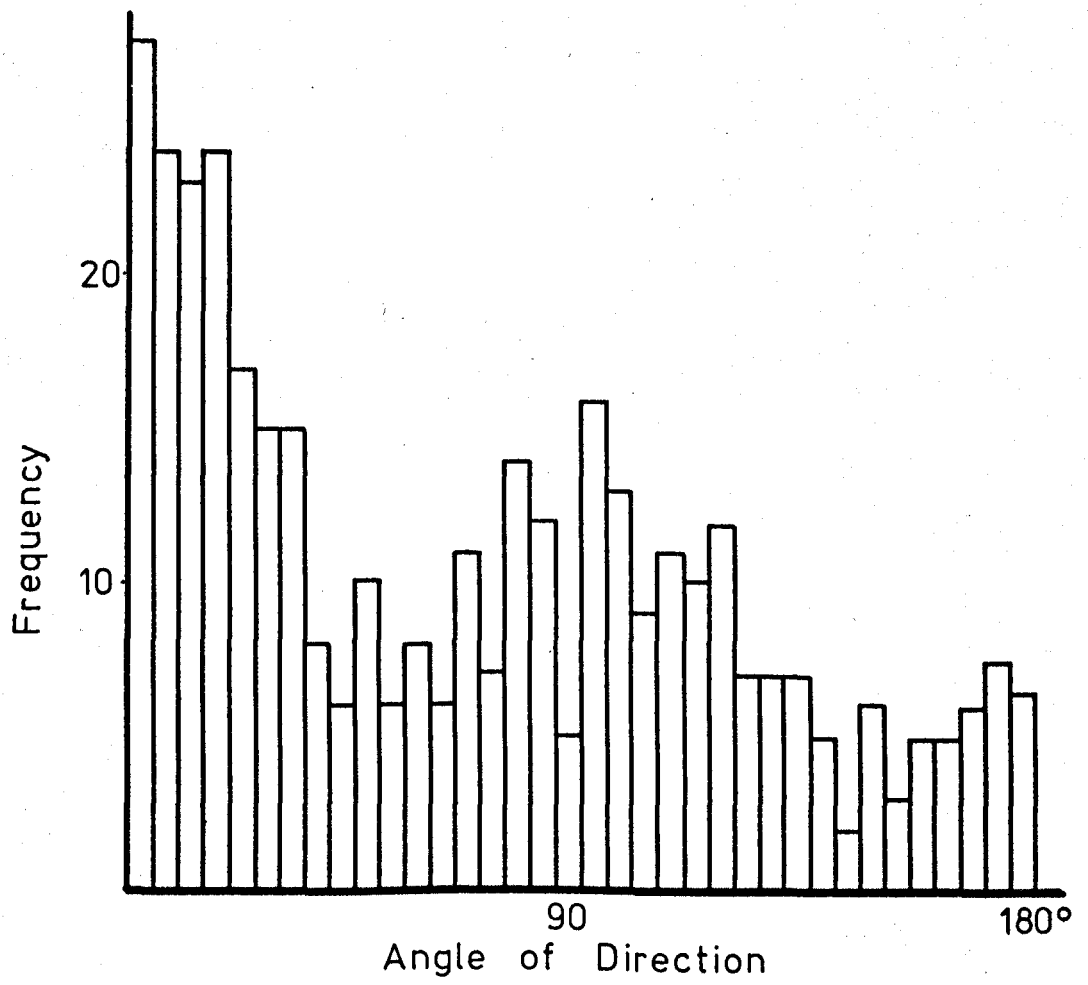

Top of Cell

FIGURE 2.5.2

The frequency histogram of the directionality of cercariae swimming within one centimetre of a mouse tail.

FIGURE 2.5.3

The frequency histogram of the directionality of cercariae swimming in HCW directly after the cell was filled with larvae.


20
10
Frequency
90
180°
Angle of Direction

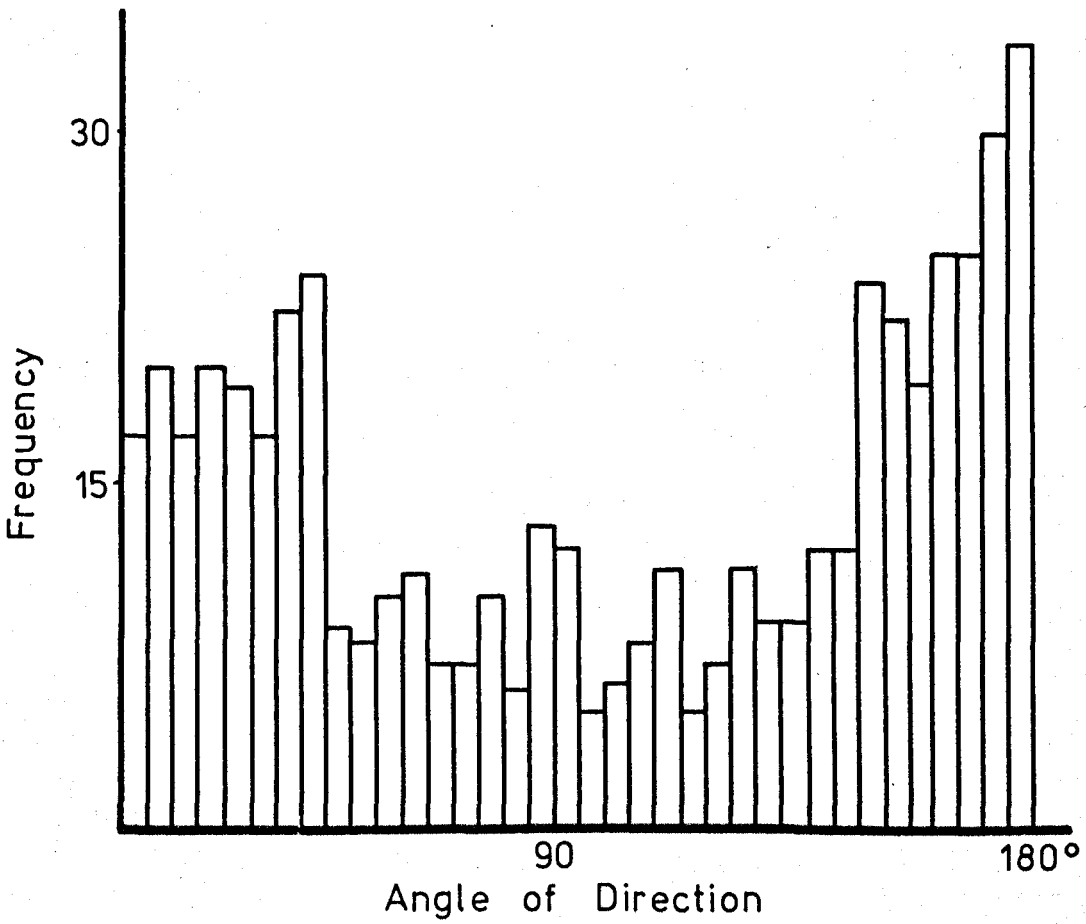
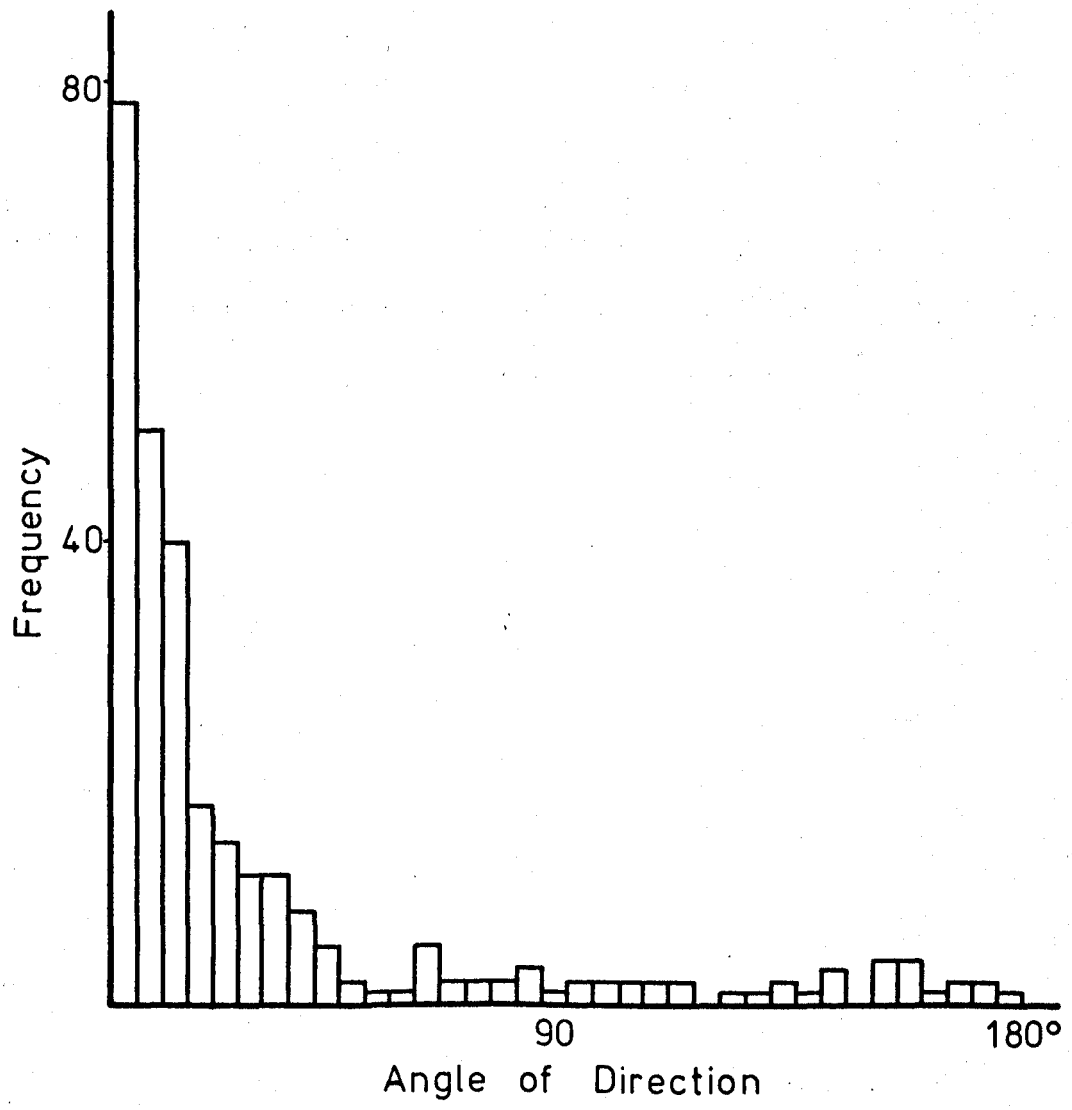

30
15
Frequency
90
180°
Angle of Direction

FIGURE 2.5.4

The frequency histogram of the directionality of cercariae swimming in HCW, 10 minutes after the cell was filled with larvae.


80
40
Frequency
90
180°
Angle of Direction

the normal tail-first swimming became briefly head-first. A few of the cercariae were also observed to attach briefly to the glass walls of the test cell.

Angularity of swimming.

The frequency histograms of the angle turned were normalised by resolving into their left and right components. The results are shown in Table 2.5.C. The results suggest that the cercariae swimming close to a mouse tail, and in HCW with rapid mixing, turn larger angles than unstimulated cercariae. However, this result might be an artefact of the increased speed of swimming.

String lengths.

The frequency histograms of string length were normalised by resolving into left and right strings. The results are shown in Table 2.5.D. There was no significant difference in the alternation of left and right turns in the presence of the chemical stimulus when compared to unstimulated cercariae.

TABLE 2.5.C

The angularity of cercariae swimming in response to skin surface chemicals.

stimulus	n	mean	se	mean of left and right	se	P_0	P_1	P_F
unstimulated	70	17.24	1.25	0.44	2.56	>.1		
mouse tail	369	30.60	1.66	1.52	2.30	>.1	>.1	<.05
HCW	506	27.60	1.38	0.62	1.85	>.1	>.1	<.05
HCW after 10 minutes	257	17.83	1.22	0.19	1.65	>.1	>.1	<.05
		degs		degs				

For both tables:-

P_0 - the probability that the difference of the resolved mean from zero would occur by chance. ("d_o" test).

P_1 - the probability that the difference between the selected and control means would occur by chance.

P_F - the probability that the ratio of the variances about the selected and control means would occur by chance.

TABLE 2.5.D

The string lengths of the angles turned by cercariae swimming in response to skin surface chemicals.

stimulus	n	mean	sd	mean of left and right	sd	P_0	P_1	P_F
unstimulated	48	1.42	0.83	-0.08	1.80	>.1		
mouse tail	226	1.63	0.90	0.02	1.80	>.1	>.1	>.05
HCW	309	1.64	1.05	-0.02	1.93	>.1	>.1	>.05
HCW after 10 minutes	162	1.59	0.89	0.09	1.78	>.1	>.1	>.05

2.5.3 SUMMARY OF SECTION 2.5

1. The duration of the swimming period of the cercariae was increased in the presence of skin surface chemicals. A rapid mixing stimulus enhanced the effect of the skin surface chemicals.

2. The percentage of the cercariae which were actively swimming was also increased by the stimuli. Even 5 minutes after the application of the stimulus, over 35% of the cercariae were actively swimming.

3. There appeared to be a slight but significant increase in the speed of swimming in the presence of the skin surface chemicals.

4. Cercariae close to a mouse tail, and in HCW directly after the cell was filled, were found to swim in all directions. Ten minutes later, the majority of the cercariae in HCW were once again swimming upwards.

5. There was some indication that the mean angle turned by cercariae close to a mouse tail or in HCW was larger than mean angle turned by unstimulated cercariae.

2.6 DISCUSSION

In this chapter, I have described and measured the activity of the cercaria of Schistosoma mansoni in the presence and absence of selected stimuli.

Immediately after shedding, the majority of the cercariae are to be found near the surface of the experimental vessel, confirming the observations of Stirewalt (1971), Pellegrino and Valle (1974) and Lawson (1977). Near the surface, the initiation of swimming is spontaneous whereas the initiation of sinking appears to be usually due to contact with the surface. For a cercaria to remain near the surface, the distance moved upwards when swimming must, on average, be equal to or greater than the distance moved downwards when sinking. This could be achieved if the capacity of the cercaria to move upwards is always greater than the actual distance moved downwards. Thus, however far the cercaria sinks, the following swimming period would continue upwards until the surface was reached, so exactly cancelling the downward movement. If the sinking period becomes greater, on average, than the distance the cercaria is able to swim upwards, then the larva will be displaced downwards in the cell. This is what appears to happen as the cercariae age. An increase in the mean sinking period was observed while the swimming period remained relatively constant as the cercariae aged. The downward displacement of the cercariae would be further aided by the decrease in the speed of swimming that was observed as the cercariae aged.

At the bottom of the cell, the initiation of swimming is usually due to contact with the substrate while the

initiation of sinking is spontaneous. In this case, I suggest that the capacity of the cercaria to sink downwards now exceeds the distance moved upwards by swimming. This situation is supported by the observation that older cercariae spend increasingly longer periods of inactivity on the bottom of the test cell (see also Lawson 1977).

Because the vertical distances moved upwards by swimming cercariae are usually cancelled by the distances moved downwards, the percentage activity of the cercarial population should be dependent only on the ratio of the swimming speed to the sum of the sinking and swimming speeds. Thus cercariae swimming four times faster than they sink will show a 20 percent level of swimming activity. It should be noted that the speed of swimming, as measured by track analysis, is the speed of swimming along the often sinusoidal track followed by the cercariae and not the absolute speed of swimming in an upward direction. Changes in the percentage activity will be observed only if the speed of swimming or sinking changes, if the cercariae are in transition from the top to the bottom, or if periods of inactivity are spent on the bottom of the cell.

The speed of swimming was found to lie within the range of 0.73 to 0.68 mm per second at the top of the cell and between 0.92 and 0.59 mm per second at the bottom of the cell as the cercariae aged. The speed of swimming of the cercariae shortly after shedding is well within the range of values reported by Nuttman (1975) and Lawson (1977) taking into account the different temperatures at which they made their measurements and that they made no distinction between cercariae at the top and bottom of the test cell.

It is interesting to note that the speed and duration of both sinking and swimming periods at the bottom of the cell were greater than for cercariae at the top of the cell (see Fig. 2.2.4 Fig 2.2.5 and Table 2.2.A). The increased duration and speed of swimming could be caused by the initiation of swimming being stimulated by contact with the bottom rather than being spontaneous. An increase in the duration of sinking would follow the increase in the duration and speed of swimming. The increased sinking speed is more difficult to explain but might be due to the change in the posture of the furcae sometimes observed in cercariae near the bottom. The altered position of the furcae could change the hydrodynamics of the sinking cercaria in such a way that the cercariae would sink faster.

The measurement of certain parameters (particularly swimming speed, swimming angularity and swimming string length) showed a greater degree of variation when cercariae at the bottom of the cell were being considered than when compared to cercariae at the top of the cell. Lawson (1977) suggested that the activity of individual cercariae was not related to their true age (time after shedding) but was a function of their physiological age. The physiological age itself was related to the amount of available food resources (ie. the concentration of glycogen). It is quite likely that the cercariae that are found at the bottom of the test cell directly after shedding are "old" in terms of the physiological age. This group of cercariae include cercariae that remain constantly suspended near the bottom of the cell, following swimming periods directly with sinking periods and vice versa, and cercariae which spend periods of

inactivity on the bottom of the cell before swimming upwards. These two groups of cercariae probably have different physiological ages and it is not surprising that a sample of cercariae at the bottom of the cell will show a considerable degree of variation.

The reactions of cercariae to a shadow stimulus which I have reported in this study agree well with the results of Lawson (1977) although she employed very small test cells. In general, three main responses were observed. Firstly, cercariae inactive on the bottom of the test cell became active and swam upwards after the application of the stimulus. Secondly, a migration of cercariae upwards in the cell was observed which appeared to be due to an increase in the time spent swimming. Thirdly, cercariae reaching the surface no longer stopped swimming but continued parallel to or away from the surface. These responses occurred with cercariae of all ages studied. If the normal site of infection is near the surface of a body of water, certainly the case in deep water, then the upward migration of the larvae should greatly increase their chance of contact with the host. Inactivity would not be a useful strategy if a host were near.

The initiation of swimming of the cercariae in response to a rapid mixing stimulus confirms the observations of Miller and Mahaffy (1930) and Haas (1969) who used direct mechanical stimulation. The initiation of swimming and the following increase in the duration of the swimming period resulted in a dramatic upward migration of cercariae in the test cell. Again, in deep water the upward migration of the larvae should increase the chance of contact with the host.

In flowing water however, currents and turbulence will occur that are not at all related to the host. An increase in the level of activity in this case would help to keep the cercariae in the top layers where the flow is greatest and so the dispersion of the parasites would be greatly enhanced, assuming adaptation to the stimulus did not take place. When the turbulence is caused by a potential host, it is possible that other host stimuli present act together with the turbulence in an additive way to increase the chance of contact.

The response of the cercariae to skin surface chemicals was by far the most obvious response when observed through a binocular microscope. The cercariae appeared to swim in a totally random, but highly excited manner with reversals in the mode of swimming and occasional attachment to the walls of the glass cell. These reactions have been observed by many authors. Bolwig (1955) described the forward and backward movement of the cercariae close to objects giving off certain chemicals and suggested that this was a klinokinetic orientation. Shiff et al (1972) simply described the response as "hyperactive" and Haas (1976) reported similar reactions in response to host substrates which led to fixation or attachment. The analysis of the swimming of cercariae responding to skin surface chemicals confirmed that the cercariae swam in all directions. A slight increase in the speed of swimming was also detected. In the light of this analysis it is obvious that the cercariae do not show a directed response to host chemicals (ie. not a taxis) whether the skin surface chemicals were diffuse (HCW) or in the form of a possible gradient (mouse tail). Indeed it

is unlikely that chemical gradients are involved in this response, as in the natural infection situation a potential host would not remain stationary long enough for gradients to be set up that could be remotely sensed by the cercariae. Gradients might be important very close to the host in attachment and penetration responses.

It is not clear if this response will increase the chance of a cercaria contacting its host. Certainly an active cercaria will have a better chance of hitting the host than an inactive one. However, as many cercariae responding to the chemical stimulus will swim away from the host as will swim towards it. The application of simulation modelling techniques in Chapter 3 will help to resolve this dilemma.

The response of the cercariae to skin surface chemicals and to rapid mixing was not studied as the cercariae aged. However, considering the age-related response of cercariae to the shadow stimulus, it is likely that cercariae of all ages will respond to these two other stimuli, although the degree to which they respond may vary.

Some of the results presented in this chapter suggest that the responses of the cercariae to host generated stimuli are additive in effect. The response of the cercariae to skin surface chemicals was enhanced by rapid mixing. Haas (1976) found that the fixation of cercariae to a host substrate was enhanced if the substrate was warmed to a temperature higher than the surrounding medium. It seems reasonable to suppose that the best situation for cercariae to locate a host is when all the specific host stimuli are present and when the responses to these stimuli all act

together.

A hierarchy of these responses to the host generated stimuli can be envisaged. Firstly, the response of the larvae to turbulence will bring them to the surface. Secondly, the response of the cercariae to shadows will also bring them to the surface, but upon reaching it, many of the cercariae continue to swim with an increased lateral component. Lastly, host surface chemicals, perhaps coupled with the other stimuli, further increases the level of activity and the randomness of the direction of swimming. It is in this last phase of the responses that the chance of contact with the host is likely to be the highest. The importance of each response and combined responses in a hierarchy is an example of a problem relatively easily assessed by computer simulation that would be particularly difficult to perform by experimentation.

In general, the response of the cercariae to host stimuli is an increase in the activity supplemented by, in some cases, an increase in the randomness of the direction of swimming. Thus, the strategy of the cercaria in the location of the host would appear to be initially to expend as little energy as possible while remaining in an area likely to be frequented by the host. When the presence of the host is detected, an active but random swimming pattern is initiated until either the host is located or adaptation reduces the degree of the response and returns the cercaria to the energy saving phase.

Why this particular strategy has evolved can be partially explained if the behaviour of the potential host is

considered. Human contact with water occurs during activities such as bathing, clothes and dish washing, fishing, irrigation and agricultural work (Jobin and Ruiz-Tibben 1968, Farooq et al 1966). Thus contact with the water is an unpredictable and often brief event. Movements, which to humans would appear slow or slight, are greatly magnified in relation to the small size of the cercaria. The host would have to be within only a few centimetres of an individual cercaria before contact would become likely or possible. This strategy, where the parasite conserves energy until the host is near and then employs an active searching pattern would appear to be an efficient method of maximising the chance of contact with a human host.

CHAPTER 3

MODELS OF BEHAVIOUR.

3.1 INTRODUCTION

Shubik (1960) wrote that "a simulation of a system or organism is the operation of a model or simulator which is a representation of the system or organism. The model is amenable to manipulations which would be impossible, too expensive or impracticable to perform on the entity it portrays. The operation of the model can be studied and from it, properties concerning the behaviour of the actual system or its sub-systems can be inferred".

This quotation summarises the properties of a computer model. Thus, the purpose of building a model is to combine in a single entity all the factors, or more aptly those factors that are considered important, in such a way that they interact as we believe them to in nature. The construction of a model thus requires a precise analysis of the processes taking place in the system under study. This requirement alone is a good reason for attempting to construct a model.

The model can be run and its predictions checked against the real situation. A model which is a good analogue of the true system can be "taken apart" and the contribution of each of its elements to the whole can be inferred. The failure of a model to closely represent the true system can lead to several lines of investigation. Firstly, additional parameters, originally considered to be of little importance, can be measured and added to the model in an attempt to bring the model's predictions closer to the real situation. Secondly, the model might indicate that the accuracy of the data used is insufficient and lead to a new

regime of experiments being undertaken to better quantify the original parameters. Lastly, the whole or part of the concepts incorporated in the model may be rejected and new hypotheses about the properties of the system formed and tested using new models.

Another feature of computer models is that manipulations on the model can be performed which, for various reasons, cannot be performed easily by experimentation. For example, in the models I will present on the host-location behaviour of the miracidium, the area around the snail, within which the observed change in behaviour occurs, can be easily varied. As the nature of the stimulant chemical within this area is largely unknown, and the diffusion or mixing of such a stimulant would be difficult to control, this area could not easily be altered in a quantifiable way by experimentation.

Simulation modelling is particularly useful in the understanding of how behavioural mechanisms involving random components can act to the adaptive advantage of the organism. In particular the effect of kineses on the distribution of organisms can be studied. The effect of a kinesis is often very difficult to visualise. For example, the observed klinokinesis of miracidia close to a snail, although increasing the time spent by the miracidia in the vicinity of the snail, results in individuals swimming away from the host as often as they swim towards it.

Few modelling studies of invertebrate behaviour have been found in the literature. An outstanding example is the study of the categories of behaviour devised by Fraenkel and Gunn (1961) carried out by Rohlf and Davenport (1969).

They simulated kineses and taxes in a simple way on a computer. The track of the simulated organism consisted of straight lines interrupted by 90 degree changes in direction. The frequency of these changes in direction and the length of the track in between turns were varied according to the response being studied. The results showed that a direct klinokinesis produced no nett displacement but that a direct klinokinesis with sensory adaptation resulted in a displacement of the organism down the gradient. They concluded that "far from increasing the probability of target-finding in organisms which have a positive taxis, a tendancy to make more frequent turns at the higher levels of stimulation decreases the efficiency of target finding". The theoretical study of Patlak (1953) however, does not agree with the results of Rohlf and Davenport. Patlak calculated that organisms responding to a gradient of a stimulant by a klinokinesis would aggregate in the region of highest concentration. It should be noted that these two studies considered organisms responding to gradients. In Chapters 1 and 2, I have shown that it is unlikely that chemical gradients are important in the location of the host by schistosome larvae, but rather that it is the response to areas of relatively uniform stimulation that are used by the larvae.

In this chapter, simulation models of the behavioural responses shown by schistosome larvae in the location of the host are constructed to test whether these responses increase the chance of contact.

3.2 GENERAL METHODS USED IN THE COMPUTER SIMULATION OF BEHAVIOUR

The basic model of behaviour employed to study the host-location mechanisms of the schistosome larvae was constructed to use the data on larval motion produced in Chapters 1 and 2 to regenerate tracks using the computer. As the model uses data from real tracks, the generated tracks should be a close fit to those originally recorded.

The parameters used for these models are :-

i) the distance moved per step

ii) the angle turned

iii) the string length

iv) the duration of the swim and sink periods (cercarial models only).

These are the same parameters as were quantified by track analysis.

For use by the computer, the frequency distributions of the distance moved and the swim and sink periods were converted into accumulated percentage distributions as is shown in Table 3.2.1. The angle turned and the string length were transformed in a similar way into accumulated percentage matrices so that, for example, for every angle turned there is an accumulated percentage distribution of the frequency of the angles which follow. These accumulated percentage distributions allow the "top hat method" to be used to randomly sample the distribution in such a way that the frequency of the selected values follows the original distribution. In the computer, this is achieved by generating a random number between 0 and 1000 and comparing

TABLE 3.2.1

Transformation of cercarial swim period frequency data for use by computer models.

Duration of period (secs)	1	2	3	4	5	6	7	8	9	10
Frequency	91	32	16	5	1	2	1	0	0	2
Percentage frequency	60.7	21.3	10.7	3.3	0.7	1.3	0.7	0.0	0.0	0.0
Accumulated % frequency	60.7	82.0	92.7	96.0	96.7	98.0	98.7	98.7	98.7	100.0
Accumulated % frequency times 10	607	820	927	960	967	980	987	987	987	1000

it with the accumulated percentage frequency (times 10), starting with the smallest value, until a value larger than the random number is detected. The value of the parameter corresponding with this accumulated percentage frequency is then assigned to that parameter in the model.

The tracks are generated in the model by simple trigonometry (see Fig. 3.2.1). Starting with an initial random position (X_1,Y_1), and with an initial random direction, a value for the string length is selected and the direction of the turn is set randomly to either the left or the right. An angle of turn is now selected and if, for example, the turn is to the right, the angle is subtracted from the initial direction to give the angle α. A value for the distance moved is finally selected and the new X and Y coordinates are calculated by the following relationships (see also Fig. 3.2.1)

$$(X_2,Y_2) = (X_1+\Delta X, Y_1+\Delta Y) \quad \text{where } \Delta X = d*\sin(\alpha)$$
$$\text{and } \Delta Y = d*\cos(\alpha)$$

If graph plotter output is being produced a line is drawn between these two points (ie. between (X_1,Y_1) and (X_2,Y_2)). New values of the X and Y coordinates are now calculated in the same way until the number of calculations equals the selected string length. A new string length is selected and the sign of the turn changed so that the selected angles are now added to the angle of direction. A simplified flow diagram of this model is shown in Fig. 3.2.2. In this way, tracks which are analogues of the movements of live schistosome larvae can be produced.

FIGURE 3.2.1.

Calculation of the coordinates of the tracks generated by the model.

α is the angle of direction relative to the top of the 'pond'.
ΔX is the change in the X coordinate.
ΔY is the change in the Y coordinate.

