A COMPARATIVE STUDY OF THE INFLUENCE OF PHYSICAL FACTORS ON THE SURVIVAL AND INFECTIVITY OF MIRACIDIA OF SCHISTOSOMA MANSONI AND ~~SCHISTOSOMA~~ HAEMATOBIUM

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ABSTRACT

In this study the influence of physical factors on the survival and infectivity of S. mansoni and S. haematobium miracidia was compared. The physical factors studied include temperature, light, gravity, depth, hydrostatic pressure, host-parasite dispersion, rate of flow of water and turbulence, ultra-violet radiation and the length of contact time.

While the influence of one physical factor was being investigated all the others were kept constant and the levels of each factor applied were chosen in order to include the limits that may be encountered in the field. The criteria for the influence of a