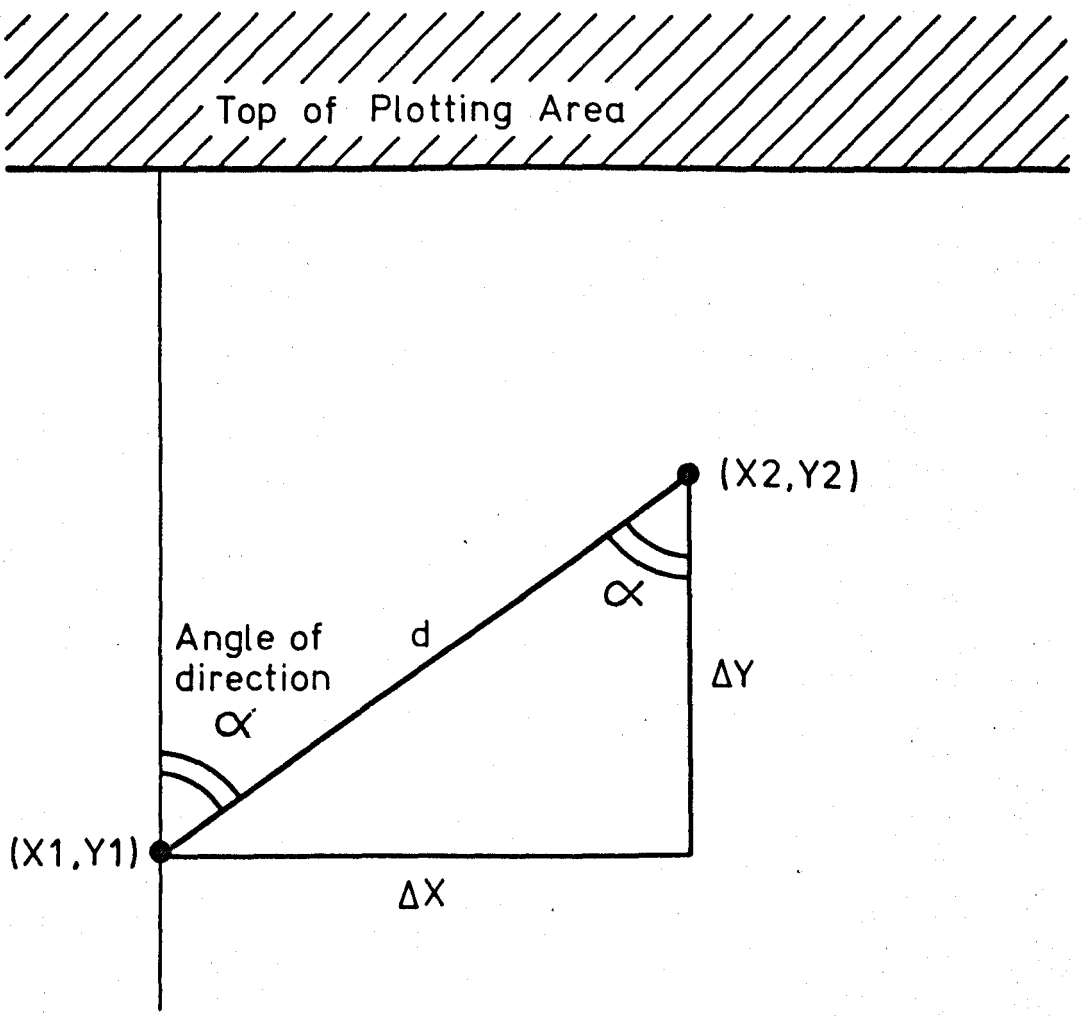
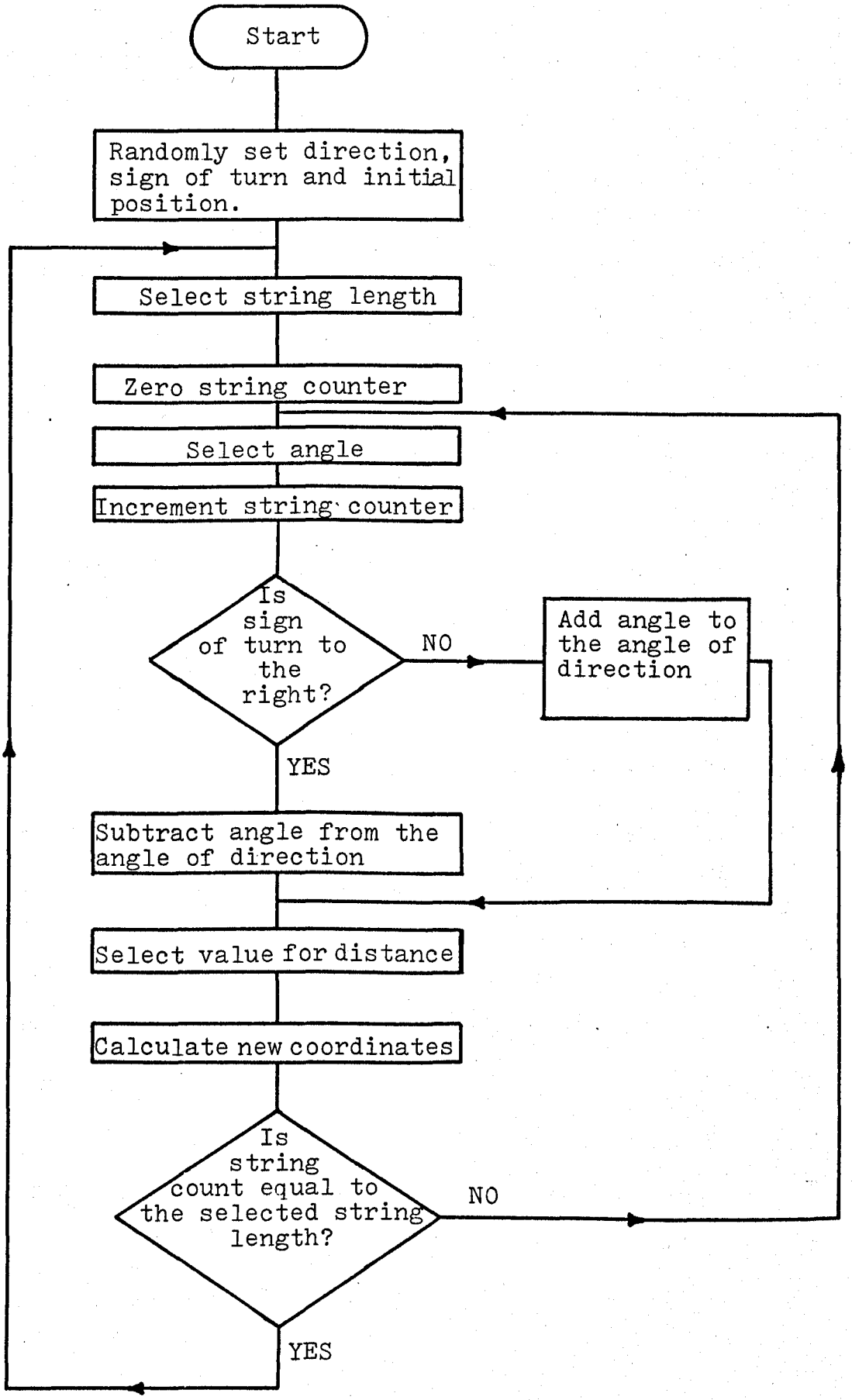

Top of Plotting Area
(X2,Y2)
Angle of
direction
α
d
α
ΔY
(X1,Y1)
ΔX

FIGURE 3.2.2.

Simplified flow diagram of the simulation model.



3.3 MODELS OF MIRACIDIAL BEHAVIOUR

3.3.1 MIRACIDIAL BEHAVIOUR - MODELS AND RESULTS

The general miracidial model consists of a square area or 'pond', in the horizontal plane, which sets the bounds within which the tracks are generated. A track hitting the sides of the 'pond' simply bounces off at an angle equal to the angle of incidence (cf. section 1.2.2). In the centre of the 'pond' is a small square area representing the snail target. Around this target is another area, called the stimulation area, within which the tracks generated represent the stimulated type of behaviour pattern. A general area of stimulation was used rather than a gradient, as the study of the currents caused by the ciliated epithelium of the snail (see section 1.3.3) produced a general zone of mixing which would prevent the formation of chemical gradients. This zone of mixing or 'active space' (see Wilson 1970) appeared to be between 5 and 10mm wide for a 5mm wide snail.

The size of the 'pond' is an important parameter of the model. If the 'pond' is too small, its edges will be close to the edges of the stimulation area and so would interfere with the simulated miracidium/snail interaction. If the 'pond' is too large, the track will rarely come close to the snail target. The 'pond' was thus initially set at 100mm.

When the model is run, the starting position is set randomly within the 'pond' but outside the stimulation area. The track is then simulated using the methods previously described, taking values for the parameters from the data produced from the track analysis of unstimulated miracidia.

Model 1.

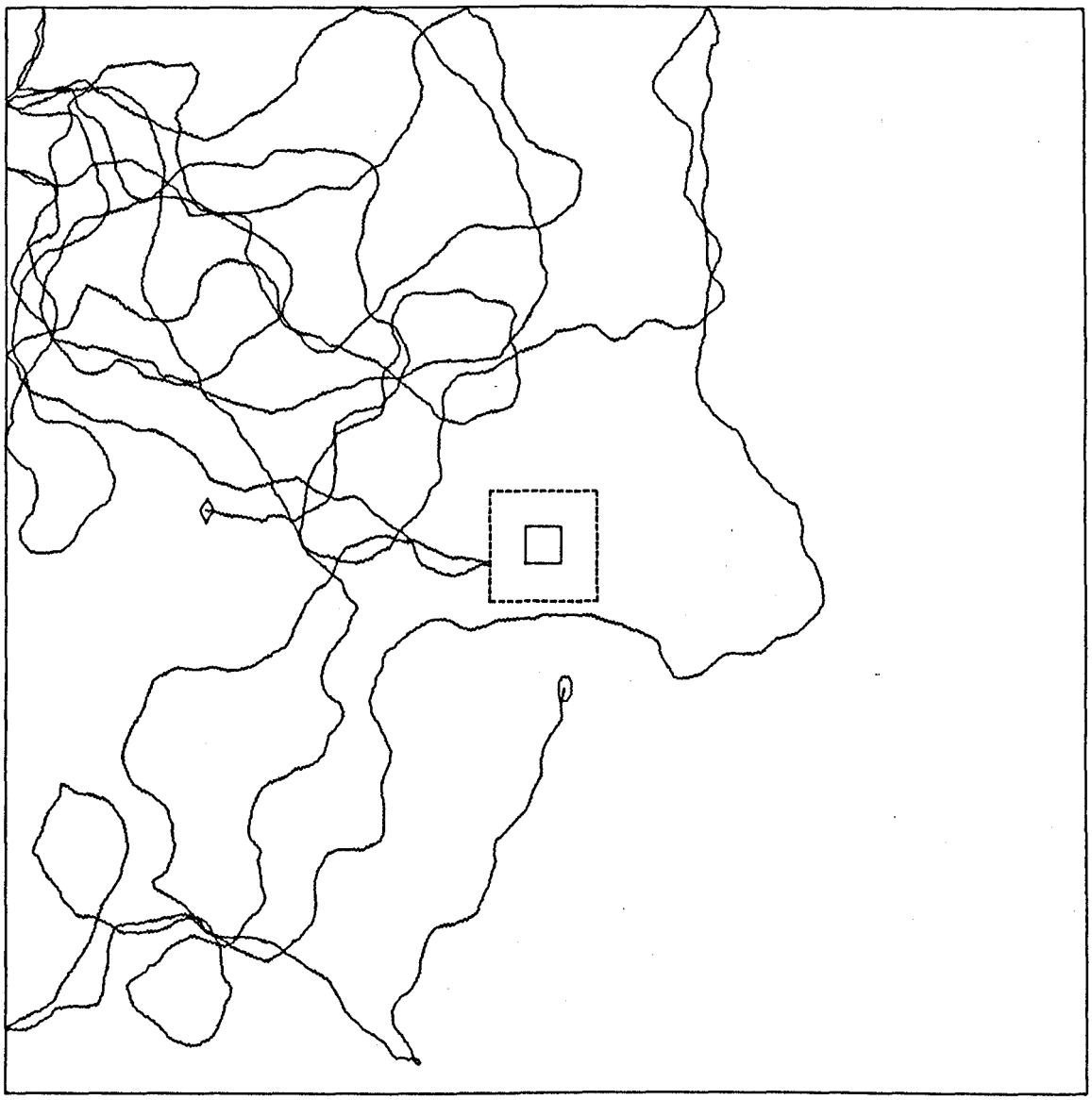
This model is basically as described above but that the size of the snail target and the stimulation area were varied. The resulting tracks of the model, run with the values of 5mm for both the snail target and the stimulation area, with 15 minutes allowed for contact are shown in Fig. 3.3.1. It would appear that contact with the snail target was a rare event because, upon entering the stimulation area, the simulated track quickly turned and left.
To quantify the proportion of hits, the model was re-run without the graph plotter output and with 100 runs for each snail target and stimulation area data set. A flow diagram of this particular model is shown in Fig. 3.3.2.

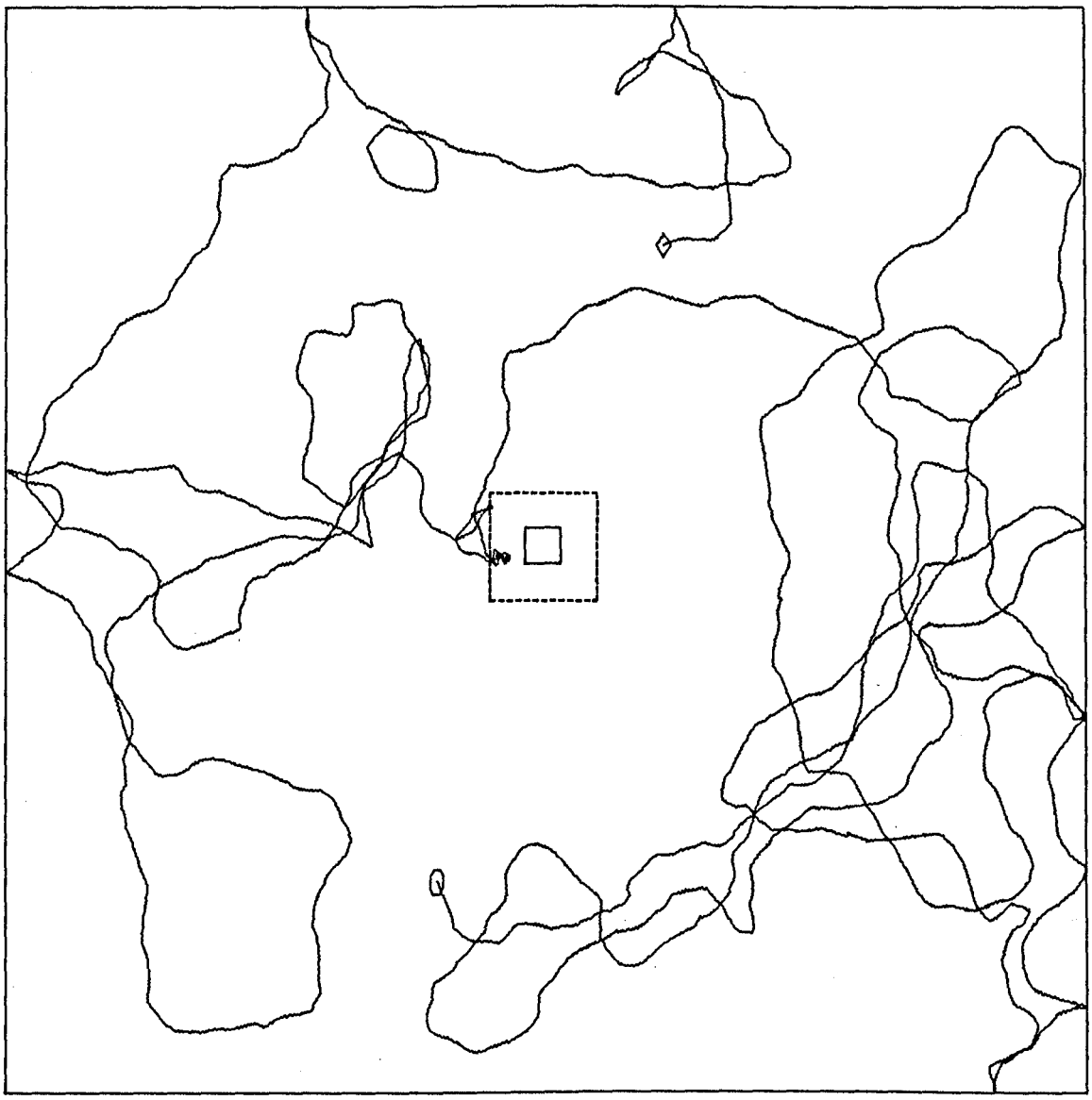
The results from this model are shown in Fig. 3.3.3. The values for a stimulation area of zero act as a control, representing the chance contacts of an unstimulated miracidium. It can be seen that the percentage of hits rapidly drops as the stimulation area increases in size. It also appears that for each snail size there is a point where further increasing the size of the stimulation area does not decrease the percentage of hits.

These results were not expected, but examination of the graph plotter output indicates that the low levels of contact with the snail target are due to problems at the interface between the "pond" and the stimulation area. As soon as a track crosses the stimulation area, the increase in the angle turned, caused by the selection of parameter values from the data taken from stimulated miracidia, turns

FIGURE 3.3.1.

Sample graph plots from Model 1.


20mm


20 mm

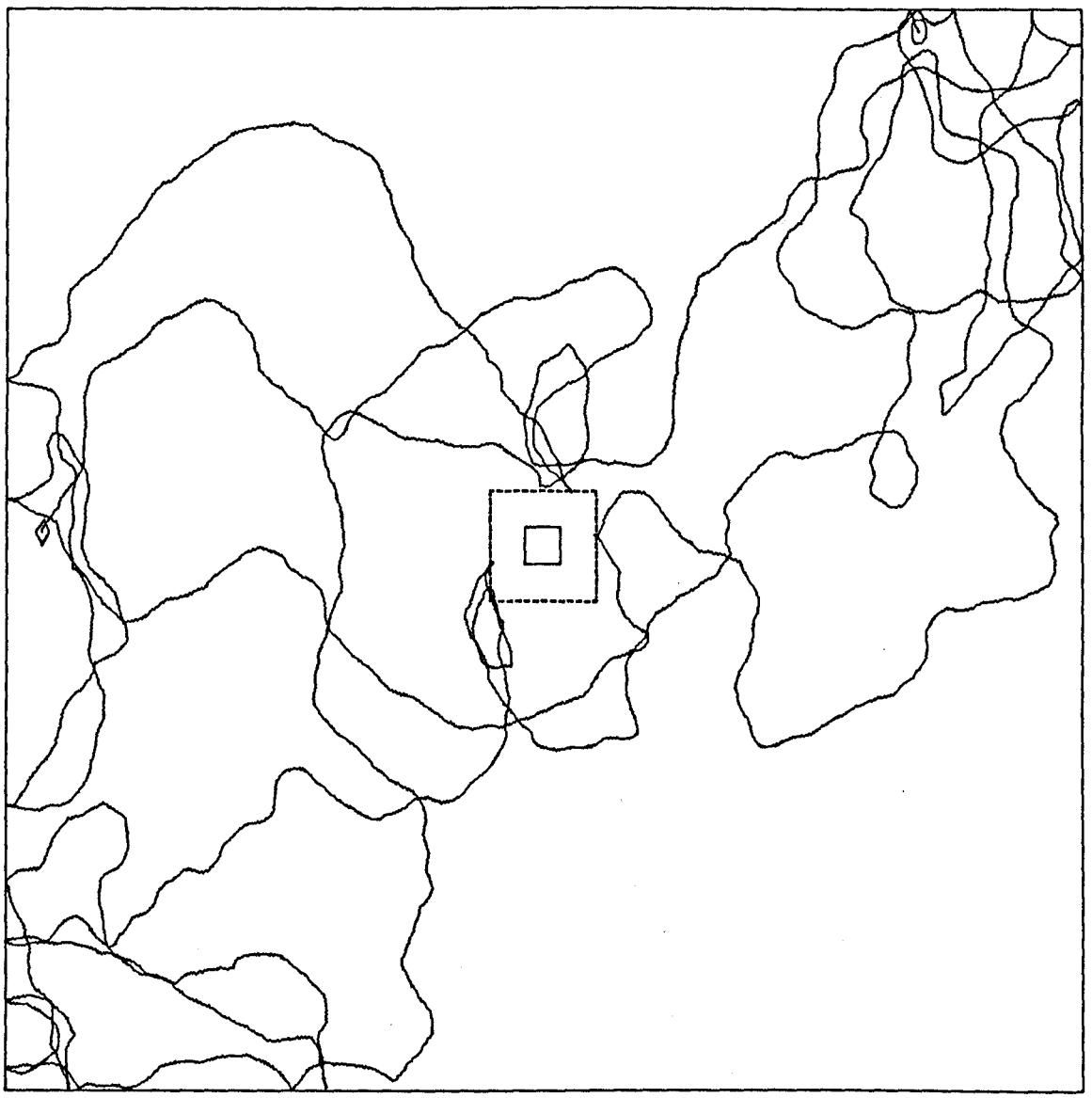
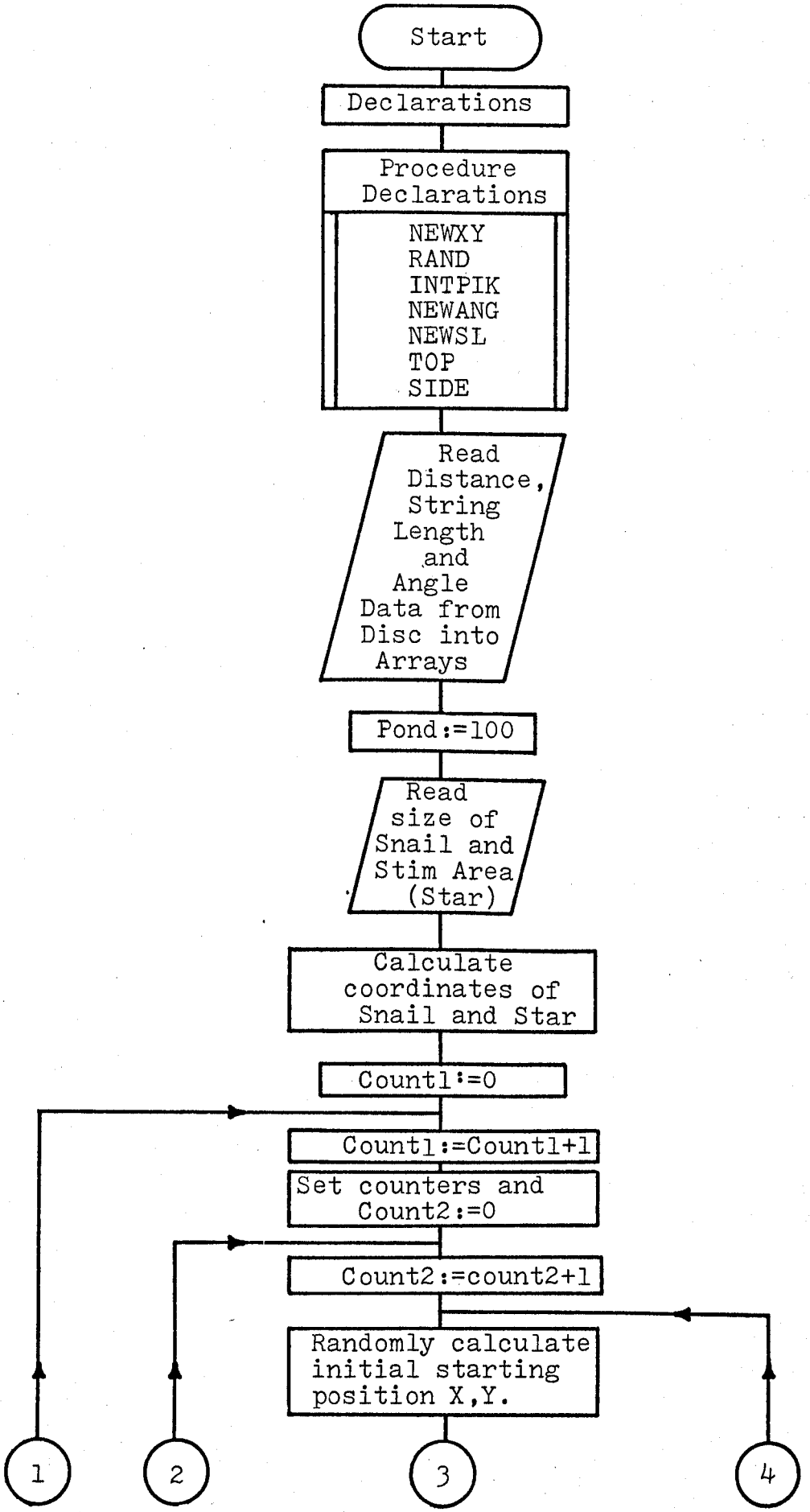
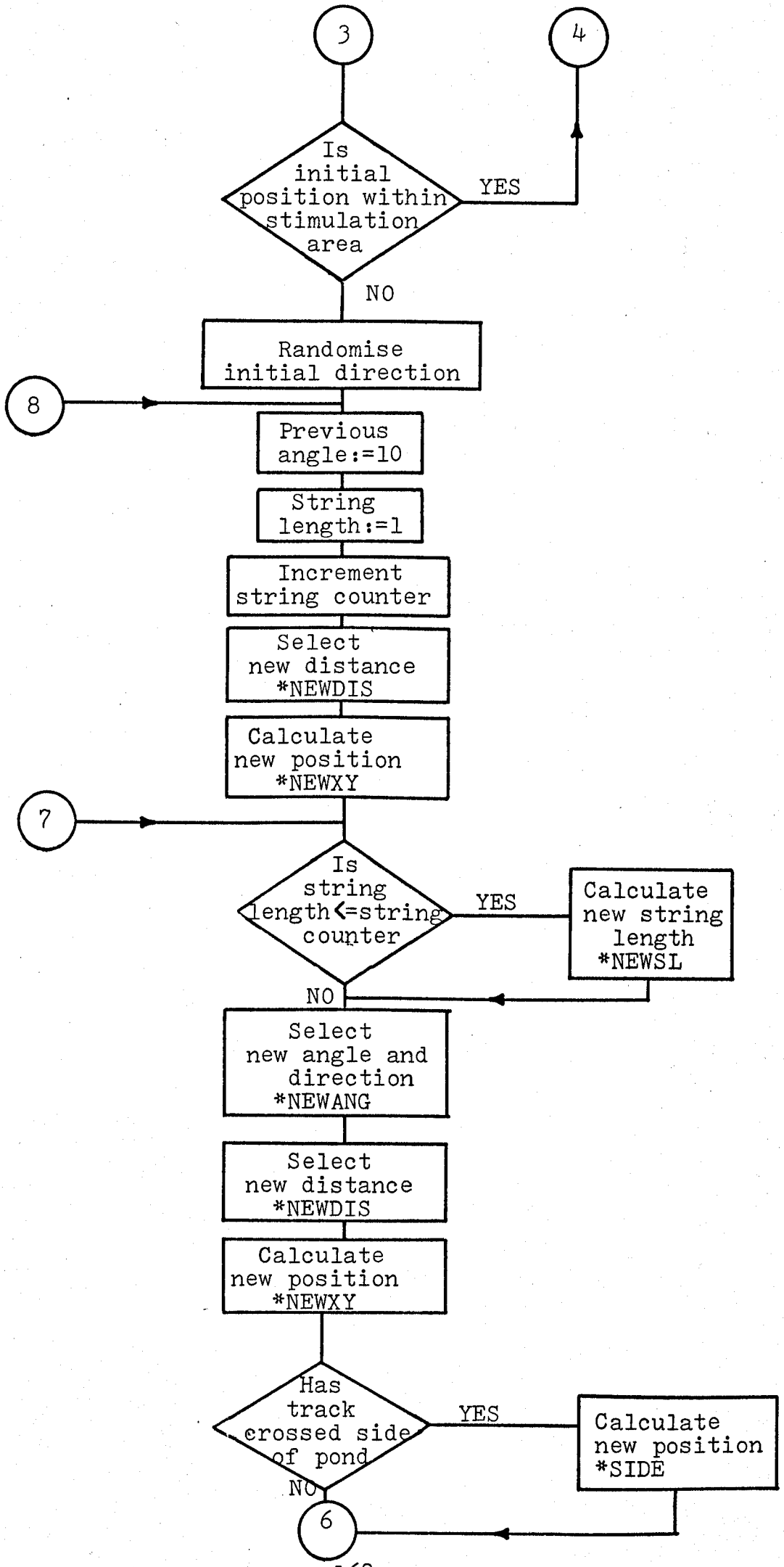
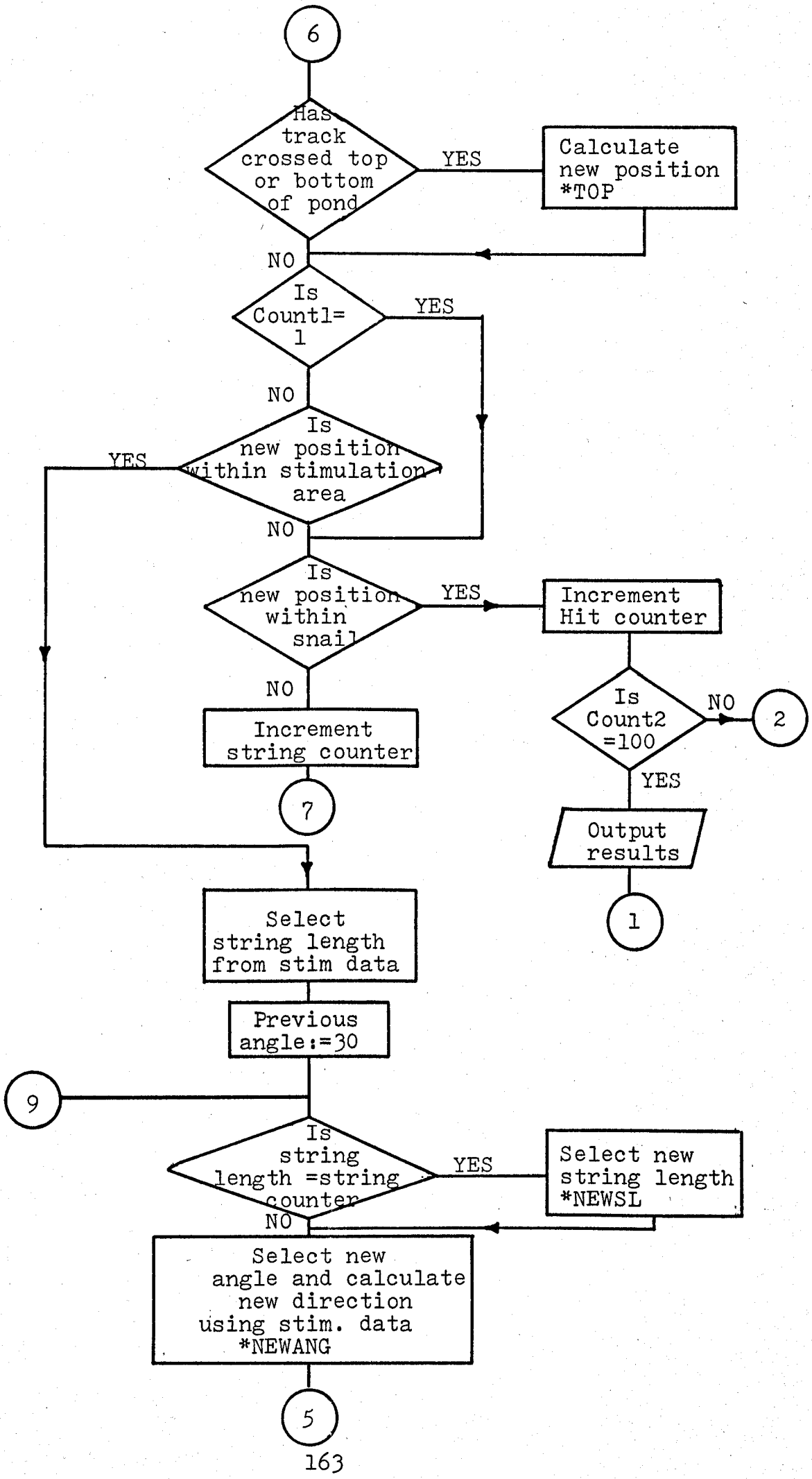

20 mm

FIGURE 3.3.2

Flow diagram of miracidial Model 1.




3
4
Is initial position within stimulation area
YES
NO
Randomise initial direction
8
Previous angle:=10
String length:=1
Increment string counter
Select new distance *NEWDIS
Calculate new position *NEWXY
7
Is string length<=string counter
YES
Calculate new string length *NEWSL
NO
Select new angle and direction *NEWANG
Select new distance *NEWDIS
Calculate new position *NEWXY
Has track crossed side of pond
YES
Calculate new position *SIDE
NO
6


6
Has track crossed top or bottom of pond
YES
Calculate new position *TOP
NO
Is Count1= 1
YES
NO
Is new position within stimulation area
YES
NO
Is new position within snail
YES
Increment Hit counter
NO
Increment string counter
7
Is Count2 =100
NO
2
YES
Output results
1
Select string length from stim data
Previous angle:=30
9
Is string length =string counter
YES
Select new string length *NEWSL
NO
Select new angle and calculate new direction using stim. data *NEWANG
5

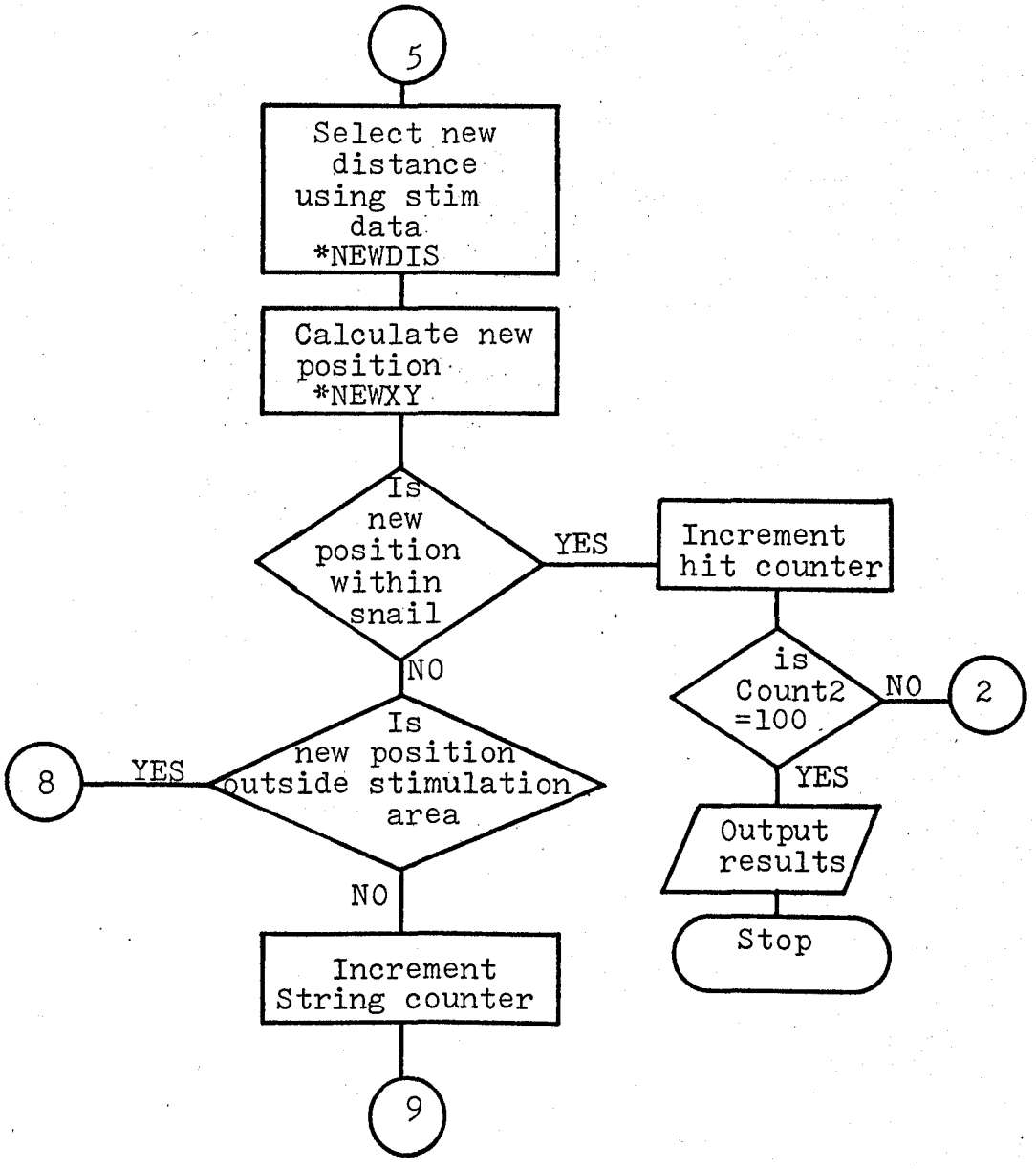
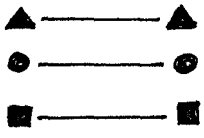
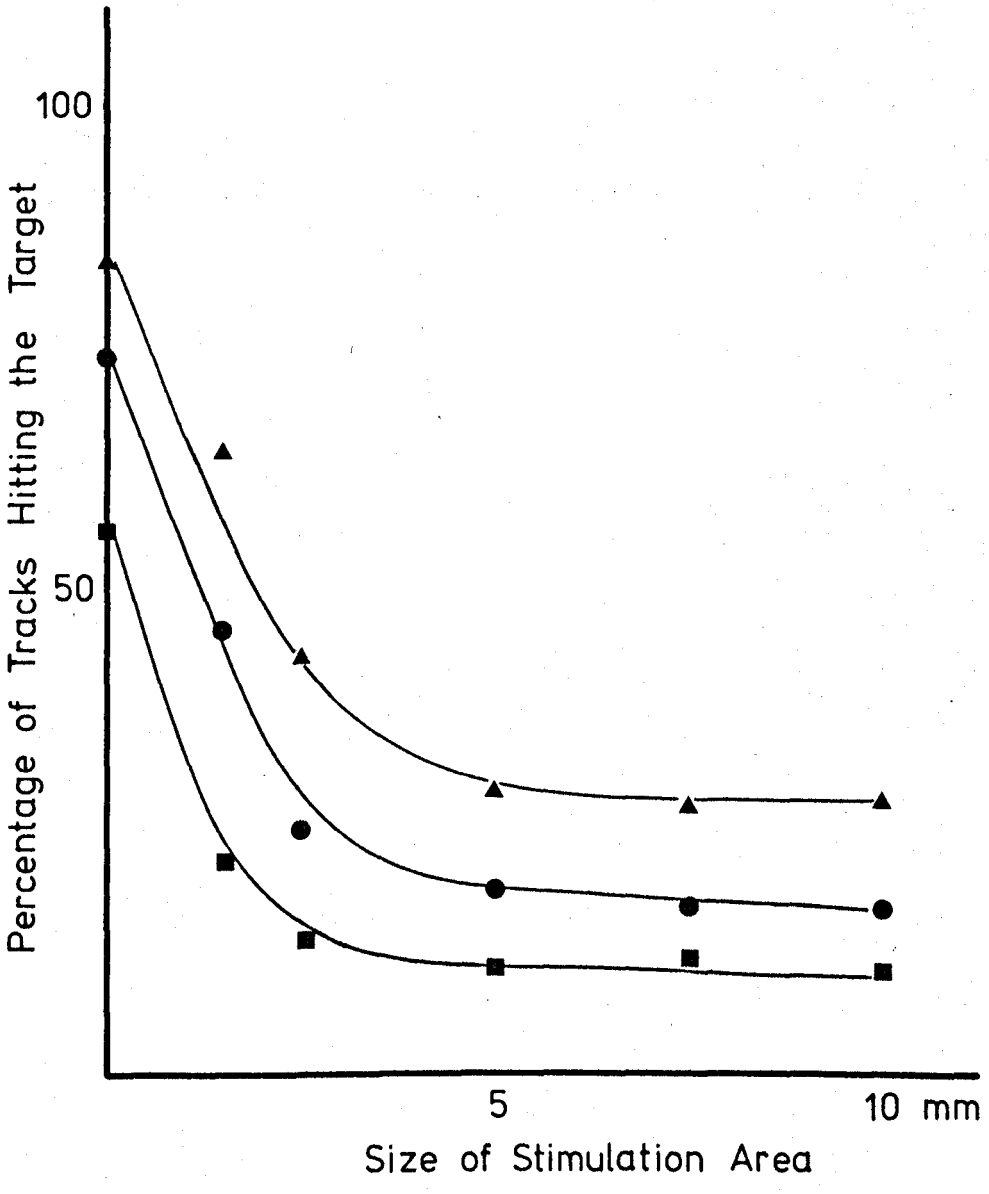

5
Select new distance using stim data *NEWDIS
Calculate new position *NEWXY
Is new position within snail
YES
Increment hit counter
is Count2 =100
NO
2
YES
Output results
Stop
NO
Is new position outside stimulation area
8
YES
NO
Increment String counter
9

FIGURE 3.3.3.

The percentage of tracks of Model 1 hitting the target for different sizes of the stimulation area.



7.5mm snail target
5.0mm snail target
2.5mm snail target


100
50
Percentage of Tracks Hitting the Target
5
10 mm
Size of Stimulation Area

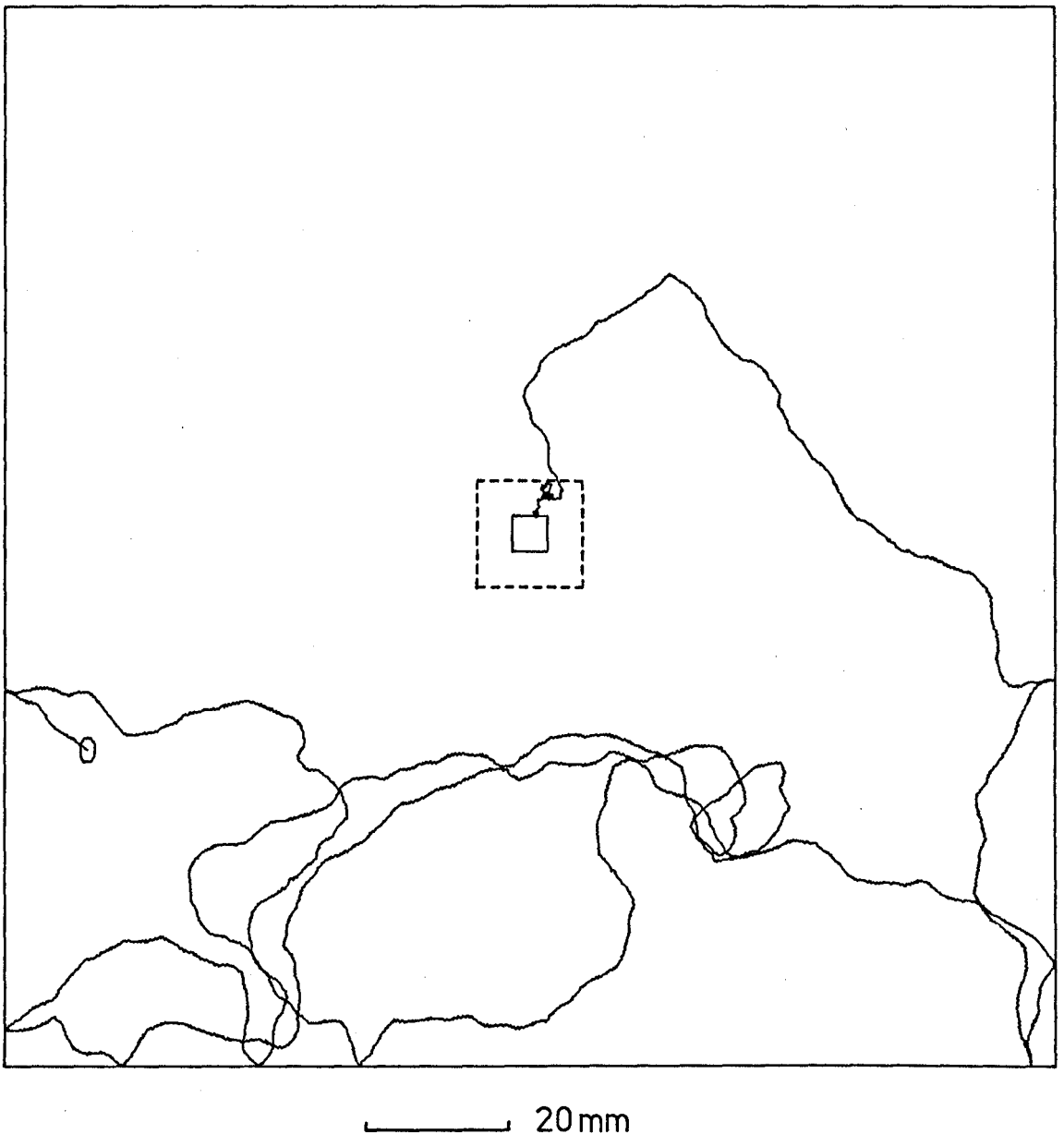
the track back out of the area where it reverts to the unstimulated type of behaviour pattern. In this model then, the stimulation area actually acts as a defence for the snail. Models 2 and 3 are attempts to overcome this interface problem.

Model 2.

This model is basically the same as Model 1 except that a delay was incorporated when the track crossed the stimulation area, so that the switch to the stimulated type of behaviour pattern did not occur for a predetermined number of steps. This situation could occur in nature if there was a sensory delay or reaction time in the response of the miracidia to snail chemicals. The resulting tracks for the model run with the values of 5mm for both the snail and the stimulation area and with a delay of 7 steps of the unstimulated type of swimming behaviour upon entry into the stimulation area are shown in Fig. 3.3.4. The proportion of hits was quantified as before, by running the model with no graph plotter output and for 100 runs for each parameter set. The results are shown in Fig. 3.3.5. An increase above the control value (no stimulation) was observed when the delay had increased to 20 steps. As the mean step of unstimulated miracidia was 0.18mm (see section 1.2.1), it follows that 20 steps of unstimulated swimming corresponds to a distance of approximately 3.6mm. This would mean that a miracidium would have to traverse over 70 percent of the distance between the edge of the stimulation area and the snail before the initiation of increased turning would result in even the same chance of hitting the snail as an unstimulated miracidium. At very long delays,

FIGURE 3.3.4.

Sample graph plots from Model 2.


20 mm

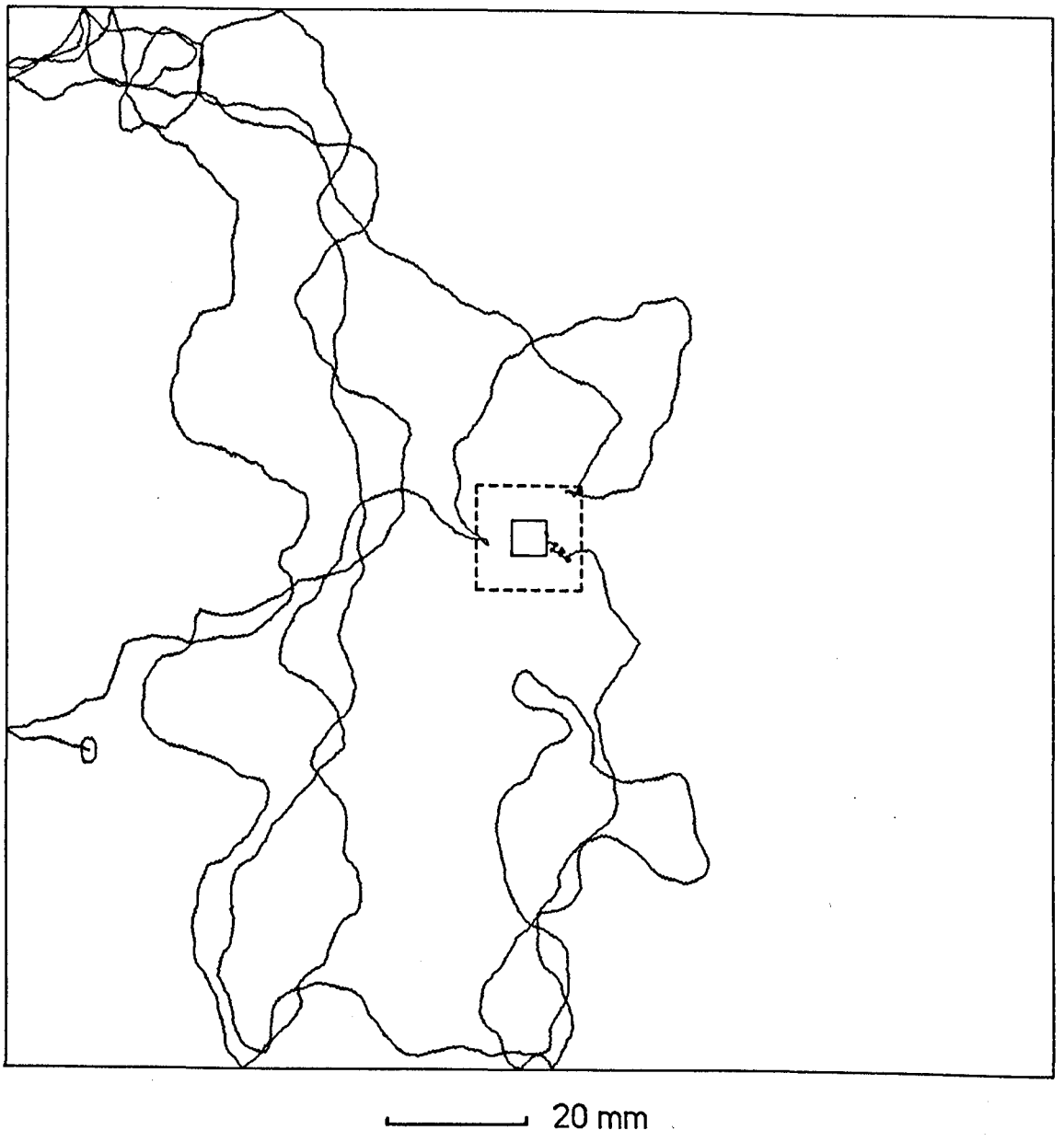
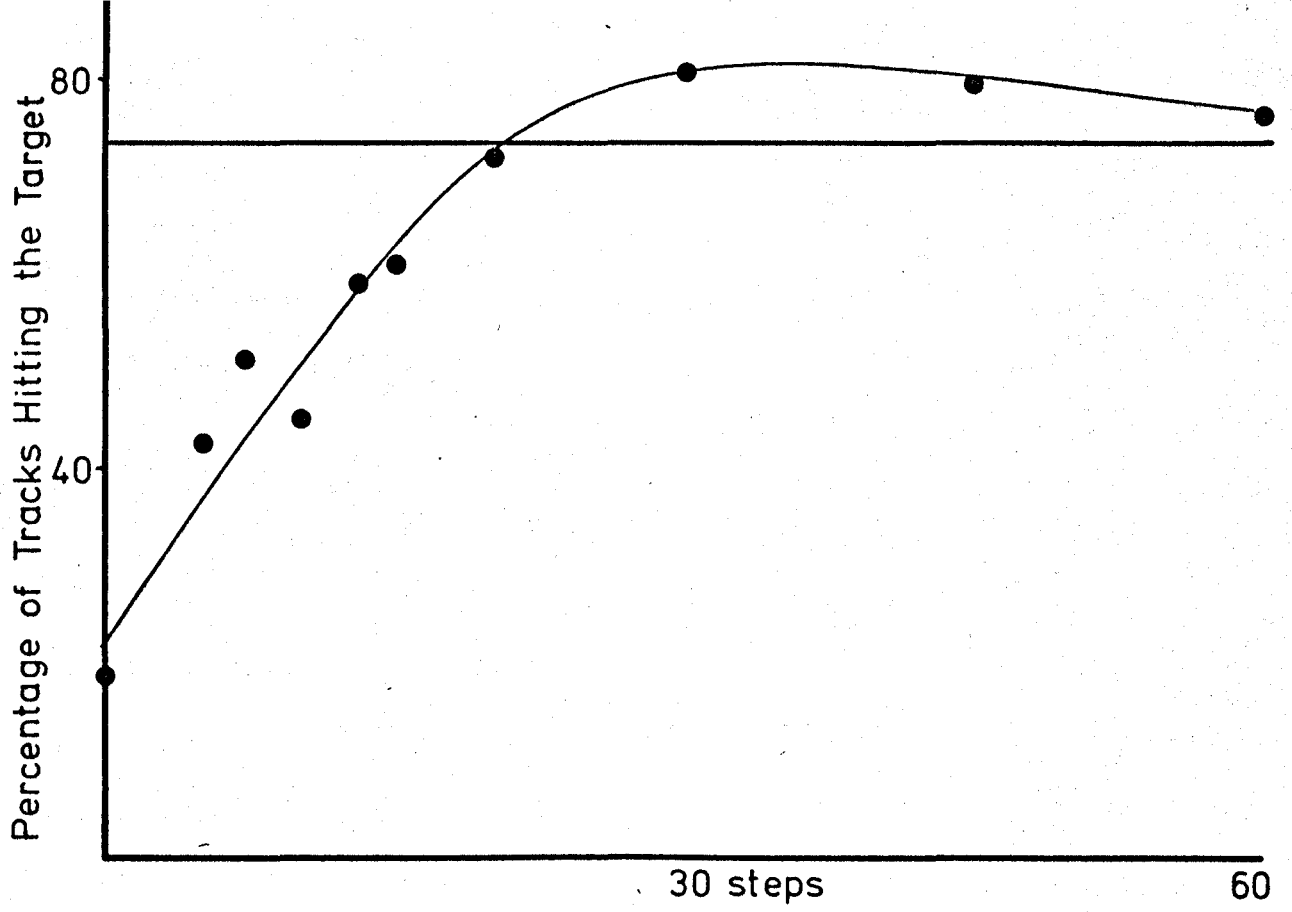

20 mm

FIGURE 3.3.5.

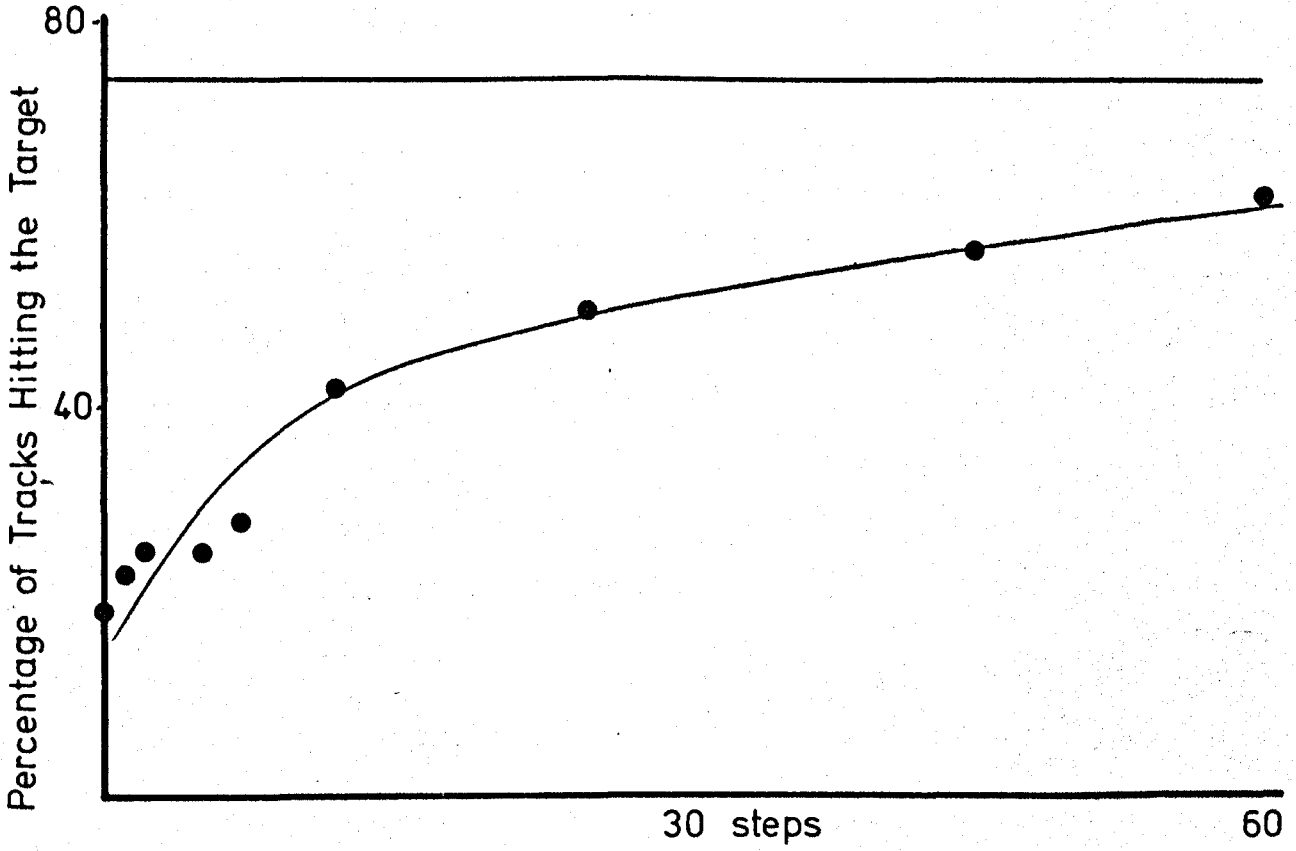
The percentage of tracks hitting the target in Model 2 as the length of the delay, before stimulated tracks were generated upon entry into the stimulation area, was increased.

FIGURE 3.3.7

The percentage of tracks hitting the target in Model 3 as the length of the delay, before unstimulated tracks were generated upon leaving the stimulation area, was increased.



Delay, after entry into the Stimulation Area, before Stimulated Tracks were generated



Delay, after leaving the Stimulation Area, before Unstimulated Tracks were generated

the percentage hit would be expected to be the same as the control, as the track will either hit the snail target or leave the stimulation area before the delay has been completed. In effect, the track would never change to the stimulated type of behaviour pattern.

Model 3.

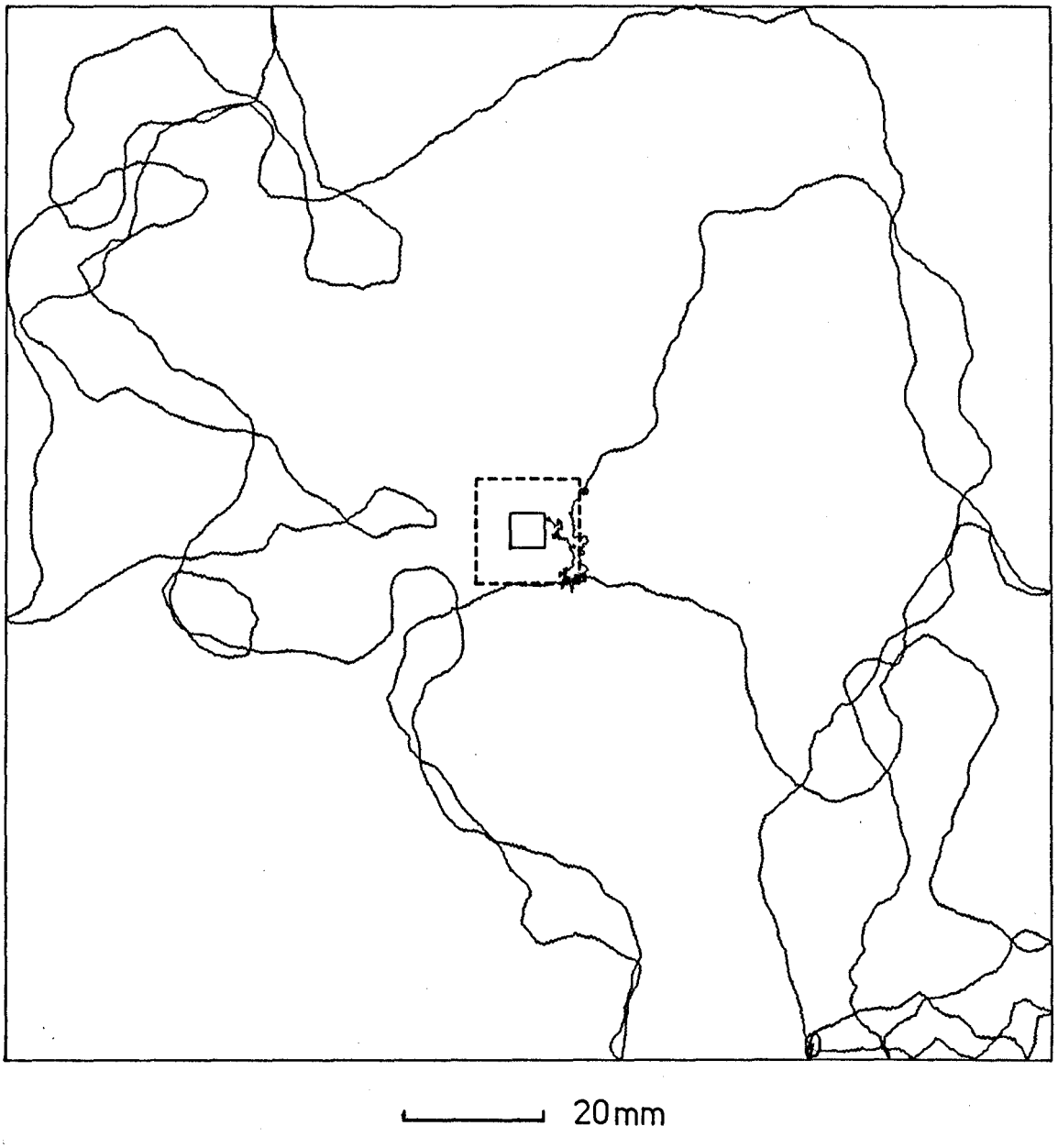
This model is basically the same as Model 1, except that the track does not revert to the unstimulated type of behaviour upon leaving the stimulation area, but remains stimulated for a predetermined number of steps. If, after this period, the track is outside the stimulation area, the swimming pattern is returned to the unstimulated form. This situation could occur in nature if the sensory cells of the miracidium quickly adapted to the presence of the stimulant, but slowly adapted back to the normal state when the stimulation ceases.

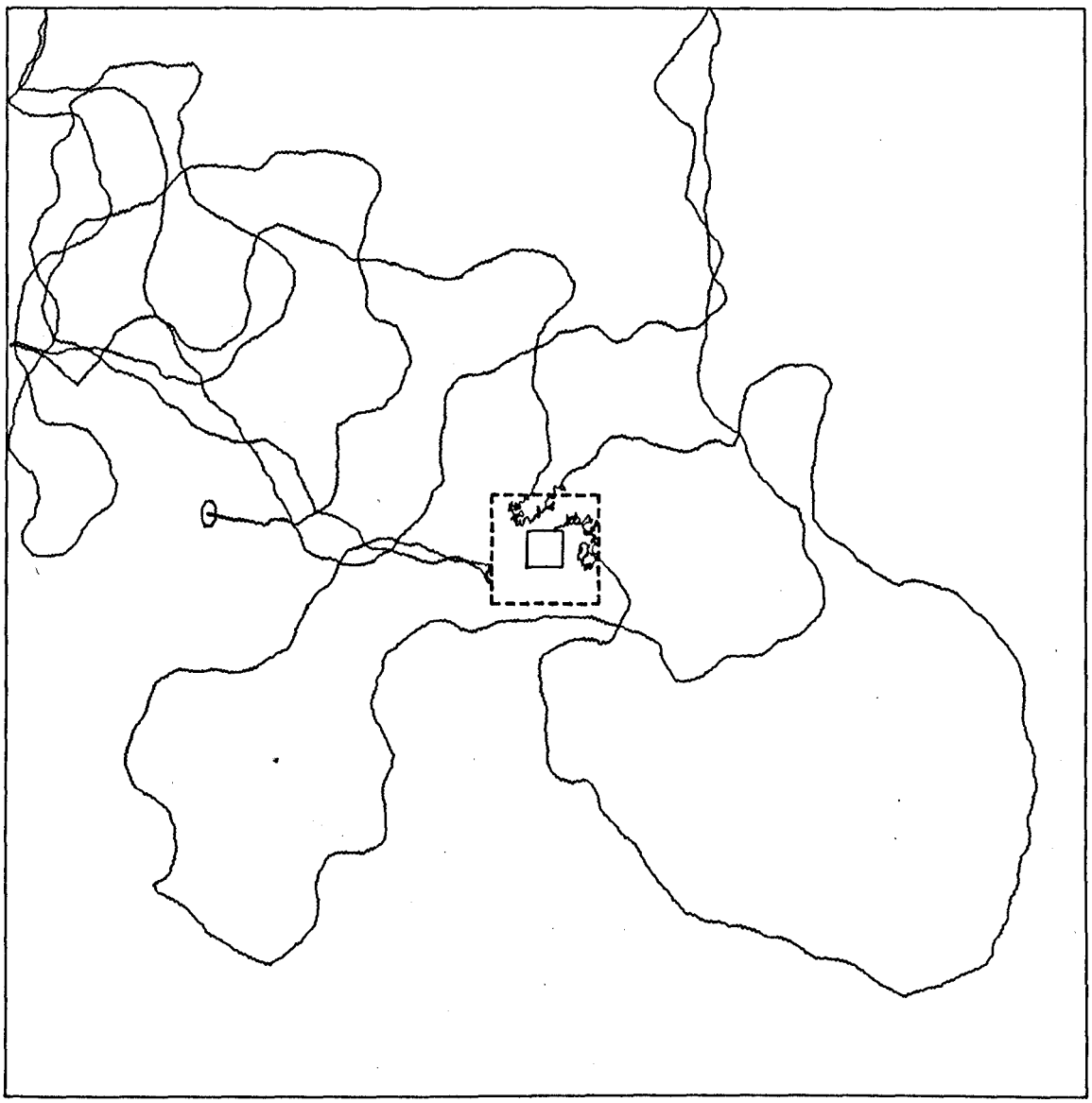
The resulting tracks from the model, run with the values of 5mm for both the snail and the stimulation area and with the track remaining in the stimulated form for 30 steps after leaving the stimulation area, are shown in Fig. 3.3.6. The proportion of hits with different values for this period of continued stimulation was quantified, as before, by running the model with no graph plotter output and with 100 runs per estimation. The results are shown in Fig. 3.3.7. It can be seen that even when the tracks remained stimulated for 60 steps (approximately 6 seconds) the control value for the percentage hit was not attained.

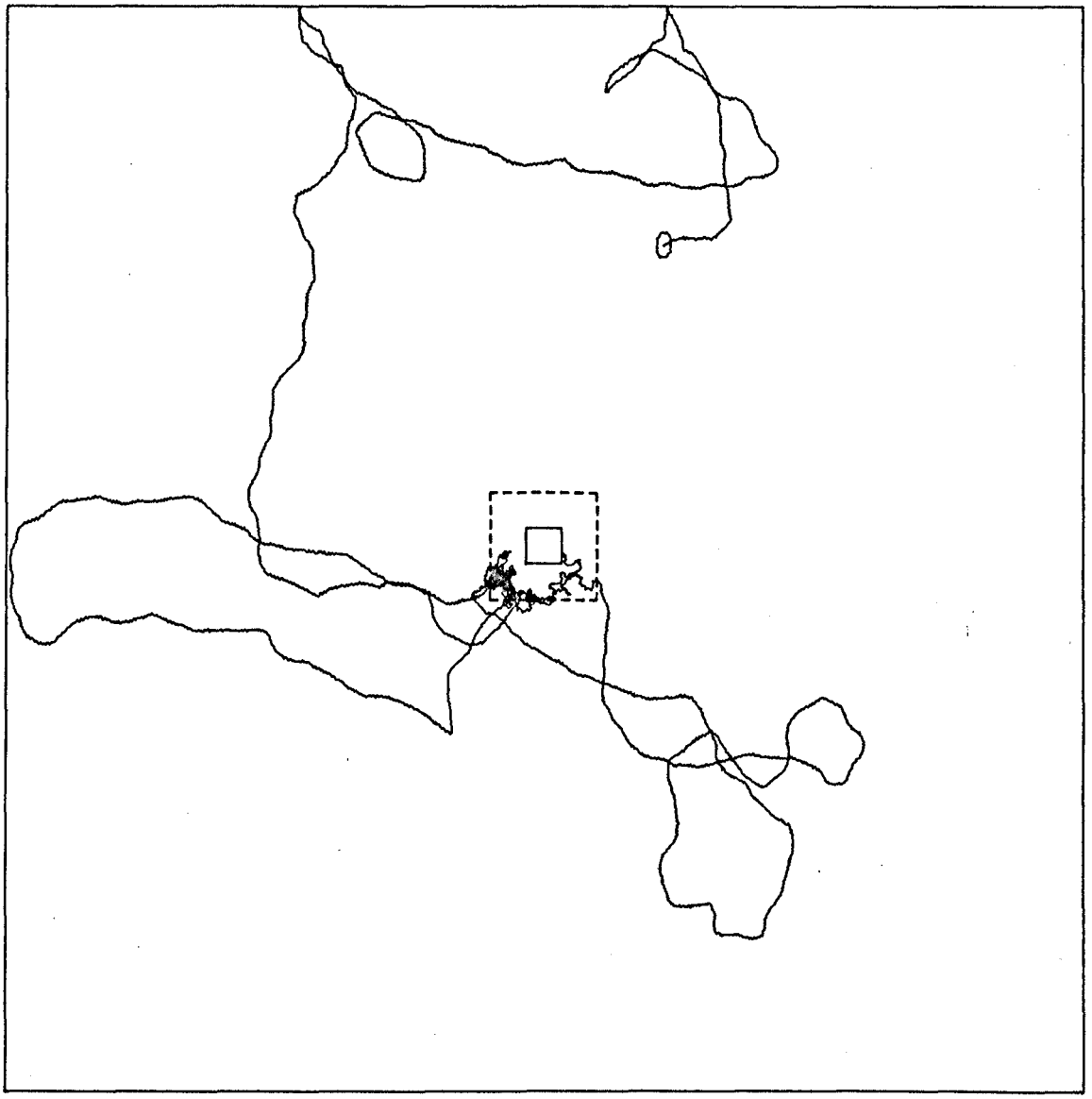
The processes occurring in this miracidial host-location situation are obviously more complex than at first

FIGURE 3.3.6.

Sample graph plots from Model 3.


20mm


20 mm


20mm

was thought. To further study the nature of this type of response to the presence of the snail, a simplified model was devised.

3.3.2 SIMPLIFIED MIRACIDIAL MODELS AND RESULTS.

For this model, the swimming pattern of the miraicidia was simplified and idealised. It was noticed, both in the tracks of miracidia swimming close to a snail and in the graph plotter output of the stimulated pattern of behaviour in section 3.3.1, that the effect of the increased level of turning was to randomise the direction of short, relatively straight lengths of swimming. Unstimulated miracidia showed a similar randomisation of direction except only that the length of the straight swimming was longer and that the angle through which the direction changed was less than for the stimulated miracidia. This reduction of the swimming pattern of the miraicidia was used as the basis of the simplified model.

In the model, the unstimulated swimming pattern was represented by long straight tracks between which the angle of direction was changed randomly by between 90 degrees to the right and 90 degrees to the left. Within the stimulation area, the swimming pattern was represented by straight tracks of a shorter length, between which the angle of direction was changed at random. Within this model, the parameters of snail size, pond size, the length of the unstimulated and stimulated step lengths, the delay in the initiation of stimulation and the length of continued stimulation could all be varied. Graph plotter output of the model run with the snail target set at 5mm and with

no continued stimulation or delay in the onset of stimulation is shown in Fig. 3.3.8. It can be seen that the same problems of the 'pond'/stimulation area interface also occurred with this model. For quantification of the percentage hit, the model was run with no graph plotter output and with 200 iterations per parameter set.

Run 1.

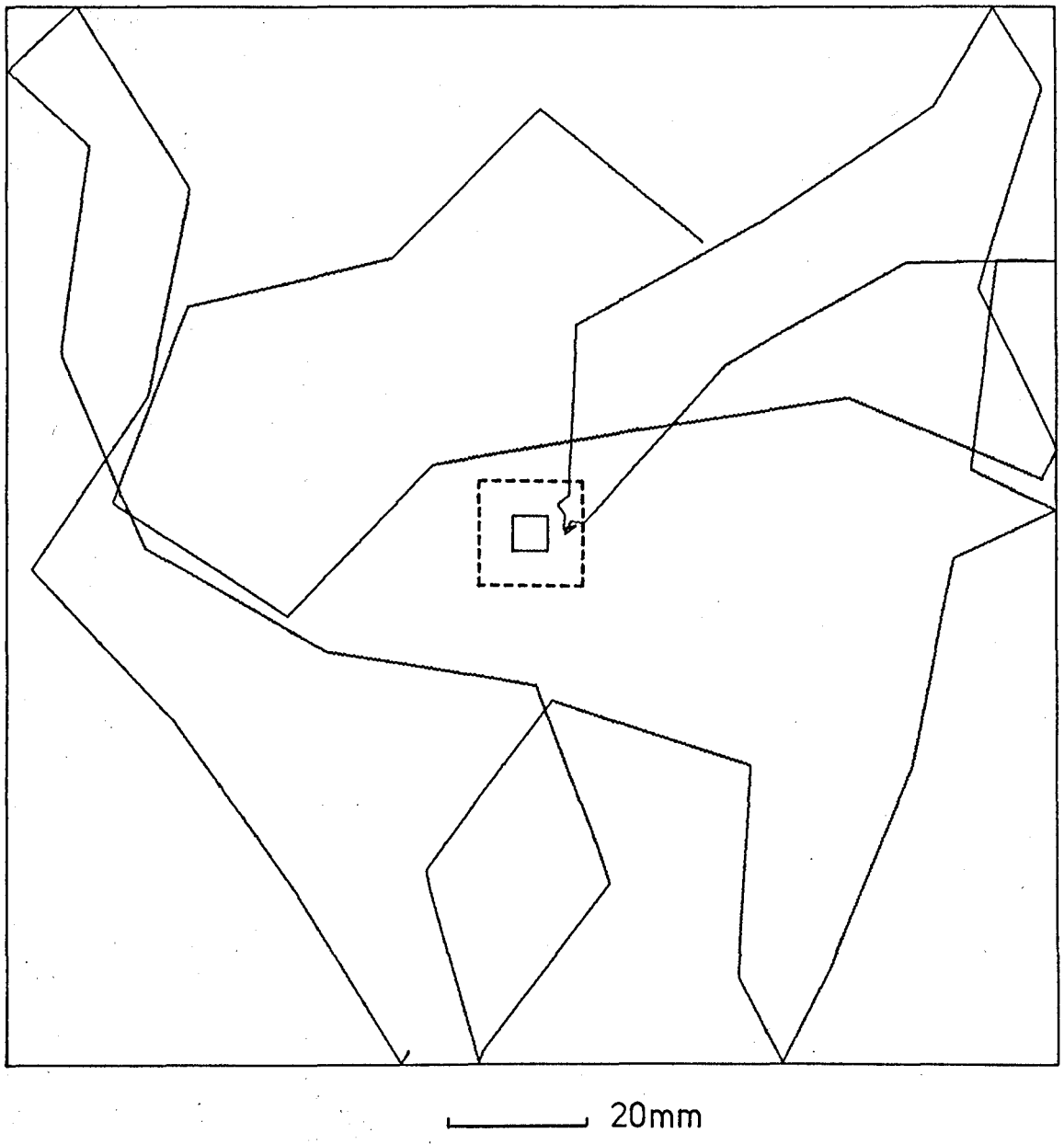
The aim of this run was to find the effect of altering the unstimulated step length on the percentage hit, when no stimulation took place close to the snail target. The snail target was set at 5mm and the'pond' at 100mm. The results are shown in Fig. 3.3.9. The value of 74 percent hit, found with a similar snail target in Model 1, was reached when the unstimulated step length had increased to between 25 and 30mm. The value of 30mm was thus used for the unstimulated step size in all other runs.

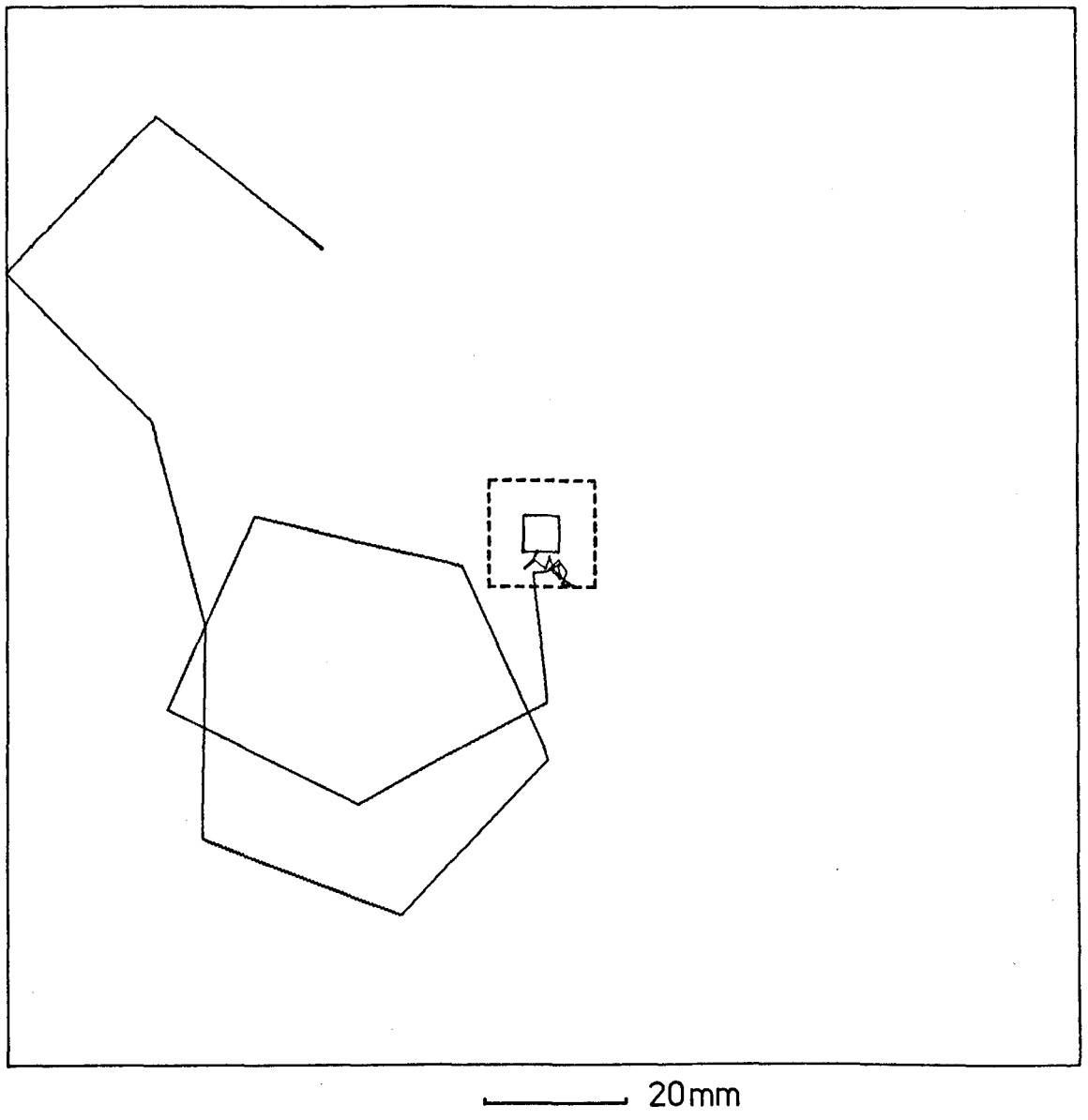
Run 2.

The aim of this run was to find the percentage hit when no stimulation took place and to measure the degree of variation in the estimation. The size of the 'pond' was increased to 150mm to reduce the percentage hit and so facilitate the detection of an increase over the control levels that might occur in other runs. The model was run twelve times with 200 iterations per run and with a snail target of 5mm. The mean percentage hit was found to be 39.5 percent with a standard deviation of $\pm$ 4.2. The range was 31.5 percent to 45.5 percent. Although many of the random elements have been eliminated from this model, it can be seen that there is still a considerable degree of

FIGURE 3.3.8.

Sample graph plots from the simplified miracidial model.


20mm


20mm

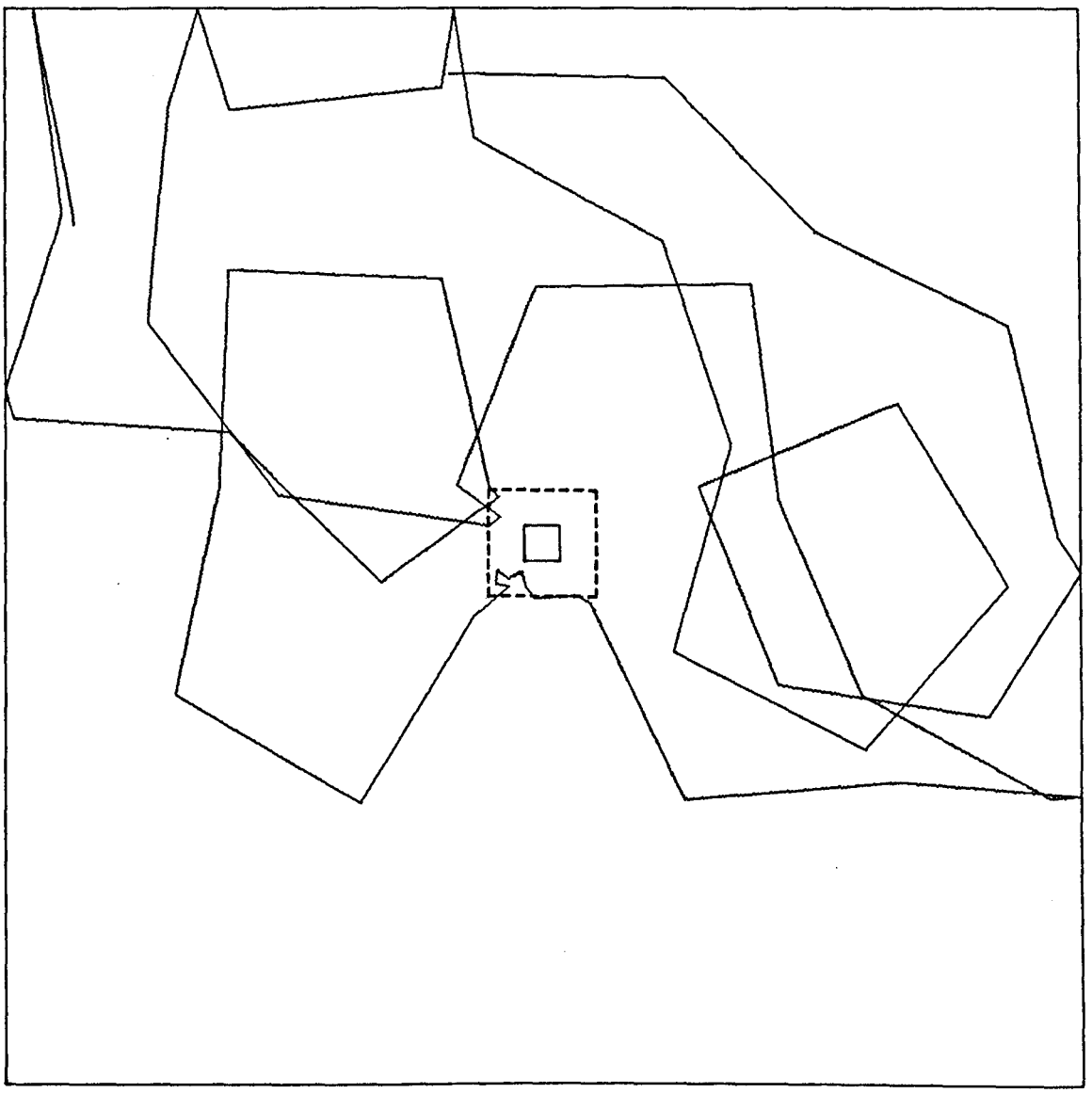
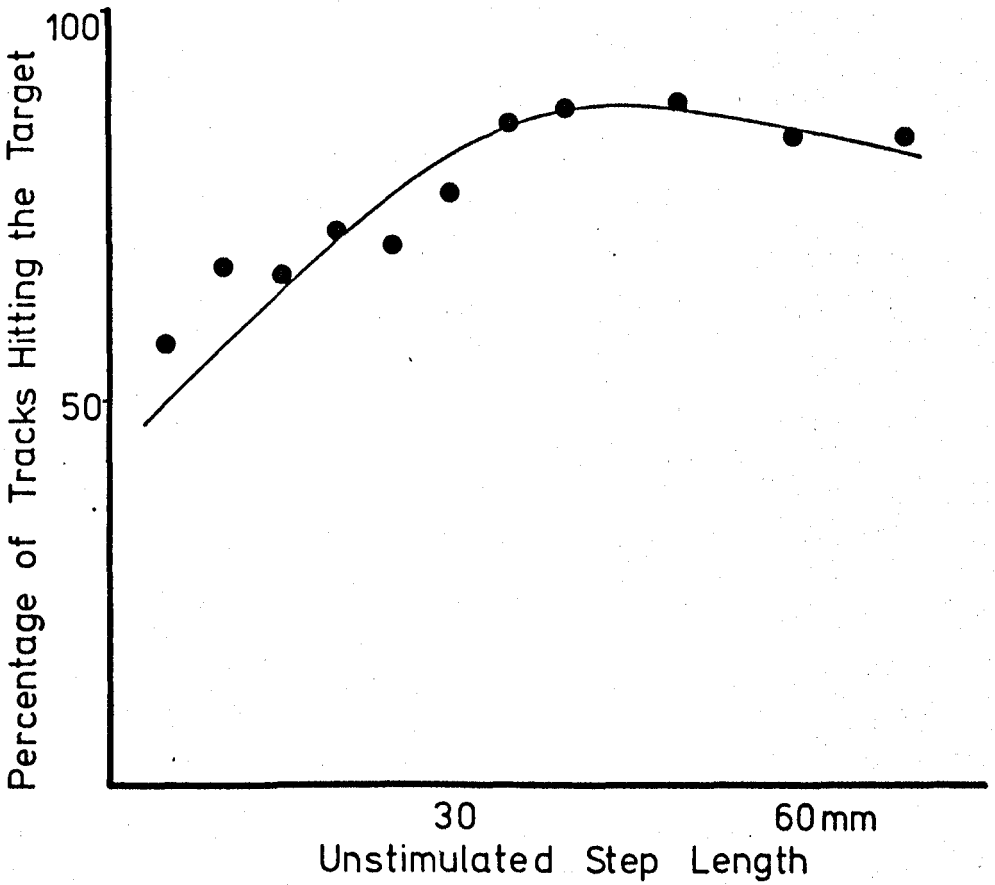

20mm

FIGURE 3.3.9.

The percentage of tracks hitting the snail target in the simplified miracidial model, Run 1. The snail target was set at 5mm and the 'pond' at 100mm.


100
50
Percentage of Tracks Hitting the Target
30
60mm
Unstimulated Step Length

variation in the results. This variation should be born in mind when the results from other runs are discussed.

Run 3.

This run was devised to assess the percentage of tracks that hit the snail target when stimulation took place in the stimulation area. The results for a stimulation area of 5mm with different stimulated step lengths is shown in Fig. 3.3.10, and in Fig. 3.3.11 for a stimulation area of 10mm. The snail target was set at 5mm in both cases. It can be seen that the percentage of tracks hitting the target rapidly rises and then gradually decreases as the length of the stimulated steps was increased. The percentage of hits does not rise above slightly more than half of the control value (the percentage of unstimulated tracks hitting the target in Run 2).

Run 4.

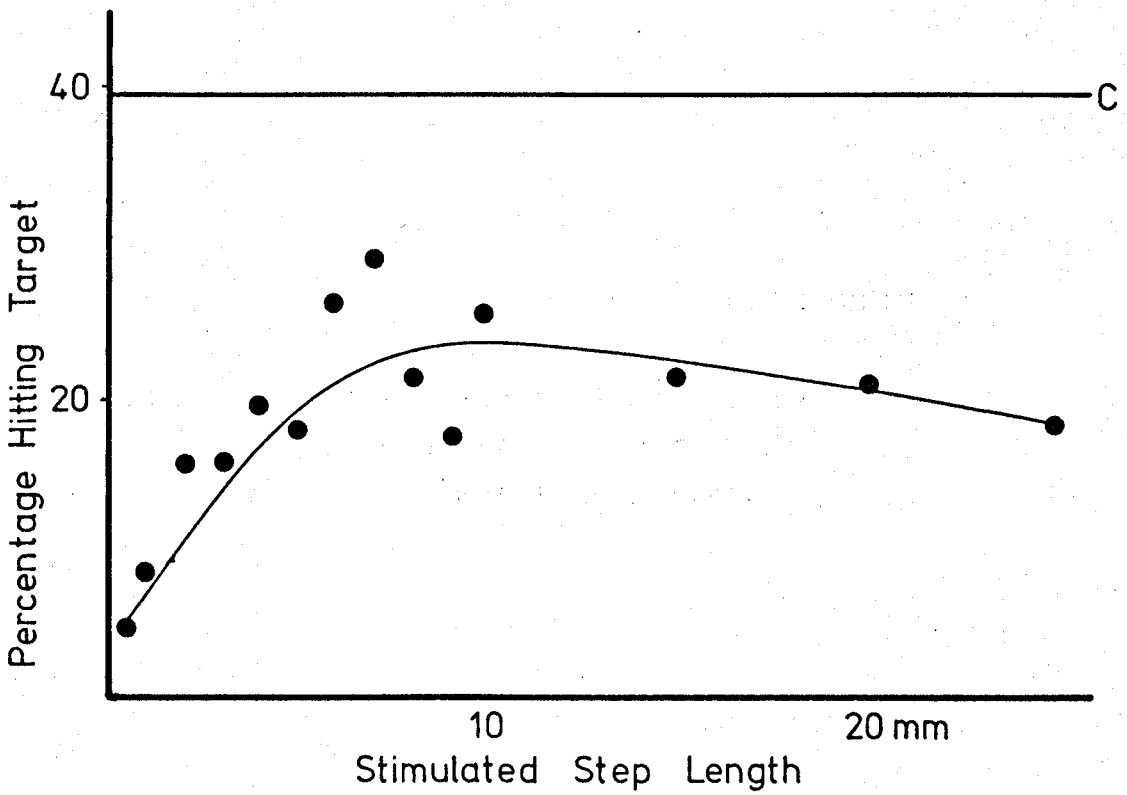
The aim of this run was to study the effect of a delay before the initiation of the stimulated pattern of behaviour when the track entered the stimulation area. This was achieved in the model by allowing the unstimulated step to pass an extra distance into the area before the switch to the stimulated type of track occurred. This situation is the equivalent of the sensory delay or slow adaptation to snail chemicals as was postulated for Model 2. The results, for a stimulation area of 5mm, and a range of stimulated step lengths and different additional distances into the area, are shown in Fig. 3.3.12 and for a stimulation area of 10mm in Fig. 3.3.13. Straight lines were fitted to the points using linear regression. All regression

FIGURE 3.3.10.

The percentage of tracks hitting the snail target in simplified miracidial model, Run 3.
The stimulation area was set at 5mm.

FIGURE 3.3.11.

The percentage of tracks hitting the snail target in the simplified miracidial model, Run 3.
The stimulation area was set at 10mm.


40
C
20
Percentage Hitting Target
10
20 mm
Stimulated Step Length

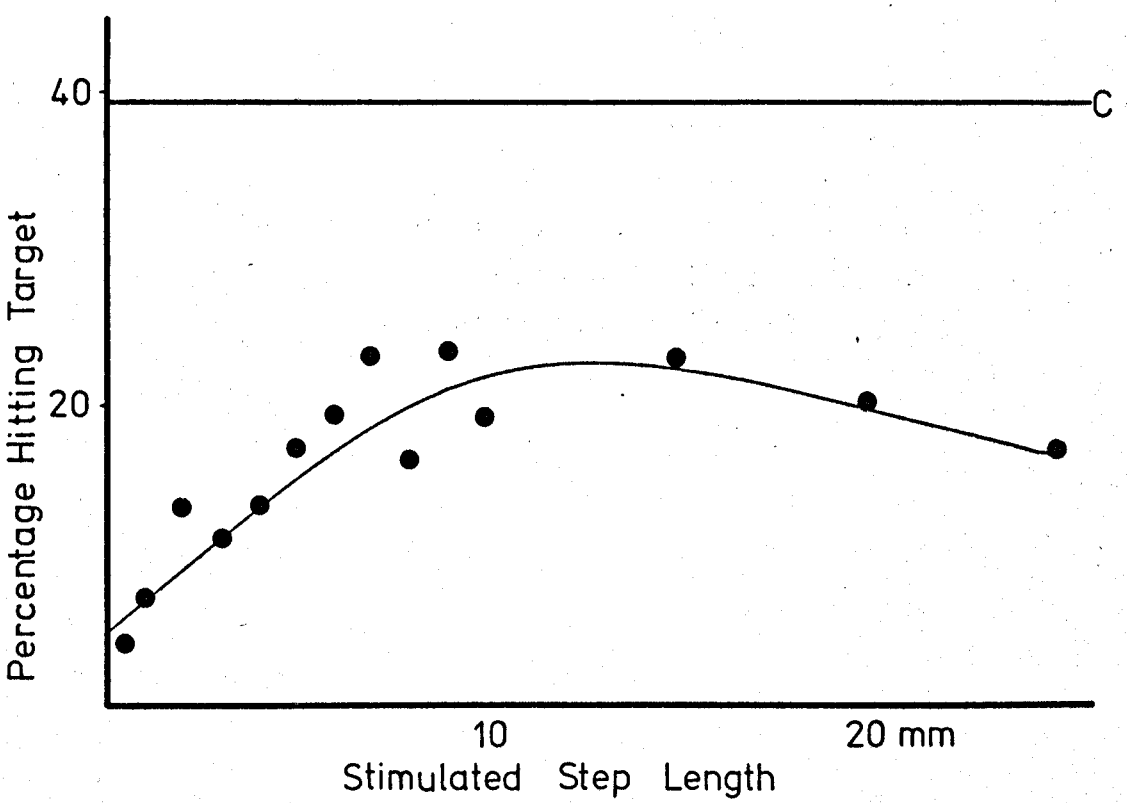

40
C
20
Percentage Hitting Target
10
20 mm
Stimulated Step Length

FIGURE 3.3.12.

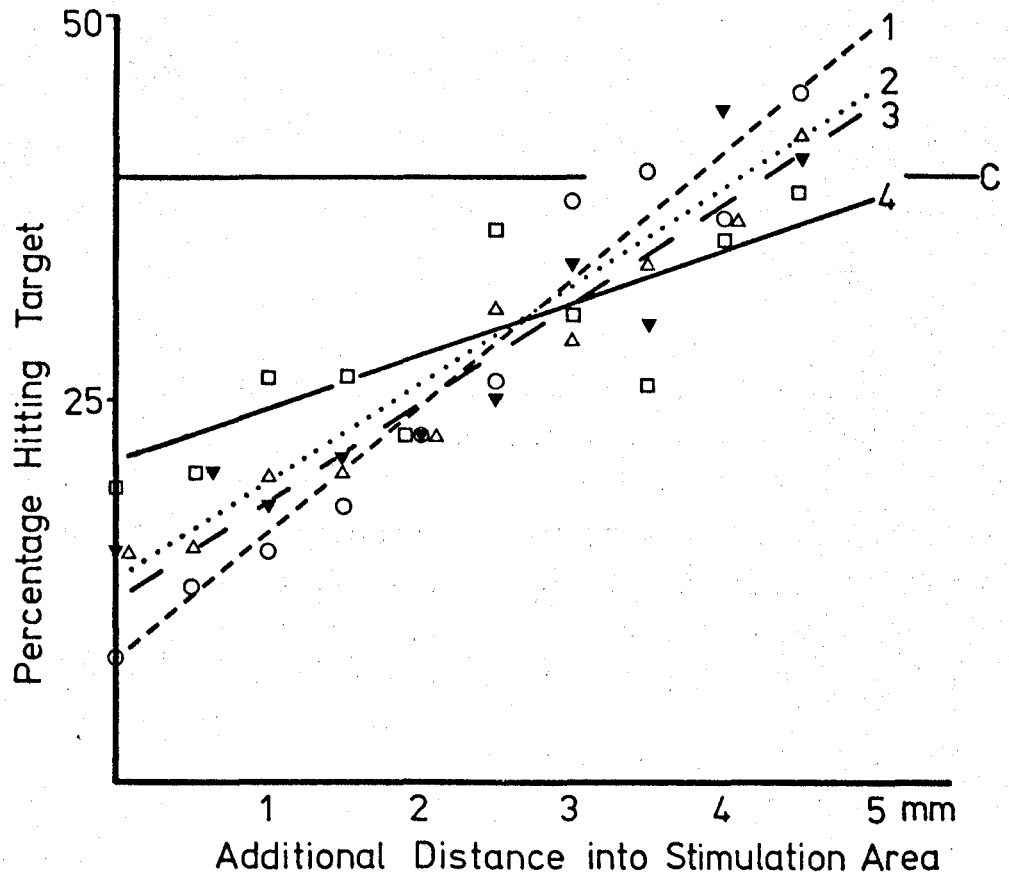
The percentage of tracks with different stimulated step lengths that hit the snail target when the delay, before stimulated tracks were generated upon entering the stimulation area, was increased. The horizontal line C represents the control level of hits (the percentage of tracks in Run 1, with a 30mm unstimulated step length and a 'pond' of 150mm, that hit the target).

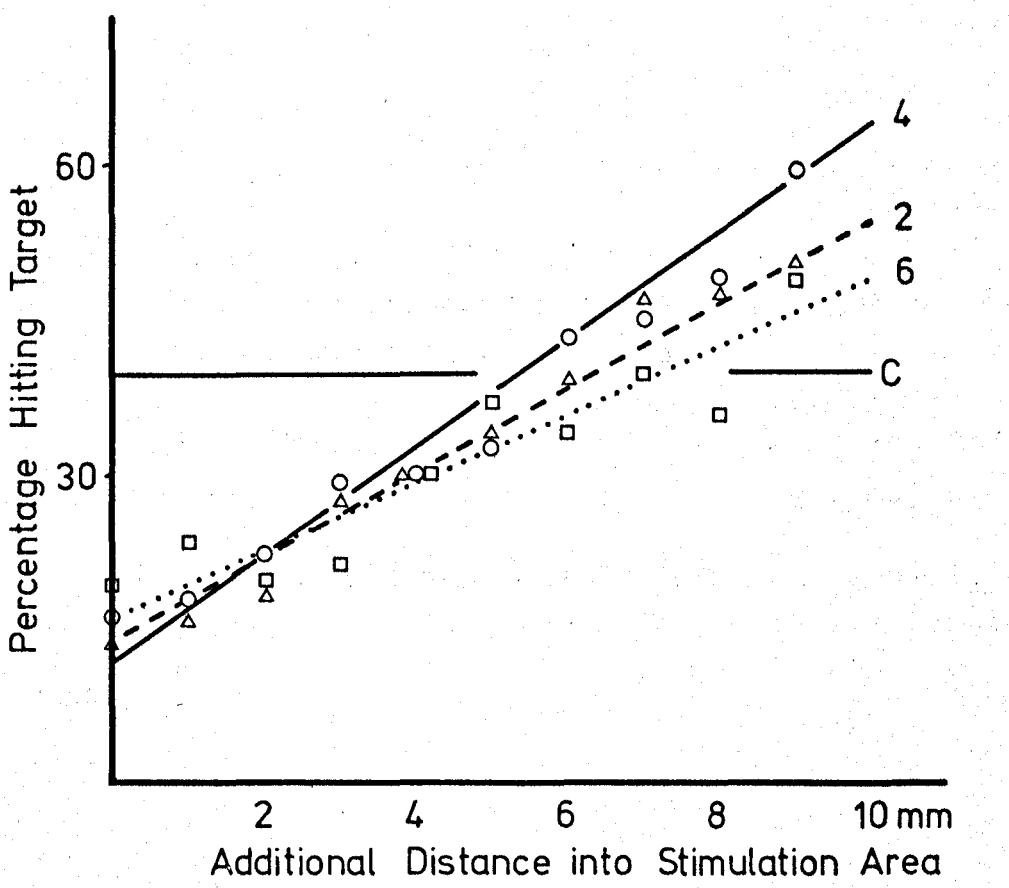
o - - - - - ostimulated step length of 1mm
△.....△ " " " " 2mm
▼— — ▼ " " " " 3mm
□————□ " " " " 4mm

FIGURE 3.3.13.

The percentage of tracks with different stimulated step lengths that hit the snail target when the delay, before stimulated tracks were generated upon entering the stimulation area, was increased. The horizontal line C, represents the control level of hits (see Fig. 3.3.12).

△ - - - - △ stimulated step length of 2mm
o———— o " " " " 4mm
□..... □ " " " " 6mm


50
25
Percentage Hitting Target
1
2
3
4
C
1
2
3
4
5 mm
Additional Distance into Stimulation Area


60
30
Percentage Hitting Target
4
2
6
C
2
4
6
8
10 mm
Additional Distance into Stimulation Area

lines were significantly different from the horizontal at the 5 percent level. In Fig. 3.3.12 (the run with a 5mm stimulation area), the slope of the regression lines for stimulated step lengths of 1, 2 and 3mm were not significantly different from one another at the 5 percent level (for statistical tests see Appendix 1). The slope of the regression line for a stimulated step length of 4mm was however, significantly different and less steep than the other three lines (at the 5 percent level). This implies that stimulated step lengths of between 1 and 3mm require less additional distance into the stimulation area than longer steps to raise the percentage of tracks hitting the snail target up to control levels. Thus, for a stimulation area of 5mm, it would appear that a distance of about 2mm between randomising turns is the most efficient pattern of behaviour for the contacting of the target. For a stimulation area of 10mm (Fig. 3.3.13), there was no significant difference between the slope of the regression lines for stimulated step lengths of 2 and 6mm and between 2 and 4mm, but a significant difference, at the 5 percent level, was detected between the slopes for step lengths of 4 and 6mm. Of all these regression lines, the graph for a stimulated step length of 4mm had the steepest gradient. Thus, for a 10mm stimulation area, it would appear that a distance of about 4mm between randomising turns is the most efficient pattern of behaviour pattern for the location of the target.

Run 5.

This run was devised to assess the effect of a period of continued stimulation after the track left the stimulation

area upon the percentage of tracks hitting the snail target. The results for a stimulation area of 5mm and a range of stimulated step lengths is shown in Fig. 3.3.14 and for a stimulation area of 10mm in Fig. 3.3.15. No significant difference at the 5 percent level, was found between the slope or intercept of the regression lines for each of the stimulation areas. It can be seen that even when the stimulated pattern of behaviour continued for over twelve seconds (64 mm) outside of the stimulation area, the control values of the number of tracks hitting the target were not reached.

Run 6.

This run combines both the delay before stimulation starts upon entering the stimulation area and the period of continued stimulation on leaving the area. This situation could occur in nature if the sensory cells of the miracidia adapted very slowly to a change in the stimulus intensity or simply if the reaction time of the sensory and motor systems of the larvae is slow. The results for a range of both of these parameters with a stimulation area of 5mm and a stimulated step length of 2mm is shown in Fig. 3.3.16, and for a stimulation area of 10mm and a stimulated step length of 4mm in Fig. 3.3.17. It can be seen that all graphs eventually rise above the control levels of hits and that it is probably the delay before stimulation is initiated that is the most important in the increasing of the chance of contacting the target.

FIGURE 3.3.14

The percentage of tracks with different stimulated step lengths that hit the snail target when the delay, before unstimulated tracks were generated upon leaving the stimulation area, was increased. The horizontal line C represents the control level of hits (see Fig. 3.3.12)

o- - - - - -ostimulated step length of 1mm
△·····△ " " " " 2mm
▼— — -▼ " " " " 3mm
□————————□ " " " " 4mm

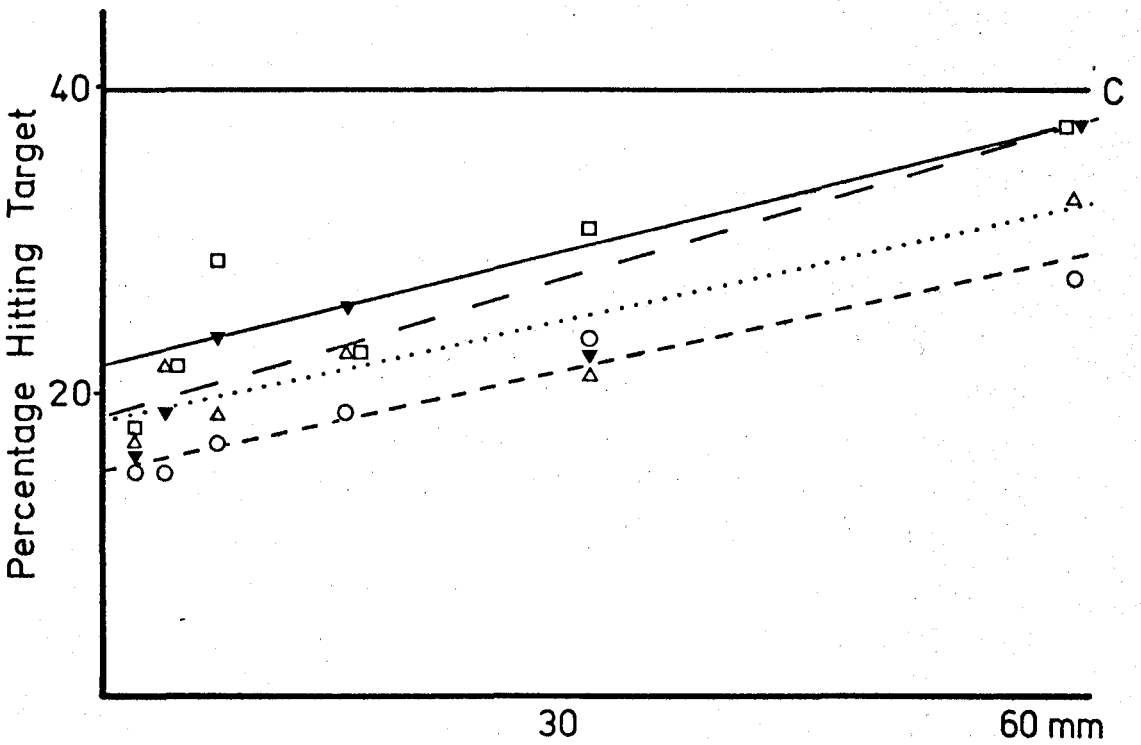
The stimulation area was set at 5mm.

FIGURE 3.3.15

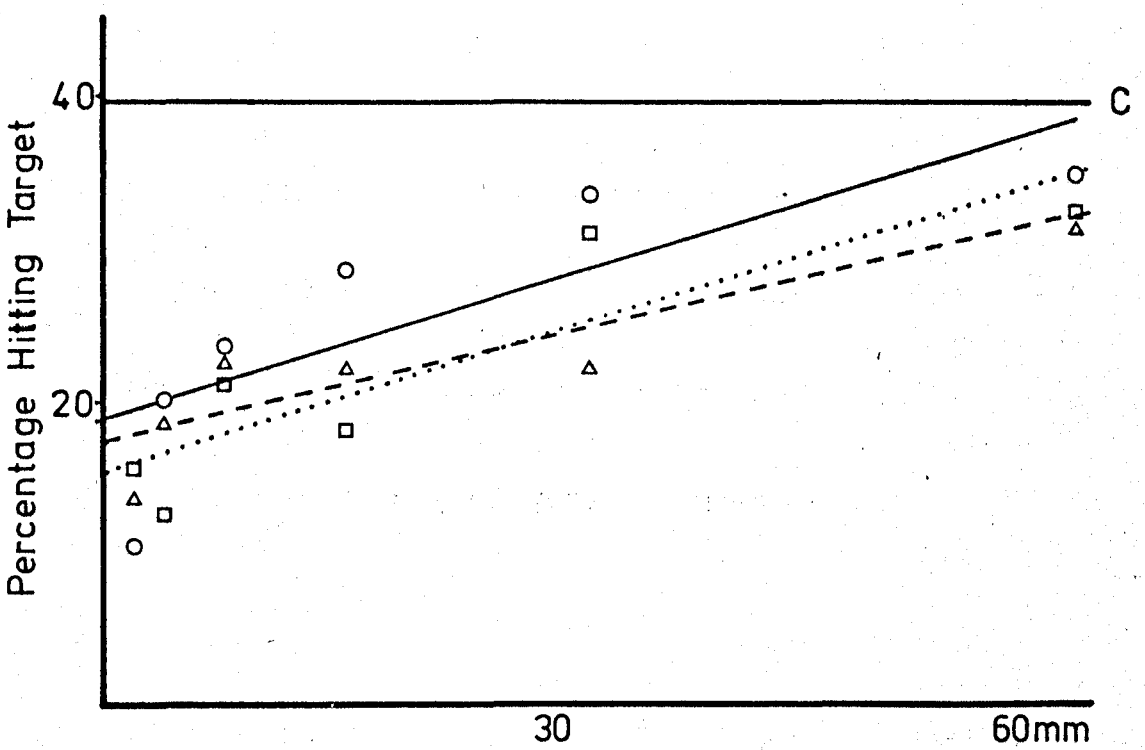
The percentage of tracks with different stimulated step lengths that hit the snail target when the delay, before unstimulated tracks were generated upon leaving the stimulation area, was increased. The horizontal line C represents the control level of hits (see Fig. 3.3.12)

△- - - - -△ stimulated step length of 2mm
o————————o " " " " 4mm
□·····□ " " " " 6mm

The stimulation area was set at 10mm.



Length of Additional Stimulated Track after Leaving the Stimulation Area



Length of Additional Stimulated Track after Leaving the Stimulation Area

FIGURE 3.3.16

The percentage of tracks with a stimulated step length of 2mm that hit the snail target for different delays before stimulated tracks were generated when the stimulation area of 5mm was entered, and as the delay, before unstimulated tracks were generated upon leaving the area, was increased.

o- - - -o1mm length of delay before initiation of stimulated tracks.

△·····△2mm " " " "

□— —□3mm " " " "

▼————▼4mm " " " "

● level of hits with no delays

FIGURE 3.3.17

The percentage of tracks with a stimulated step length of 4mm that hit the target for different delays before stimulated tracks were generated when the stimulation area of 10mm was entered, and as the delay, before unstimulated tracks were generated upon leaving the area, was increased.

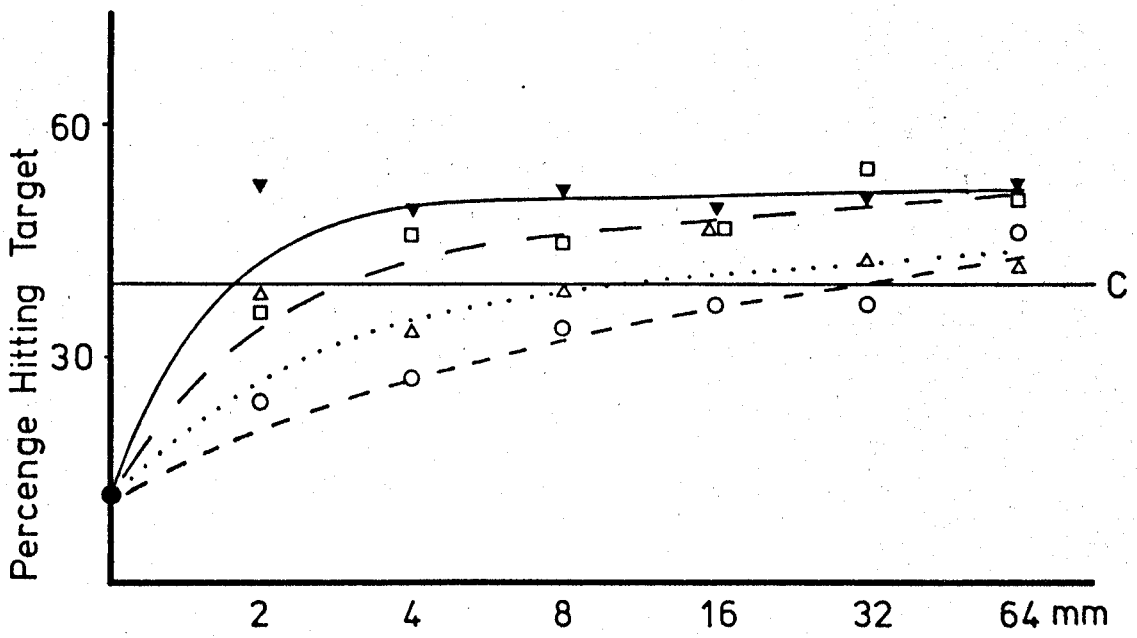
o- - - o2mm length of delay before initiation of stimulated tracks.

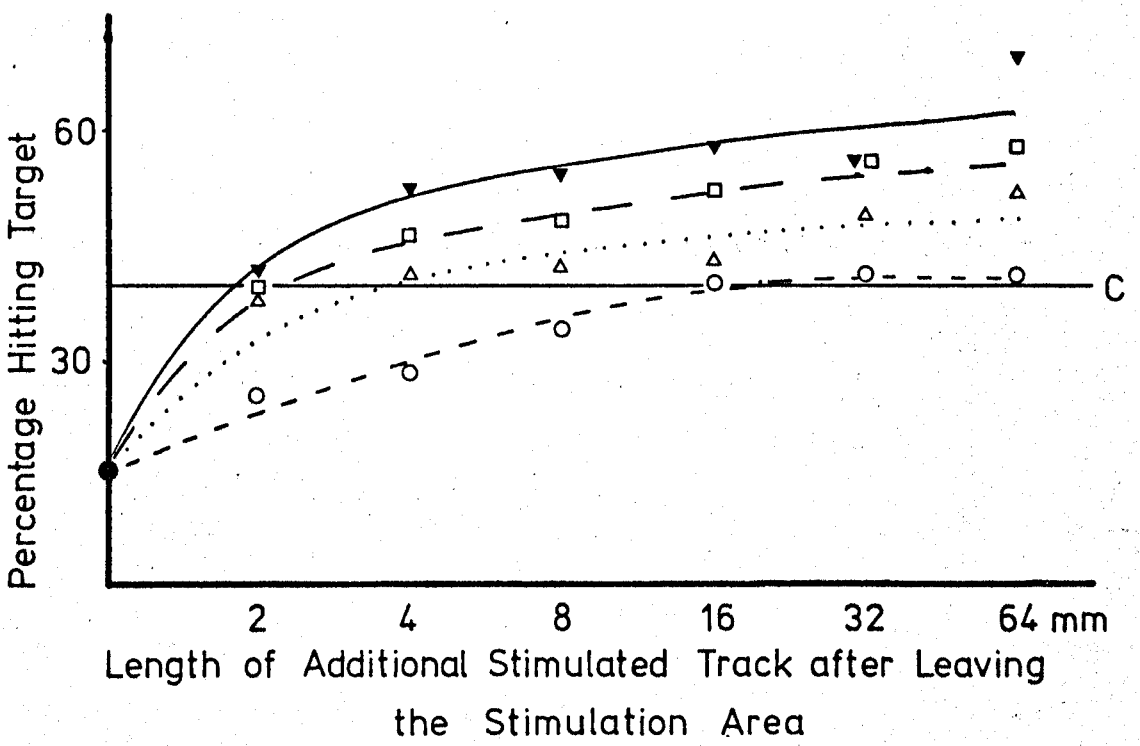
△····· △4mm " " " "

□— —□6mm " " " "

▼————▼8mm " " " "

● level of hits with no delays


Percenge Hitting Target
60
30
C
2
4
8
16
32
64 mm
Length of Additional Stimulated Track after Leaving the Stimulation Area


Percentage Hitting Target
60
30
C
2
4
8
16
32
64 mm
Length of Additional Stimulated Track after Leaving the Stimulation Area

3.4 MODELS OF CERCARIAL BEHAVIOUR.

3.4.1 CERCARIAL BEHAVIOUR - MODELS AND RESULTS.

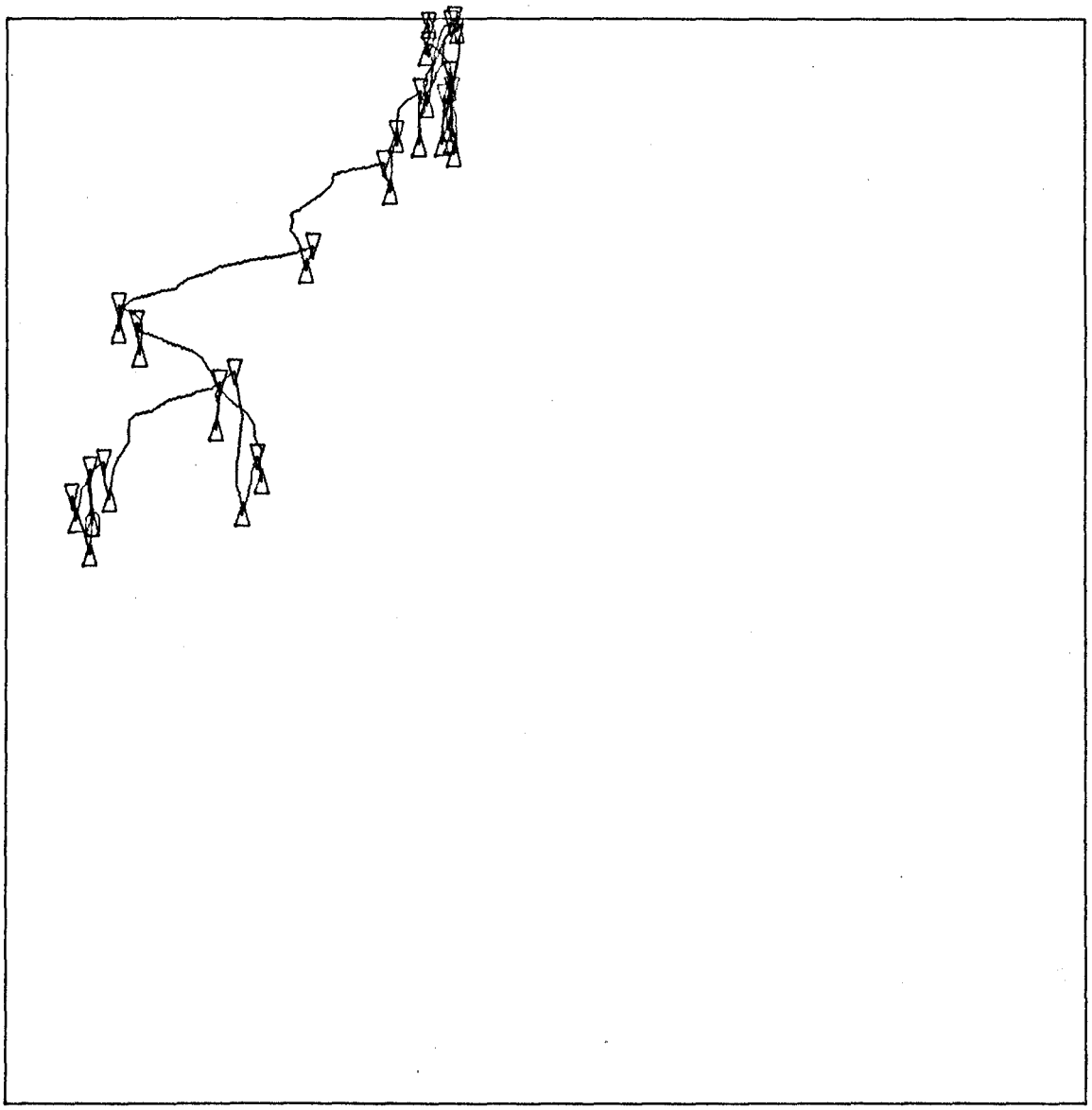
The general model of cercarial behaviour consists of a square area or 'pond' in the vertical plane which sets the bounds within which the tracks are generated. The model generates tracks of the unstimulated activity of the cercariae in two alternating phases, corresponding to the swimming and sinking of live larvae. Tracks in the swimming phase move upwards in the 'pond' within an angle of direction of between 90 degrees to the left and right of the vertical (see section 2.2.3). Tracks in the sinking phase move downwards in the cell within 20 degrees to the right and left of the vertical. If the track hits the top of the 'pond' while in the swimming phase, a switch to the sinking phase takes place. If the track hits the bottom of the 'pond' while in the sinking phase, a similar switch to the swimming phase takes place.

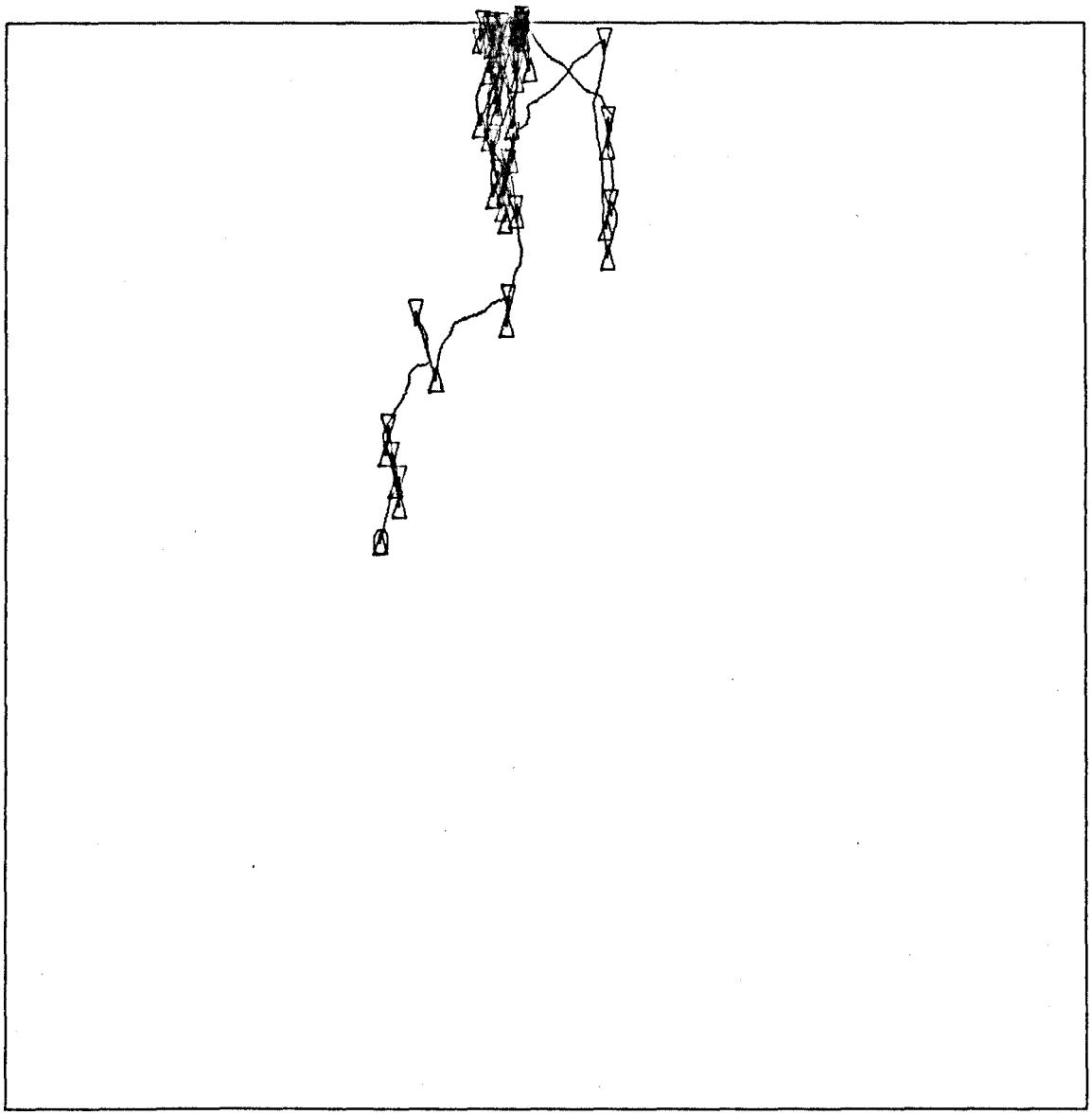
The data used to calculate the coordinates of the tracks in the swimming phase were taken from the track analysis of swimming cercariae. Similarly, the data used to calculate the coordinates of the sinking phase were taken from the track analysis of sinking cercariae. Sample graph plotter output of the tracks produced by the model of the unstimulated activity of the cercariae is shown in Fig. 3.4.1.

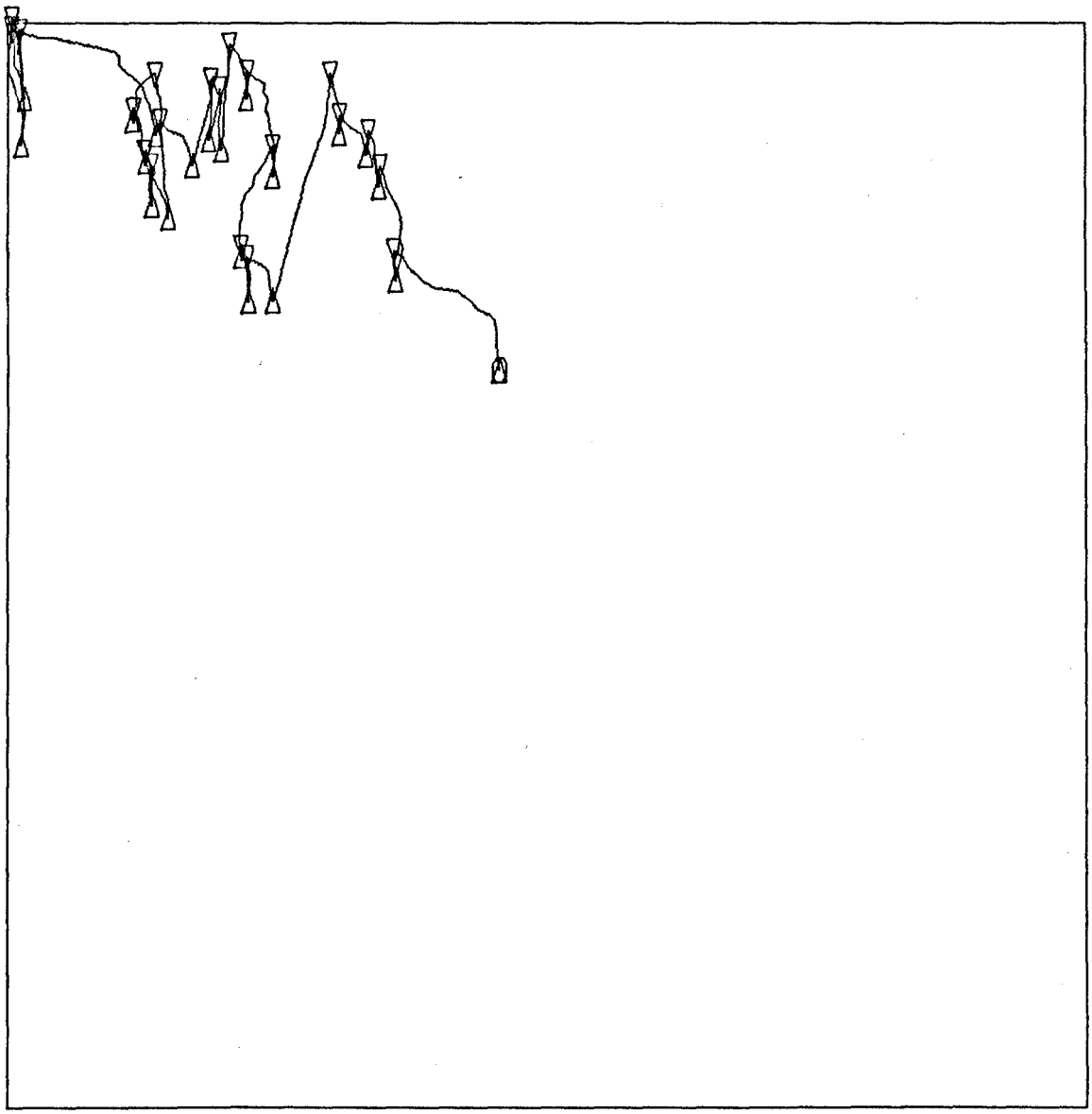
A model incorporating all the responses of the cercariae to host-generated stimuli was not attempted at this stage. The most important response in the short-range location of the host is the response of the cercariae to skin surface chemicals. The other responses all act to bring the

FIGURE 3.4.1

Sample graph plotter outputs from a model of unstimulated cercarial activity rhythms.


5mm


5mm


5mm

cercariae towards the surface, but do so with little lateral displacement (except the shadow response close to the surface). Assuming that the most common infection surface would be the limbs of a man standing in shallow water, the main areas of the exposed skin will be vertical. It is thus only the responses incorporating considerable lateral displacements which are likely to result in contact with the host. The models thus start as if the cercariae have already been brought towards the surface.

In the models, the host surface is represented by the left side of the 'pond' with a stimulation area extending away from it into the 'pond'. The stimulation area width was set at 10mm as, in the absence of information on the true distance from the host surface that chemicals could be detected by the larvae, this was the distance from a mouse tail in which the stimulated behaviour pattern was detected (see section 2.5). If a track enters this area, the model switches the data used to calculate the coordinates to the data produced by the track analysis of cercariae swimming in HCW with rapid mixing. The starting point for all tracks was towards the top of the 'pond' and at the edge of the stimulation area. The tracks were started in the unstimulated swimming phase and randomly set to initially move to either the left or the right of the vertical. This model thus represents the situation where a cercaria is suspended in the water when the limb of a man suddenly moves to within 10mm. As no information was available on the reaction of the cercariae to the skin chemical/water interface, two models were built, each representing different responses at the interface.

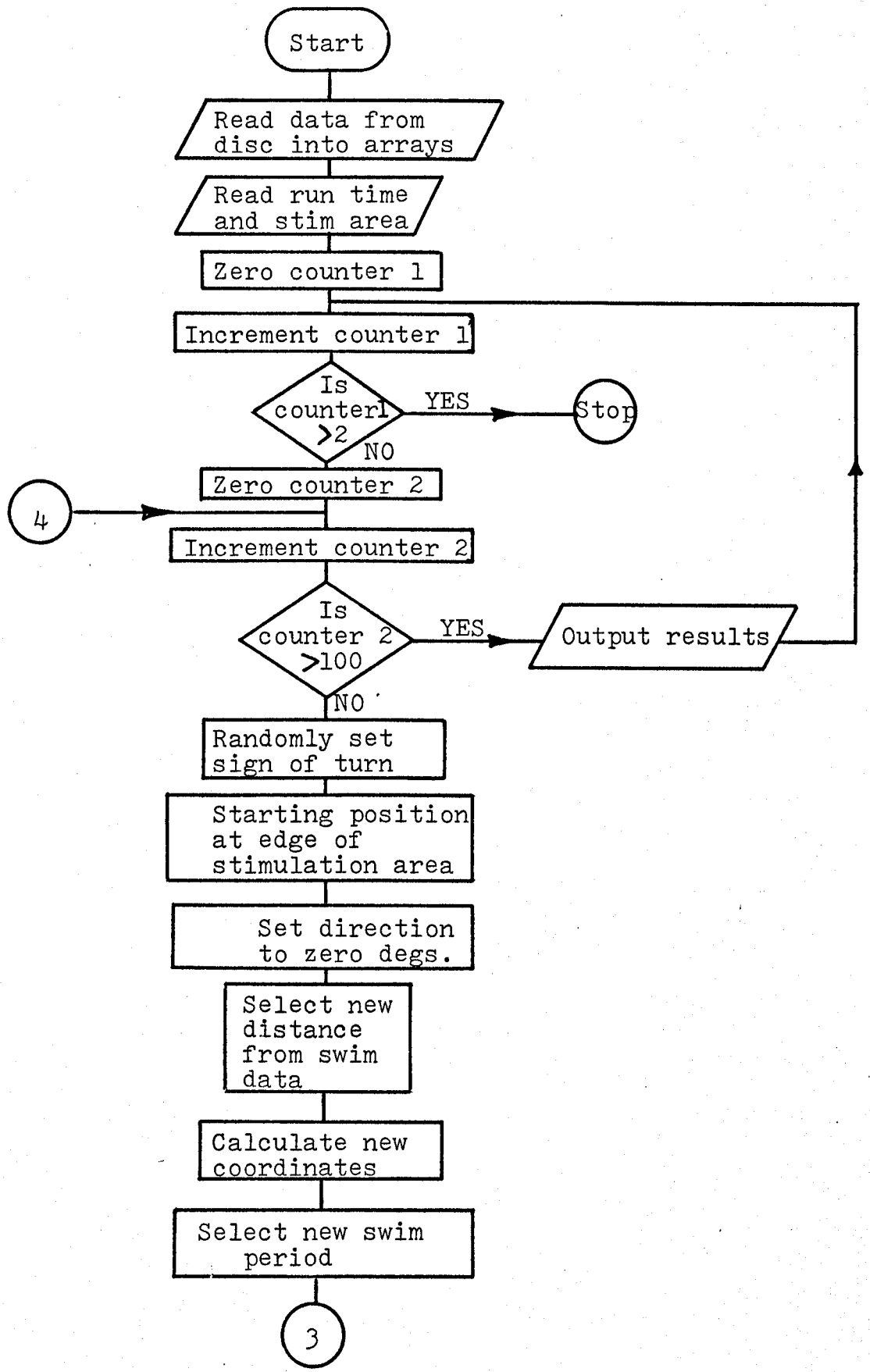
Model 1.

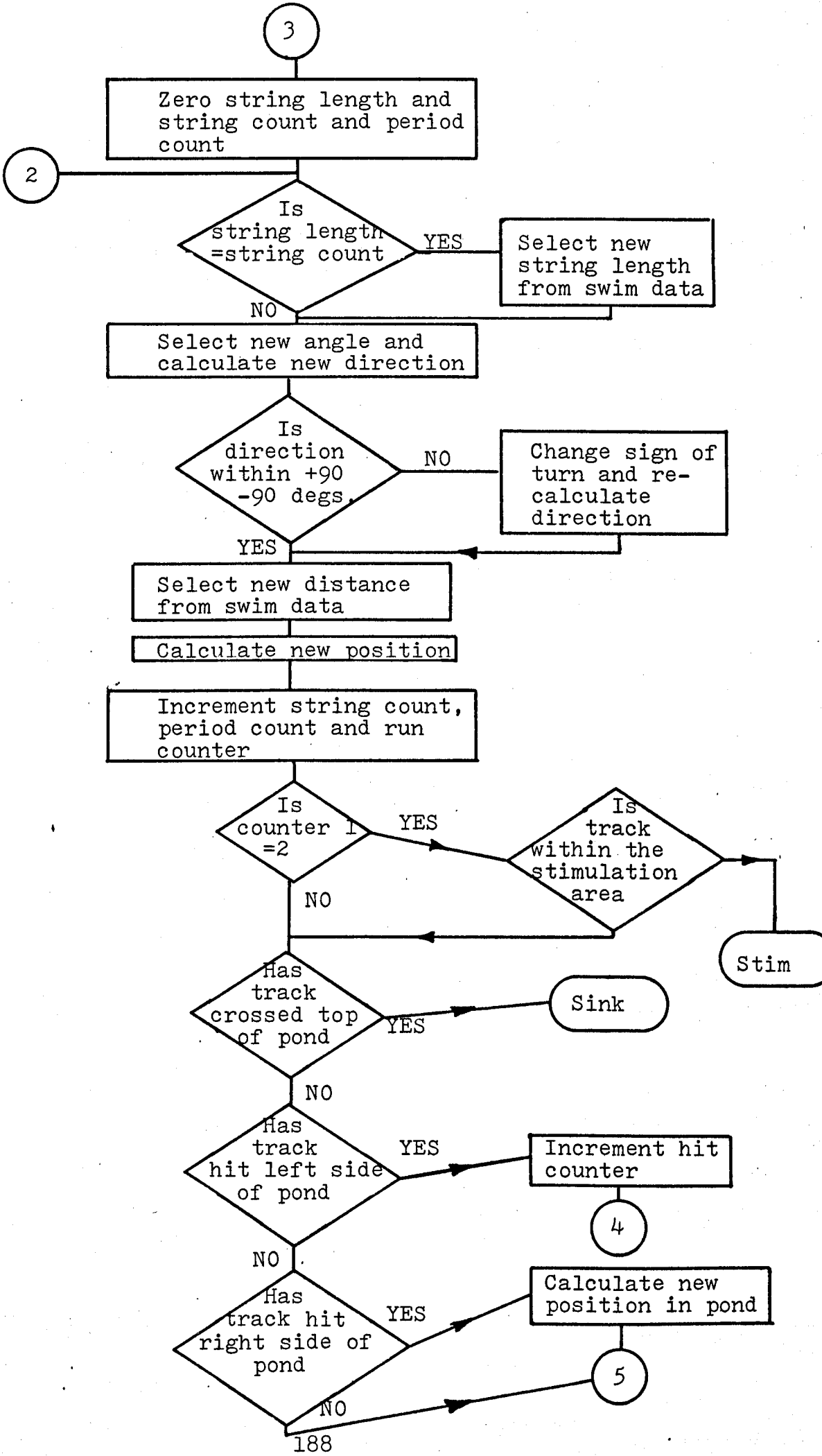
In this model, the track upon leaving the stimulation area reverted to the sinking phase of the unstimulated type of activity pattern. The track only changed to the stimulated pattern again, if the stimulation area was re-entered. The model represents the situation where a limb has entered the water within one centimetre of a cercaria and the resulting turbulence has produced an area of relatively uniform skin chemical concentration around the limb. A simplified flow diagram of the model is shown in Fig. 3.4.2 and graph plotter output is shown in Fig. 3.4.3. The proportion of tracks hitting the target surface was estimated by running the model 100 times and recording the time taken for each track to hit the left side of the "pond" within an equivalent of ten minutes. Ten minutes is probably much longer than the host is likely to remain close to a cercaria in nature, but as the time taken to hit the target was recorded, the results can be analysed for any period of host exposure up to ten minutes. Thus, if the proportion of hits within one minute is considered, all runs in which the track took longer than one minute to hit the target are scored as misses (because, in nature, the host would no longer be present). The results are shown in Fig. 3.4.4. The same model, when run with no stimulation occurring in the stimulation area (the tracks always stayed in the normal swimming and sinking phases) resulted in only 10 percent of the tracks hitting the target within the equivalent of ten minutes. However, over 25 percent of the tracks incorporating the response to skin surface chemicals contacted the target within the equivalent of

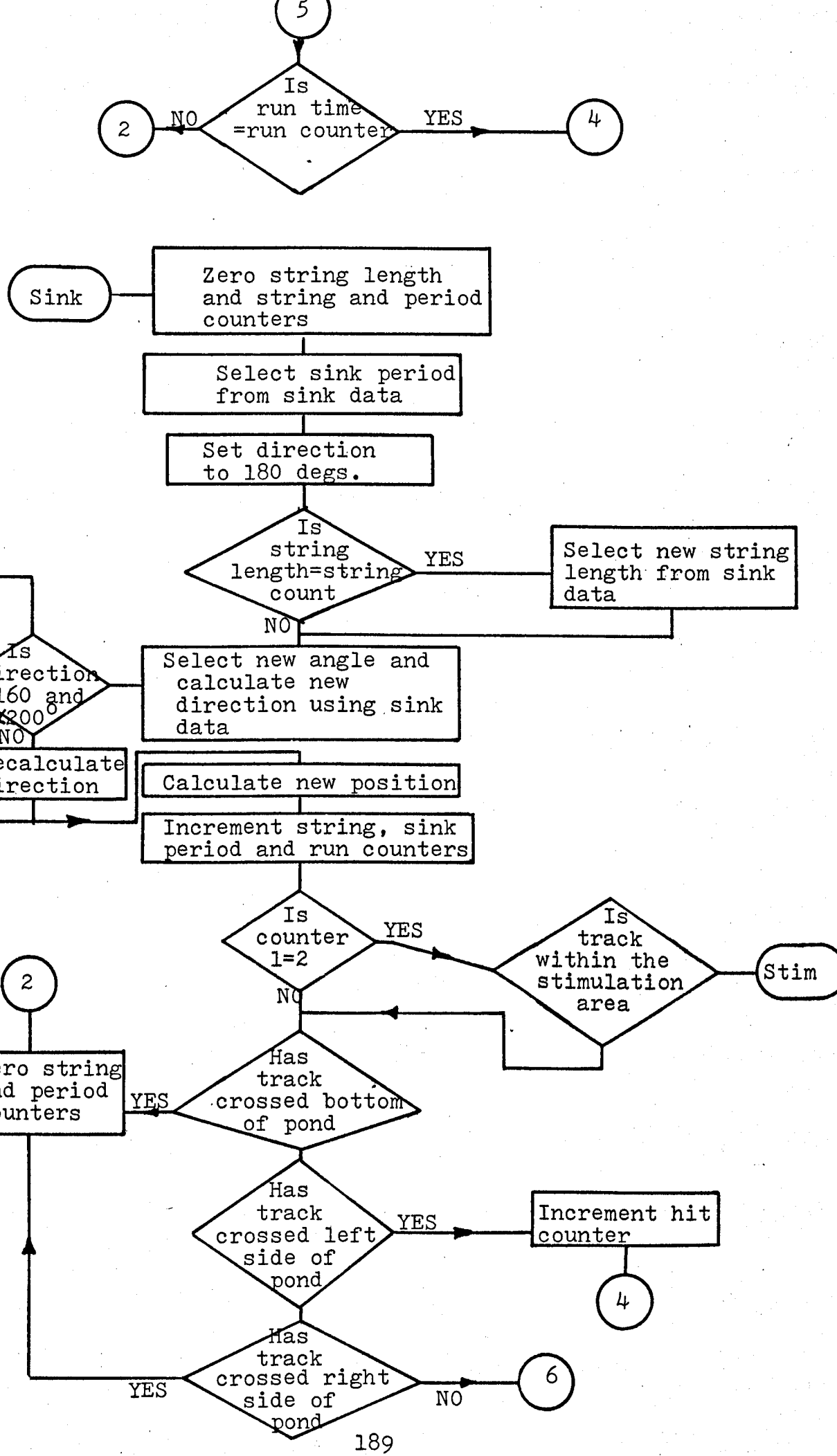
two minutes. Clearly the response to the presence of skin surface chemicals increases the chance of a cercaria contacting the host.

FIGURE 3.4.2

Simplified flow diagram of cercarial Model 1




3
Zero string length and string count and period count
2
Is string length =string count
YES
Select new string length from swim data
NO
Select new angle and calculate new direction
Is direction within +90 -90 degs.
NO
Change sign of turn and re-calculate direction
YES
Select new distance from swim data
Calculate new position
Increment string count, period count and run counter
Is counter 1 =2
YES
Is track within the stimulation area
NO
Stim
Has track crossed top of pond
YES
Sink
NO
Has track hit left side of pond
YES
Increment hit counter
4
NO
Has track hit right side of pond
YES
Calculate new position in pond
5
NO


5
Is run time =run counter
NO
YES
2
4
Sink
Zero string length and string and period counters
Select sink period from sink data
Set direction to 180 degs.
Is string length=string count
YES
NO
Select new string length from sink data
YES
Is direction >160 and <200
NO
Select new angle and calculate new direction using sink data
Recalculate direction
Calculate new position
Increment string, sink period and run counters
Is counter 1=2
YES
NO
Is track within the stimulation area
Stim
2
Zero string and period counters
Has track crossed bottom of pond
YES
Has track crossed left side of pond
YES
Increment hit counter
4
Has track crossed right side of pond
YES
NO
6

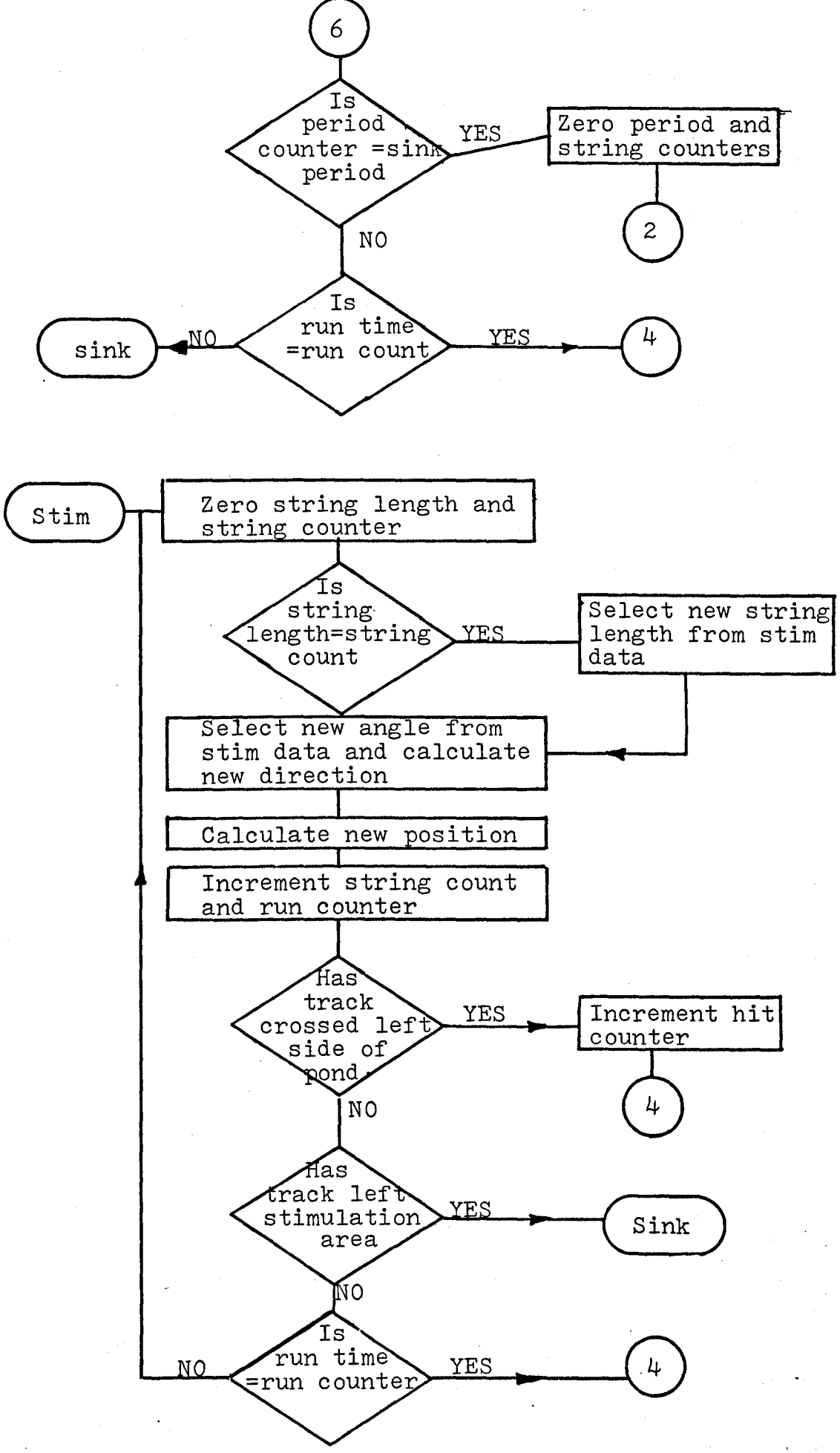
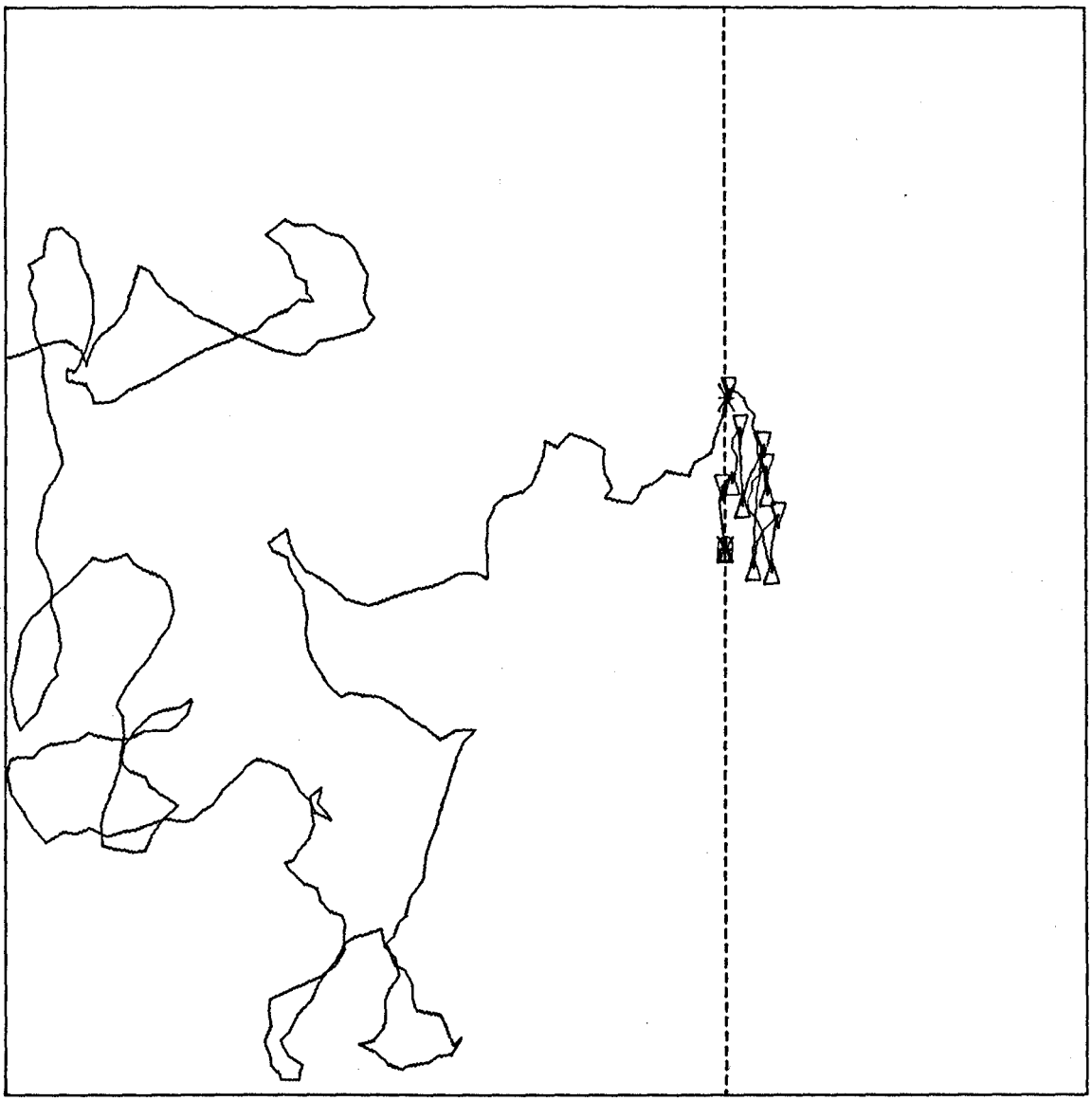
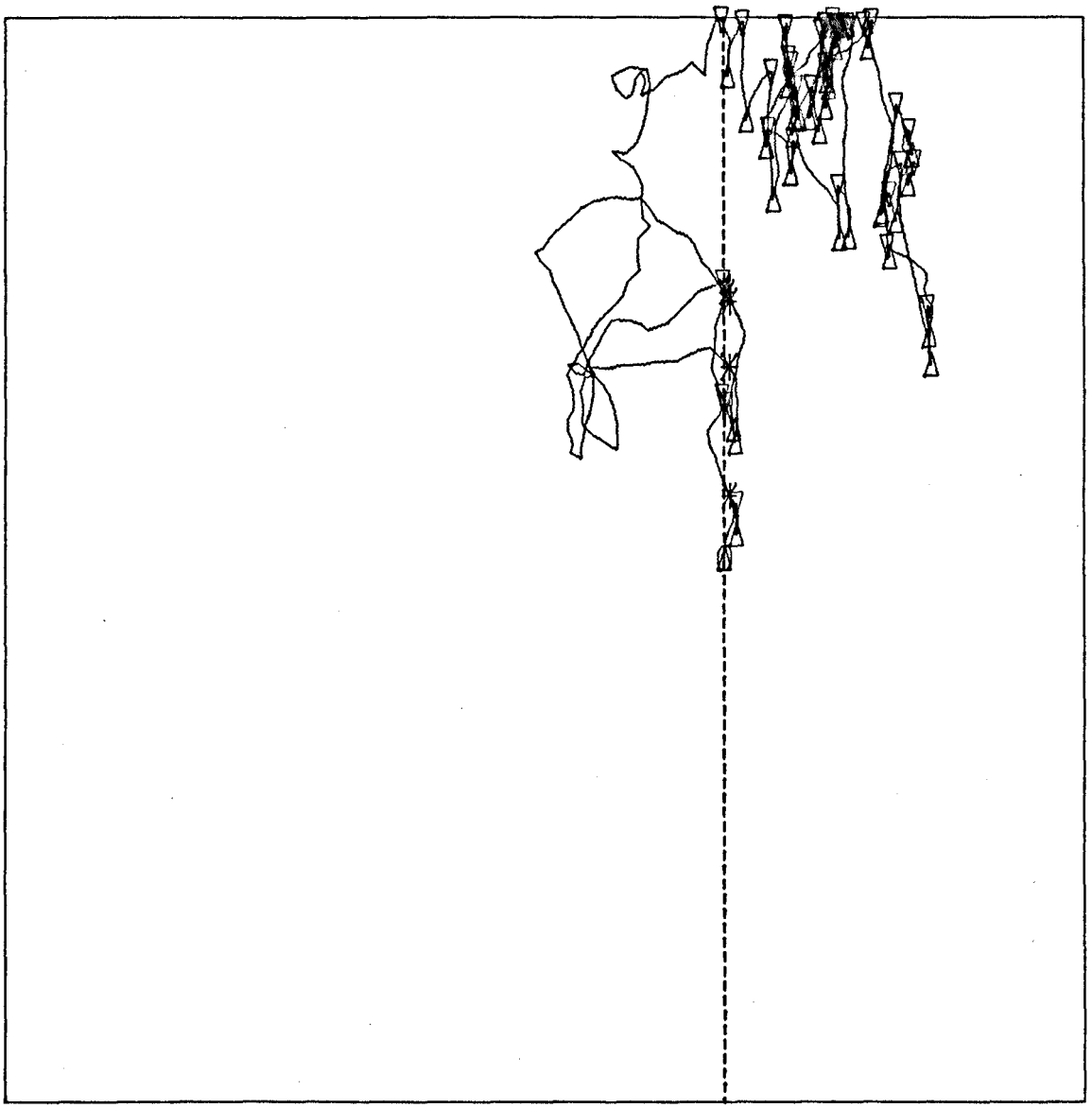

6
Is period counter =sink period
YES
Zero period and string counters
2
NO
Is run time =run count
NO
sink
YES
4
Stim
Zero string length and string counter
Is string length=string count
YES
Select new string length from stim data
Select new angle from stim data and calculate new direction
Calculate new position
Increment string count and run counter
Has track crossed left side of pond
YES
Increment hit counter
4
NO
Has track left stimulation area
YES
Sink
NO
Is run time =run counter
NO
YES
4

FIGURE 3.4.3

Typical graph plotter output from cercarial Model 1.


5mm


5mm

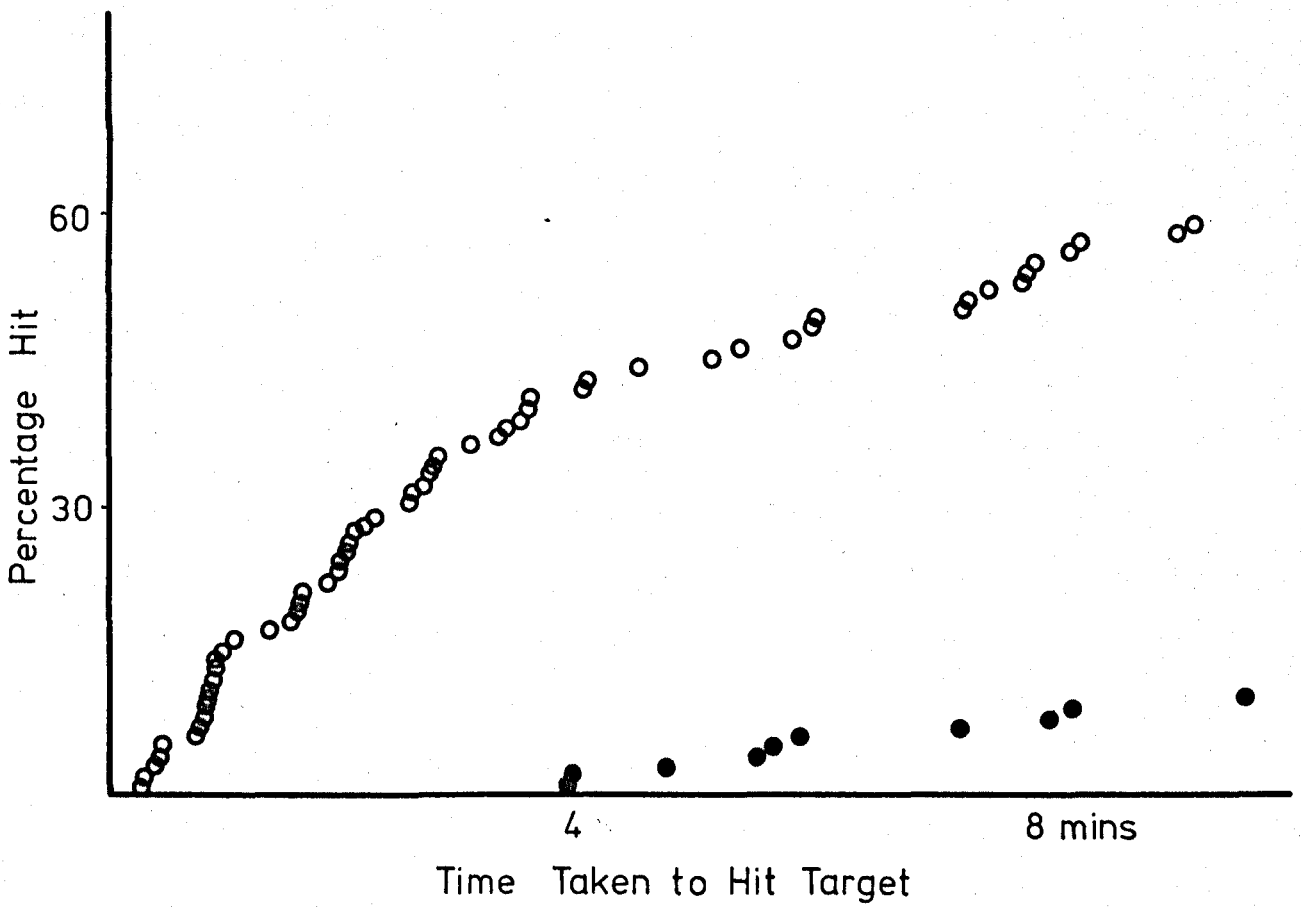

5 mm

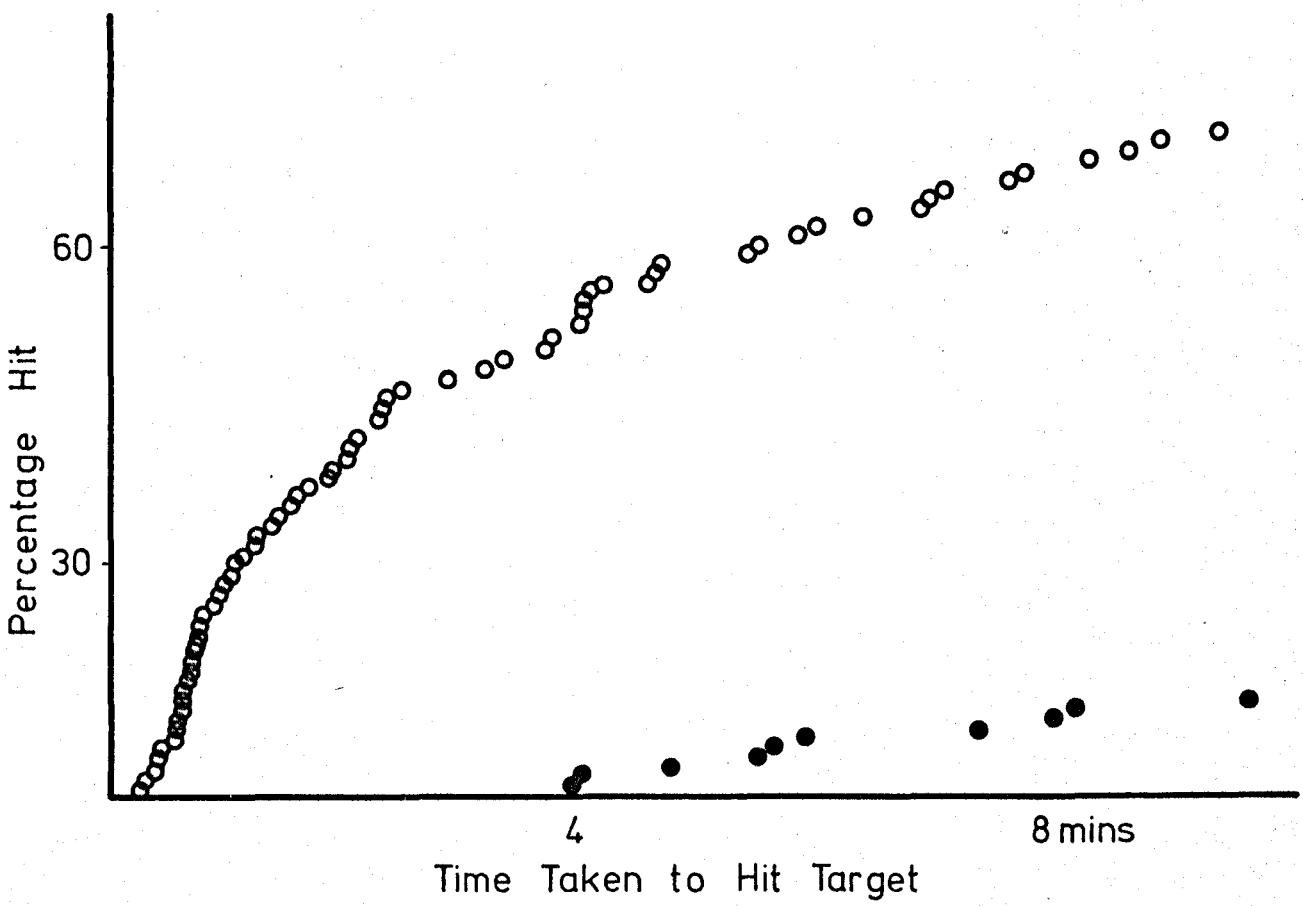
FIGURE 3.4.4

The percentage of tracks hitting the target surface for cercarial Model 1. The filled circles show the percentage of hits of unstimulated tracks.

FIGURE 3.4.6

The percentage of tracks hitting the target surface for cercarial Model 2. The filled circles show the percentage of hits of unstimulated tracks.


60
30
Percentage Hit
4
8 mins
Time Taken to Hit Target


60
30
Percentage Hit
4
8 mins
Time Taken to Hit Target

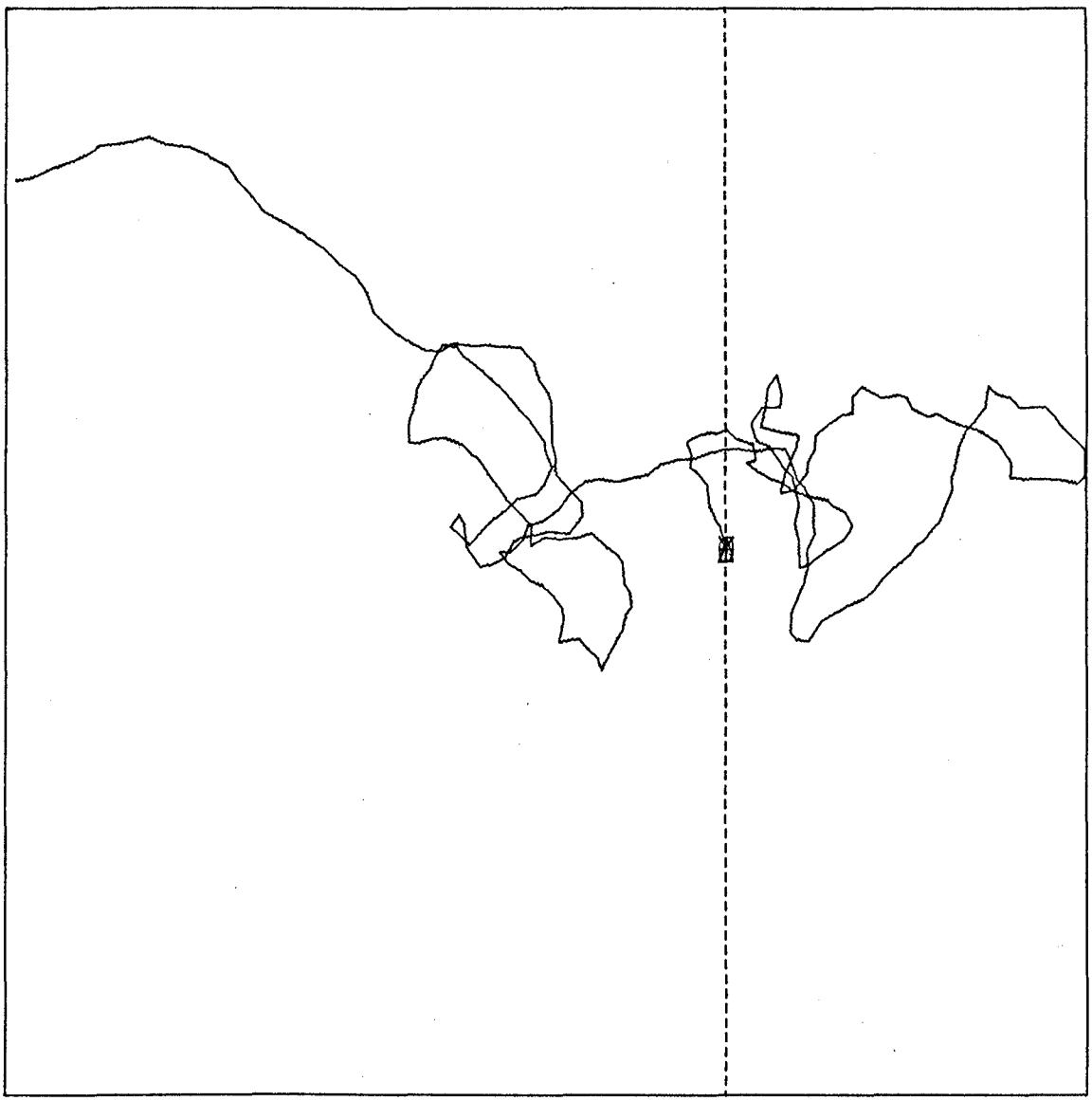
Model 2.

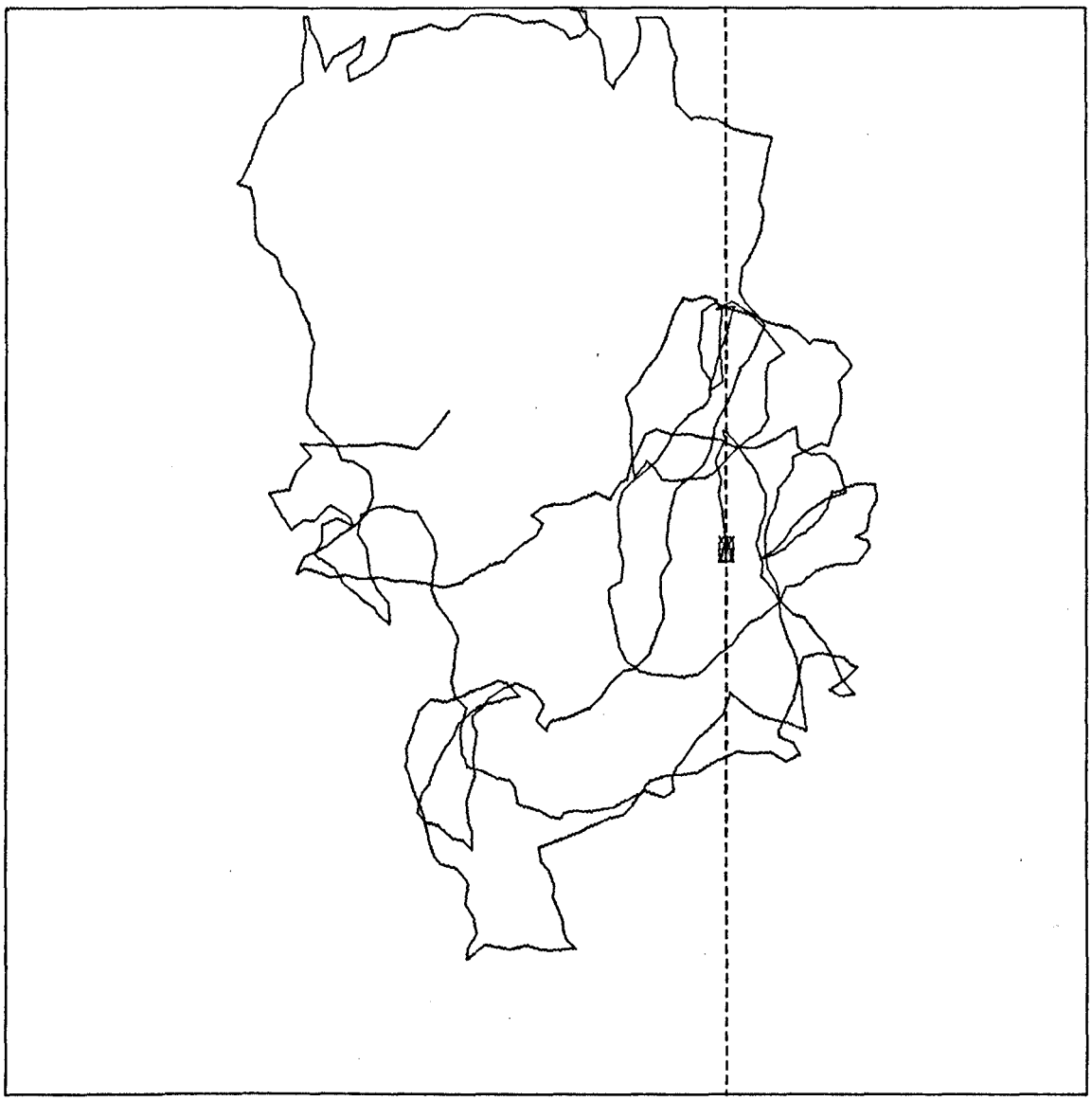
In this model, the track remains in the stimulated phase even when it leaves the stimulation area. This corresponds to the situation where either the cercariae are triggered into activity and remain stimulated until adaptation returns them to the basal level of activity or where the turbulence results in the stimulant chemical being present over greater distances than 10mm from the skin surface. Again, a long run time was used, but as the time taken for each track to hit the target was measured, the effect of shorter run times can be assessed. A shorter run time could represent the situation where either the host moves out of the area or the cercaria quickly adapts back to its unstimulated state.

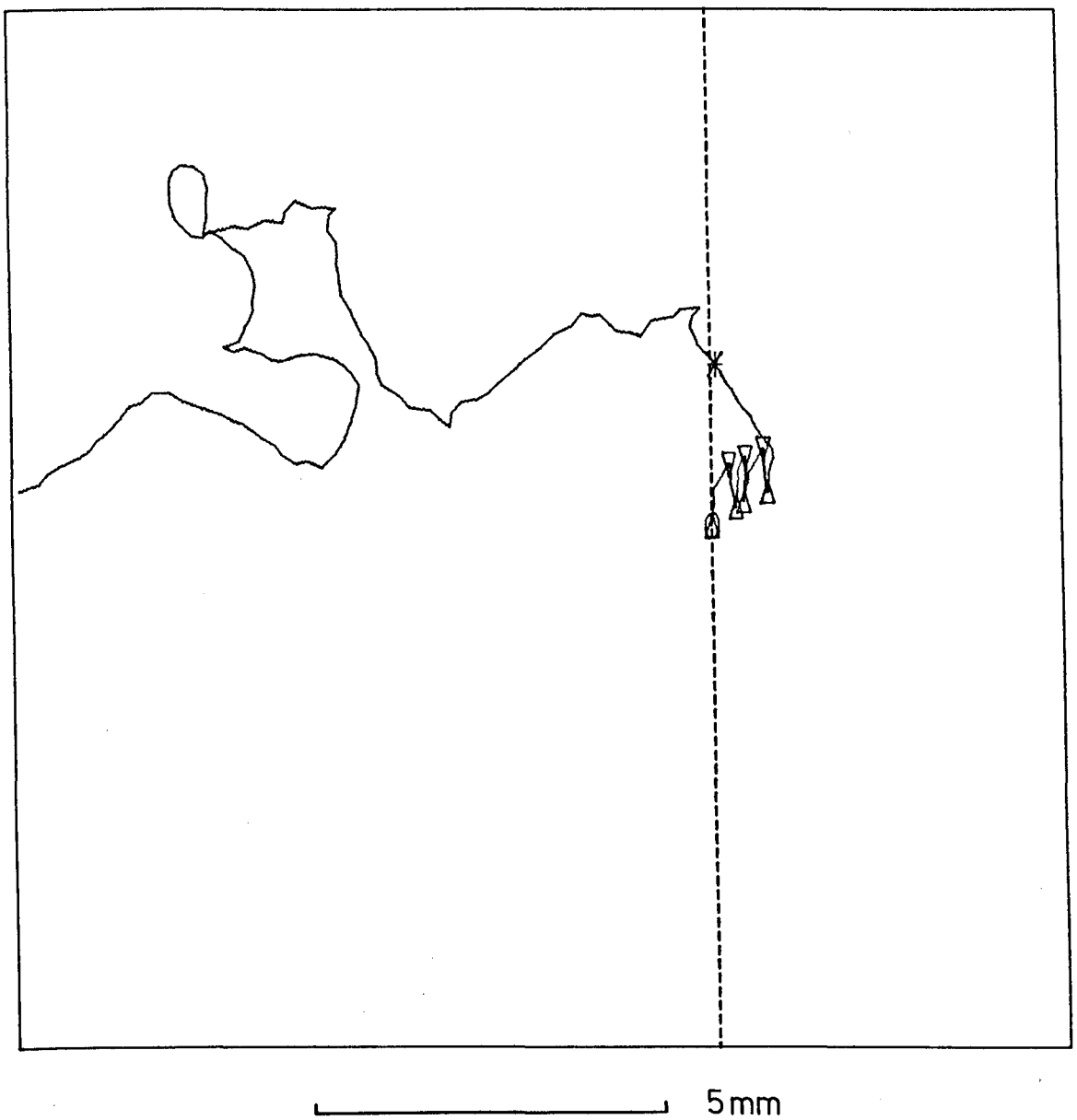
Graph plotter output from this model is shown in Fig. 3.4.5 and the results from the model run to assess the proportion of tracks hitting the target are shown in Fig. 3.4.6. It appears that in this situation, more tracks contacting the target surface did so in a shorter time than the tracks of Model 1. 25 percent of the tracks contacted the surface within the equivalent of one minute of the host appearing. Once again, the incorporation of the response to host skin surface chemicals results in an increased chance of contact.

FIGURE 3.4.5

Typical graph plotter output from cercarial Model 2.


5mm


5mm


5mm

3.5 DISCUSSION AND CONCLUSIONS.

In this chapter, I have demonstrated that simulation modelling using the computer, will recreate tracks of the swimming of larvae that bear a close resemblence to natural tracks. Tracks can be generated that correspond to the response of the larvae to a stimulus. However, the combination of different behaviour patterns into a model of the host location process has indicated that certain behavioural responses (ie. sensory adaptation or reaction time) are involved. These sort of responses would not normally be included in a proposed mechanism of host-location.

The models of the behaviour of miracidia showed that the unstimulated swimming is essentially a series of straight tracks between which the direction varied within limits. Close to a snail, the swimming is essentially a series of short straight tracks between which the change in direction is totally random. However, the increased turning close to the snail did not seem to act as an efficient host-location mechanism. In most cases, the inclusion of this turning response in the model, decreased the chance of the track hitting the snail target. I feel that it is very unlikely that this reflects the true situation. For a behavioural response to evolve, it must, at some time in the evolution of the organism, have a selective advantage. Thus the turning response of the miracidium in the vicinity of the snail ought to increase its chance of contact.

There are two main areas where the model is a poor analogue of nature These are, that the model only operates in two dimensions and also that the response of a miracidium as it crosses into or out of an area of stimulation was unknown.

The models only consider the infection process as if it occurred in the horizontal plane. As a result, any track which is moving towards the snail target will stand a good chance of hitting the snail target. In three dimensions, the same track might pass above or below the target and contact would not be made. This lack of the third dimension would, in particular, increase the proportion of the unstimulated tracks which hit the target. However, it is quite possible that the turning of the stimulated tracks when passing directly above or below the target might increase the chance of contact. A series of experiments where miracidia are tracked and analysed while swimming in the vertical plane would clearly help to resolve this problem.

The models I have used were seriously hampered by the lack of a detailed analysis of the response of the miracidia as they entered or left the area of stimulation. As the stimulant chemical has not been clearly identified, the position of the stimulant/water interface cannot be measured. The models however, suggested that a delay, both before a reaction occurred upon entering the area of stimulation and before stimulation ceases after leaving the area, would act together to increase the chance of contact. Two physiological processes could cause such an effect.

Firstly, the sensory cells of the miracidium could show a slow rate of adaptation to the change in the intensity of the stimulus. Secondly, there could be a long reaction time between the detection of the change in the stimulus intensity and the contraction of the muscle fibres responsible for the alteration of the swimming pattern.

I would suggest that it is the reaction time of the motor systems of the miracidia that is responsible for delays in the reaction of miracidia to snail chemicals. Miracidia propel themselves through the water using their ciliated epithelium. Changes in direction are not caused by the differential beating of the cilia, but by the bending of the body. Here, I should point out that MacInnis (1965), Sponholtz and Short (1975) and Mason and Fripp (1976) all refer to the spiralling of the miracidia as they swim. Certainly miracidia follow an undulating path as they swim, but I have not been able to detect any complete rotations of the miracidium on its longitudinal axis. A slight oscillation about this axis does occur, suggesting that the larvae do have a positional sense. The miracidium of Fasciola hepatica also behaves in this way as can be easily demonstrated by observing the position of its pigmented eye spots. At a very low temperature, these miracidia will however, start to spiral (R.A.Wilson, personal communication). The undulating pattern of swimming can be seen in the dark-ground photographs of Wilson and Denison (1970a), Mason and Fripp (1976) and Mason (1977).

These undulations in the swimming pattern of miracidia provide some evidence for the reaction time hypothesis.

The tracks presented by Wilson and Denison (1970a), for the miracidium of F.hepatica, show larvae orientating to a directional light source. These tracks show approximately three undulations per second. Each undulation represents a deviation of about 20 degrees from a straight path. The undulations are probably caused by the bending of the body to correct for deviations away from the intended direction of movement. A correction for a displacement to the right will eventually lead to a displacement to the left of the true path and vice versa. The undulations are thus a property of the speed at which the miracidium can detect a change in the intensity or direction of the stimulus and then change its direction by bending its body. At low temperatures, the wavelength of these undulations should be long, as the reaction time will also be slow. The converse should be true at high temperatures. The rate of change of direction of the above miracidia is thus 60 degrees per second (three times 20 degrees). If miracidia respond to the presence of the stimulant chemical in a similar way, but by turning through, for example 90 degrees, the reaction time would be in the order of one to two seconds. It is quite possible that the reactions of miracidia to a non-directional stimulus would be slower than the response to a directional stimulus.

A future experiment, to study the reactions of miracidia to non-directional stimuli, could be performed by recording the behaviour of the larvae as they swim from an area of low light intensity into an area of high light intensity. A low temperature would be used for this experiment, as this should increase the length of any reaction time and so make

the detection and measurement of this delay easier. The changes in the possible reaction time at different temperatures might not necessarily affect the host location response. As the temperature drops, the reaction time would increase and the swimming speed would decrease. Thus the distance travelled by a miracidium into the area of stimulation due to the reaction time would probably be similar to the distance travelled by a miracidium swimming faster but with a shorter reaction time, at a higher temperature.

There is some evidence for the existence of reaction time at a physiological level. Wilson (1969) found that the musculature of the miracidium of F.hepatica was unstriated. The longitudinal musculature (which would be responsible for the bending of the body) consisted of 12 to 15 muscle fibres which branched towards the anterior end. Each individual fibre was about 130μ in length. The muscle fibres were similar in appearance to smooth muscle. Smooth muscle contracts at a much slower rate than striated muscle and a period of one second or more for an unloaded muscle to shorten to two thirds of its original length is not uncommon (see Bülbring et al, 1970). Assuming that the musculature of the miracidium of S.mansoni is similar to that of F.hepatica, the contraction of the fibres while a miracidium was turning could easily be in the region of a few seconds.

At a particular temperature and in the same infection situation, the duration of the delay before a response occurred would probably be a constant. Thus, there should be an optimum size for the area of stimulation in which this delay would be the most efficient at increasing the

chance of contact (simplified model, Run 4). According to the results of the model, this stimulation area should be twice the distance travelled during the reaction delay. The models also predicted that the optimum stimulated step length (distance between periods of continued turning) was when the step length was half the distance between the snail and the edge of the stimulation area. Examination of the graph plotter output of Model 3, suggests that the stimulated step length is about 2 or 3mm. This gives a value of between 4 and 6mm for the size of the stimulation area. Clearly these values are only a rough estimate of the size of the area of stimulation but are in the order of the area of mixing found at the sides of a snail in section 1.3.3. Several factors will affect the size of this area of mixing, of which snail size and environmental temperature are probably the most important. However, the true nature of the relationship of the behaviour of the miracidia and the size of the area of stimulation cannot properly be studied until the stimulant chemical has been identified.

The models have thus shown that an increase in turning, upon the detection of host chemicals, does not in itself increase the chance of a miracidium contacting its host. The importance of sensory delays, adaptation or reaction time was also indicated. The hypothesis that the increase in the rate of turning close to the snail keeps the miracidium in the vicinity of the host and so increases its chance of contact has not been rejected, but the details of the supposed mechanism of host-location has been subjected to considerable modification.

The results from the models of cercarial behaviour have shown that the change from the normal pattern of upward swimming and downward sinking to the random direction of the prolonged swimming in the presence of host skin surface chemicals, increased the chance of the cercariae contacting a vertical skin surface. The models were again hampered by the lack of information of the reaction of the cercariae as they swam into or out of an area of stimulation. Until the stimulant chemical (or chemicals) has been identified, the response of the cercariae to the true stimulant/water interface cannot be recorded.

The two models, incorporating two different responses at this interface, both showed a great increase in the number of tracks contacting the target when compared to the situation where no stimulation took place. Thus, it is the increase in the duration and randomness of direction of swimming that is important in the contacting of a vertical surface, even though many of the cercariae will swim away from the host and not towards it.

It is interesting to note that, in Model 2, many of the tracks hit the target surface faster than the tracks of Model 1. It was expected that the reverse would have been true because when the tracks of Model 1 left the stimulation area, a sinking phase was initiated, so 'trapping' the track close to the interface. When the tracks of Model 2 left the stimulation area they continued to be generated as if the stimulation continued. The tracks would thus move on, away from the target surface and the chance of them then contacting the target was expected to be decreased. It is probably the long time taken to complete

a sinking phase and so reenter the stimulation area in Model 1 that was responsible for the increase in the time taken for these tracks to contact the target.

The percentage of the tracks hitting the target is obviously highly dependant upon the length of time which is allowed for contact. However, the majority of the tracks which hit the target within the equivalent of ten minutes, did so in a reasonably short time (<4 minutes). This prediction of the model agrees well with the behaviour of the host. Human contact with the water is a brief and unpredictable event and the presence of the host near to a cercaria would occur for only a short time. On this scale, even four minutes would be rather a long time. It is thus of the utmost importance for a cercaria, having detected the presence of the host, to contact the skin surface in the shortest time possible. In the absence of directional stimuli, the response of the cercariae to the host, in particular to skin surface chemicals, works well to achieve this end.

These models were constructed with a vertical surface as the host target. It is most likely that vertical surfaces do represent the most common infection sites for the cercariae. The majority of human contact with the water will be in small streams or ponds or at the edges of deeper rivers or lakes. Generally, it will be in the shallower areas where human activity will be concentrated, snails will become infected and the highest concentrations of cercariae will be found. At these sites, humans would be wading or bending with their arms in the water. Thus, most of the accessible areas of skin will be vertical in

the water. If the skin surface was horizontal, the normal behaviour pattern of the cercariae and the responses to shadows and turbulence would make the chance of contact very likely. Attachment would then occur in response to skin chemicals and temperature by the behaviour patterns described by Haas (1976).

The location of the host in moving water is a more complex situation to try and understand. As already mentioned, it is at the edges of rivers or in small streams that infection is likely to take place and here the rate of flow will be fairly gentle. Because the cercariae will be carried along with the stream, the leg of a man standing in the water will appear as if it is moving relative to the larvae. Thus the time that the host will be available for contact to the cercariae will be much shorter than in still water.

The type of model of cercarial behaviour I have used could be developed to include all the responses of the cercariae to host stimuli as the larvae aged. Thus a model of the complete cercarial life could be produced and the probability of contact with any specific host target could be assessed at any cercarial age. In this way, the relative importance of each response throughout the life of the cercaria could be determined.

The total host-location response of the cercariae thus appears to be as follows. The unstimulated behaviour pattern of the cercariae results in the larvae remaining suspended in the water This is the situation where as little energy as possible is expended while remaining in an area likely to be frequented by the host. When a host

does enter the vicinity, the turbulence and shadows cast stimulate the cercariae to swim upwards at a higher than normal level of activity. Shadows will also make some of the cercariae swim in a horizontal direction at the surface of the water. Finally, if the cercariae enter an area containing skin surface chemicals, the swimming pattern again changes so that the larvae swim in all directions. Although cercariae will swim away from the host as well as towards it, this response increases the chance of an individual contacting the skin surface. If at any of these stages the stimulation ceases, the cercariae will eventually revert to the unstimulated pattern of swimming and sinking, so conserving energy until another host appears or until death occurs.

In conclusion, a contrast between the mechanisms of host-location of the different free-living larval stages of the schistosome life cycle can be demonstrated. The miracidium infects a slowly moving host which is constantly present in the water. This larva appears to employ an active but random searching pattern until host stimuli are detected. An increase in the rate of turning then follows which increases the time spent by the miracidia in the region of the host, so decreasing the search area and supposedly increasing the probabilty of contact. The cercaria, however, infects an unpredictably present, quickly moving host and so appears to employ a "sit and wait" strategy, minimising energy consumption while remaining in an area which the host is likely to frequent. When host stimuli are detected, an active but random searching pattern of behaviour is utilised to increase the

chance of contact. The two different mechanisms of host-location shown by the free-living larvae of S.mansoni are thus related to the behaviour of their respective hosts.

APPENDICES.

APPENDIX 1.

Statistical tests were generally taken from Bailey (1959) and Sokal and Rohlf (1969).

The "d_o" test refers to "Comparing the mean of a single large sample size with a known standard, assuming a normal distribution" in Bailey, page 170.

The "d_1" test refers to "Comparing the means of two large samples from normal populations" in Bailey, page 171.

The variance ratio is taken from Bailey, page 172. The 5 percent points of the variance ratio (entered at the 2.5 percent level for a two-tailed test) were calculated for large degrees of freedom using a formula after those given in pages 48-57 in Statistical Tables, R.A. Fisher and F. Yates (1963, Oliver and Boyd).

The "t_1" test refers to "Student's t" for comparing the means of small samples, unknown variances of the population not assumed to be equal in Bailey, page 173.

The arcsine transformation was taken from Sokal and Rohlf, pages 386-387.

Linear regression was performed using a statistical package on the Dec System 10 computer at the University of York. Comparison of regression coefficients was taken from Bailey, page 183 using "Student's t".

APPENDIX 2 Distribution of cercariae in cell as they aged.

Data expressed as mean of 13 observations calculated using the arcsine transformation and converted back to percentage suspended ± standard error of transformed data.

SECTION OF CELL		HOURS AFTER SHEDDING											
		1	2	3	4	5	6	7	8	9	10	11.5	13
Top	1	57.60 ±3.37	47.12 ±2.41	46.82 ±2.28	40.07 ±2.60	33.47 ±2.91	27.99 ±3.12	24.76 ±3.08	19.84 ±2.94	16.87 ±3.10	12.64 ±3.59	6.39 ±3.35	3.35 ±2.82
	2	6.35 ±1.16	5.20 ±0.76	3.05 ±1.11	4.06 ±0.69	2.39 ±1.07	2.63 ±1.19	1.56 ±1.04	1.33 ±0.84	1.54 ±0.98	0.50 ±0.87	0.39 ±0.87	0.21 ±0.84
	3	3.30 ±1.54	3.74 ±1.02	2.75 ±0.91	2.81 ±0.76	2.16 ±0.99	2.38 ±0.99	1.83 ±0.55	1.09 ±1.02	0.73 ±1.04	0.52 ±1.03	0.24 ±0.79	0.09 ±0.76
	4	2.50 ±0.78	3.36 ±0.67	2.80 ±1.07	2.53 ±1.08	2.45 ±0.64	2.66 ±0.50	2.25 ±0.50	1.09 ±1.07	1.06 ±1.16	1.10 ±0.79	0.74 ±0.91	0.17 ±0.93
	5	2.03 ±0.97	3.29 ±0.54	2.80 ±0.87	3.28 ±0.78	2.73 ±0.69	3.02 ±0.86	2.86 ±0.50	2.05 ±o.89	1.53 ±1.17	1.03 ±0.93	1.16 ±0.90	0.12 ±0.76
	6	1.98 ±0.90	3.12 ±0.62	3.47 ±0.64	2.66 ±0.76	3.13 ±0.62	3.00 ±0.95	3.31 ±0.86	2.18 ±0.64	2.18 ±0.78	1.28 ±0.71	0.53 ±1.09	0.30 ±0.91
	7	2.05 ±0.84	2.89 ±0.50	3.42 ±0.87	3.42 ±0.83	3.19 ±0.59	3.05 ±0.62	2.82 ±0.66	1.94 ±1.02	2.41 ±0.67	1.38 ±0.90	0.87 ±0.78	0.58 ±0.91
	8	1.65 ±1.03	2.53 ±0.55	3.28 ±0.76	3.62 ±0.73	3.15 ±0.67	3.54 ±0.69	3.09 ±0.64	3.13 ±0.71	2.39 ±0.73	1.71 ±0.93	1.37 ±0.81	0.77 ±1.01
	9	1.84 ±1.09	3.08 ±0.69	3.22 ±0.98	3.70 ±0.64	4.10 ±0.83	4.27 ±0.86	3.31 ±1.15	3.53 ±0.55	3.80 ±0.71	2.00 ±0.64	1.73 ±1.11	0.77 ±1.01
	10	2.78 ±1.31	3.83 ±0.66	3.68 ±0.75	4.32 ±0.69	4.91 ±0.95	5.00 ±1.01	4.87 ±0.59	5.78 ±0.48	4.32 ±0.76	3.99 ±0.83	1.48 ±1.11	1.18 ±1.12
Bottom	11	3.46 ±1.69	5.44 ±1.15	4.15 ±0.73	4.98 ±0.91	6.02 ±1.20	6.08 ±0.91	5.83 ±0.90	6.80 ±0.81	5.77 ±0.78	5.47 ±1.04	3.02 ±1.43	1.93 ±1.17
	12	6.66 ±2.52	9.32 ±1.24	7.22 ±1.81	7.75 ±1.32	10.09 ±1.56	10.76 ±1.92	14.98 ±1.79	14.40 ±1.70	14.27 ±1.54	12.99 ±1.93	8.43 ±2.46	6.36 ±2.07
Total		99.31 ±1.90	97.83 ±2.55	91.08 ±1.35	87.27 ±1.76	83.62 ±1.62	80.06 ±1.85	77.00 ±1.60	68.73 ±1.57	63.18 ±2.29	53.57 ±2.93	37.42 ±3.36	25.01 ±2.94

APPENDIX 3.

The distribution of cercariae in the test cell after the application of a shadow stimulus.

The results are expressed as the mean of four observations of the number of cercariae suspended in each section of the cell, calculated as a percentage of the total number of larvae suspended in the cell before the application of the stimulus (calculated using the arcsine transformation). The standard error of the transformed data is also given. The distribution of the cercariae was measured before and 0.5, 1, 5 and 10 minutes after the application of the stimulus.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Top	1	59.3 ±9.0	70.1 ±9.5	64.9 ±8.8	61.8 ±6.9	61.2 ±2.6
	2	17.5 ±5.1	18.0 ±5.2	29.3 ±7.0	20.5 ±1.7	19.5 ±3.5
	3	10.5 ±1.9	11.5 ±3.7	11.0 ±3.8	11.5 ±2.8	11.0 ±2.0
	4	3.8 ±1.1	2.9 ±0.8	2.4 ±0.8	3.1 ±0.6	2.7 ±0.3
	5	8.3 ±2.1	5.8 ±1.9	4.3 ±2.0	7.0 ±1.5	5.0 ±1.0
	6	6.3 ±2.1	4.0 ±1.8	3.5 ±1.3	6.5 ±1.7	5.0 ±3.0
	7	2.7 ±0.7	1.3 ±0.5	1.6 ±0.3	2.5 ±0.5	1.9 ±0.7
	8	5.0 ±1.8	4.8 ±1.1	3.5 ±0.7	6.8 ±1.3	3.0 ±1.0
	9	6.3 ±2.3	6.8 ±1.4	4.0 ±1.2	4.5 ±1.7	2.5 ±0.5
	10	3.7 ±0.7	2.5 ±0.6	2.2 ±0.6	1.4 ±0.5	1.9 ±0.7
	11	7.0 ±2.4	4.8 ±2.4	5.6 ±1.7	4.0 ±2.0	2.5 ±0.5
Bottom	12	12.0 ±4.6	8.0 ±2.4	9.8 ±3.2	10.5 ±2.6	3.0 ±0.0
	Total	100.0 ±0.0	103.5 ±1.5	101.2 ±1.7	99.0 ±1.2	92.9 ±5.4

Section of Cell

The distribution of cercariae one hour after shedding.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Top	1	58.1 ±5.8	69.4 ±6.1	68.4 ±7.0	55.8 ±10.4	54.2 ±8.5
	2	14.0 ±7.7	12.0 ±6.7	15.3 ±6.8	10.3 ±6.6	14.5 ±8.5
	3	6.3 ±1.0	5.8 ±3.5	7.0 ±5.4	5.5 ±2.5	10.0 ±5.0
	4	2.3 ±0.7	2.3 ±0.3	2.3 ±0.6	3.2 ±0.4	2.8 ±0.9
Section of Cell	5	6.3 ±3.3	5.5 ±2.0	4.3 ±1.7	6.5 ±3.8	9.0 ±7.0
	6	5.3 ±1.6	5.3 ±1.7	4.3 ±2.0	7.0 ±3.7	10.5 ±3.5
	7	3.6 ±1.3	2.6 ±0.9	2.7 ±0.4	3.8 ±0.6	5.6 ±1.7
	8	4.3 ±1.6	5.5 ±2.3	3.8 ±0.8	6.3 ±3.0	5.5 ±2.5
	9	4.0 ±0.9	4.3 ±1.9	4.0 ±1.8	5.3 ±2.0	6.0 ±5.0
	10	3.4 ±0.6	2.5 ±0.6	2.0 ±0.4	3.4 ±1.3	2.4 ±2.4
	11	5.3 ±2.0	4.5 ±1.2	4.5 ±1.9	6.3 ±3.9	11.0 ±10.0
Bottom	12	9.5 ±3.6	7.3 ±1.0	5.5 ±0.7	8.5 ±1.9	22.5 ±13.5
	Total	100.0 ±0.0	106.9 ±1.7	103.6 ±3.5	96.0 ±6.7	102.4 ±0.3

The distribution of cercariae three hours after shedding.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Section of cell: Top	1	43.1 ±14.7	48.9 ±14.2	52.8 ±14.7	50.4 ±10.4	44.4 ±11.3
	2	8.8 ± 5.4	12.0 ±9.4	10.8 ±6.8	4.3 ±7.8	22.5 ±14.5
	3	8.8 ±6.5	8.8 ±5.5	7.8 ±4.9	7.8 ±3.0	15.0 ±9.0
	4	4.5 ±1.4	4.7 ±0.9	6.0 ±1.1	4.5 ±0.6	3.1 ±1.3
	5	6.0 ±3.4	6.8 ±3.4	7.5 ±3.3	8.8 ±5.8	10.0 ±6.0
	6	6.3 ±3.0	5.8 ±2.8	6.3 ±2.7	8.0 ±4.7	10.0 ±6.0
	7	6.2 ±2.6	5.3 ±1.9	5.8 ±2.7	3.9 ±0.7	4.7 ±0.3
	8	7.0 ±2.5	5.5 ±1.6	4.0 ±1.7	6.0 ±3.4	11.5 ±6.5
	9	8.0 ±3.1	6.3 ±2.5	4.3 ±1.0	4.8 ±2.8	8.5 ±4.5
	10	5.4 ±2.4	3.1 ±0.6	4.0 ±1.3	3.6 ±0.8	4.3 ±0.7
	11	8.0 ±2.2	2.5 ±0.7	3.8 ±1.7	5.0 ±1.4	11.0 ±7.0
Bottom	12	8.3 ±4.2	7.0 ±1.8	4.0 ±1.7	7.3 ±2.3	17.0 ±8.0
Total		100.0 ±0.0	99.0 ±5.9	103.0 ±4.2	100.8 ±3.6	103.9 ±0.5

The distribution of cercariae five hours after shedding.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Top	1	33.0 ±7.0	42.1 ±9.4	46.0 ±9.6	38.9 ±6.2	35.4 ±4.8
	2	7.3 ±4.2	8.5 ±5.6	10.0 ±5.7	9.5 ±3.8	9.5 ±5.5
	3	4.5 ±2.3	6.3 ±3.6	7.3 ±3.3	9.0 ±4.0	8.0 ±6.0
	4	3.8 ±1.1	3.3 ±0.9	3.9 ±0.5	7.5 ±2.8	5.9 ±2.2
	5	4.5 ±2.2	5.3 ±2.6	5.5 ±2.9	9.8 ±4.7	10.5 ±7.5
	6	5.0 ±3.3	7.0 ±3.4	6.8 ±2.2	10.0 ±5.6	9.0 ±8.0
	7	4.4 ±1.4	6.2 ±1.5	5.9 ±2.2	5.7 ±2.0	5.6 ±3.7
	8	6.3 ±2.1	9.3 ±2.7	9.0 ±2.7	6.5 ±2.7	12.0 ±9.0
	9	6.0 ±2.4	7.3 ±1.6	7.0 ±2.5	3.0 ±0.7	12.0 ±8.0
	10	7.0 ±2.2	5.2 ±1.6	6.5 ±2.7	3.3 ±1.0	7.5 ±1.9
	11	9.3 ±2.2	8.8 ±1.6	8.0 ±2.9	6.8 ±1.0	13.0 ±5.0
Bottom	12	21.5 ±5.1	11.5 ±3.8	10.3 ±2.8	11.5 ±3.4	21.5 ±1.5
Total		100.0 ±0.0	108.0 ±4.4	113.8 ±7.7	107.3 ±8.2	107.0 ±10.8

Section of Cell

The distribution of cercariae seven hours after shedding.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Section of Cell: Top	1	20.8 ±4.3	24.9 ±7.3	25.8 ±8.0	35.6 ±3.8	22.0 ±3.0
	2	4.0 ±3.3	4.3 ±2.9	7.0 ±4.1	8.3 ±3.1	7.0 ±4.0
	3	4.0 ±2.7	4.0 ±2.7	6.0 ±3.7	4.5 ±2.3	8.0 ±5.0
	4	1.8 ±0.9	3.9 ±1.0	3.5 ±0.5	3.8 ±0.9	6.0 ±1.4
	5	4.5 ±2.2	5.8 ±3.1	5.0 ±2.4	7.0 ±2.7	5.5 ±3.5
	6	6.5 ±3.5	7.0 ±4.3	8.8 ±3.5	7.5 ±3.3	7.0 ±5.0
	7	7.2 ±1.0	7.9 ±1.5	10.1 ±2.0	6.2 ±1.1	5.9 ±0.0
	8	6.0 ±3.1	9.3 ±2.3	7.0 ±1.1	6.8 ±3.0	10.5 ±7.5
	9	7.5 ±2.6	11.5 ±2.7	7.8 ±1.8	7.8 ±2.3	10.5 ±7.5
	10	8.5 ±0.4	12.9 ±3.4	11.6 ±5.4	6.6 ±2.5	13.3 ±1.5
	11	11.3 ±3.6	9.8 ±1.8	8.3 ±2.3	10.0 ±3.3	17.5 ±8.5
Bottom	12	21.3 ±4.0	16.3 ±6.1	15.5 ±6.0	14.3 ±4.0	22.0 ±0.0
Total		100.0 ±0.0	120.4 ±12.0	119.4 ±13.8	118.0 ±9.0	108.9 ±13.1

The distribution of cercariae nine hours after shedding.

		Time after application of stimulus				
		0	0.5	1.0	5.0	10.0
Top	1	14.5 ±3.2	14.2 ±6.2	17.9 ±5.4	22.8 ±3.0	17.3 ±1.4
	2	2.8 ±1.8	7.3 ±6.3	4.0 ±3.0	4.8 ±1.8	9.5 ±7.5
	3	3.3 ±2.6	4.5 ±3.3	4.0 ±2.0	4.0 ±2.7	7.5 ±5.5
	4	4.4 ±2.2	2.4 ±1.5	3.0 ±1.1	4.5 ±1.0	4.8 ±0.2
	5	4.8 ±3.4	3.8 ±3.1	3.8 ±2.8	3.8 ±3.1	6.0 ±4.0
	6	4.3 ±2.4	4.3 ±3.0	6.8 ±3.3	5.3 ±2.6	6.5 ±5.5
	7	6.9 ±3.0	4.3 ±2.1	8.5 ±1.5	7.5 ±1.4	4.3 ±2.0
	8	5.8 ±2.8	5.8 ±3.6	7.3 ±3.3	5.8 ±2.2	9.5 ±8.5
	9	7.3 ±2.6	5.5 ±2.8	6.8 ±2.5	8.3 ±5.3	12.5 ±11.5
	10	10.4 ±1.4	7.7 ±3.1	14.3 ±4.4	9.5 ±0.9	7.4 ±0.6
	11	11.3 ±6.2	6.3 ±2.8	9.5 ±3.0	9.0 ±4.2	11.5 ±4.5
Bottom	12	18.5 ±4.9	11.0 ±5.4	13.0 ±3.7	20.3 ±6.8	24.0 ±5.0
Total		100.0 ±0.0	79.9 ±27.2	108.5 ±10.7	108.6 ±5.9	102.5 ±6.6

Section of Cell

The distribution of cercariae eleven hours after shedding.

APPENDIX 4.

The distribution of cercariae in the test cell after the application of a rapid mixing stimulus.

The results are expressed as the mean of three observations of the number of cercariae suspended in each section of the cell, calculated as the percentage of the total number of larvae suspended in the cell 15 seconds after the cell was filled (calculated using the arcsine transformation). The standard error of the transformed data is also given.

			Seconds after the application of the stimulus					
		15	30	45	75	135	195	315
Section of Cell: Top	1	33.0 ±2.7	49.5 ±1.3	59.3 ±0.5	77.4 ±5.9	81.2 ±2.0	82.3 ±1.3	46.7 ±3.2
	2	10.9 ±1.5	11.2 ±0.8	10.2 ±0.6	7.0 ±0.8	3.7 ±2.0	3.1 ±1.8	6.8 ±0.9
	3	11.0 ±1.6	8.8 ±0.4	8.3 ±2.1	4.2 ±4.6	1.9 ±2.3	1.6 ±2.5	4.9 ±3.0
	4	9.2 ±1.5	8.2 ±1.5	5.3 ±1.9	1.9 ±4.6	1.1 ±1.9	1.3 ±1.3	3.8 ±0.7
	5	9.0 ±1.3	7.2 ±0.9	4.6 ±0.8	1.1 ±2.5	1.1 ±1.2	0.6 ±1.0	1.9 ±2.3
	6	7.5 ±0.3	5.5 ±1.5	2.5 ±1.8	1.3 ±2.4	1.7 ±3.4	1.2 ±0.6	2.4 ±1.7
	7	7.4 ±3.6	3.4 ±±2.2	1.6 ±0.7	1.0 ±0.7	0.2 ±2.9	0.4 ±3.0	2.2 ±0.7
	8	3.3 ±4.6	1.7 ±1.6	1.3 ±0.5	0.7 ±4.1	0.3 ±2.9	0.8 ±2.1	3.4 ±2.1
	9	3.0 ±3.2	0.5 ±1.6	0.6 ±1.1	0.7 ±0.9	0.6 ±2.0	0.1 ±2.7	1.8 ±1.6
	10	2.0 ±1.0	0.4 ±3.5	0.9 ±2.3	0.2 ±2.4	0.1 ±2.3	0.0 ±1.9	2.9 ±1.4
	11	1.4 ±2.4	0.8 ±1.1	1.1 ±1.8	0.7 ±0.9	0.3 ±3.8	0.2 ±4.3	3.1 ±3.7
Bottom	12	1.2 ±1.9	0.4 ±3.7	0.7 ±4.2	1.1 ±2.5	0.4 ±3.7	0.4 ±3.7	4.8 ±3.6
Total		100.0 ±0.0	98.7 ±4.3	97.1 ±2.4	95.4 ±2.1	94.3 ±0.9	93.2 ±1.6	88.8 ±3.3

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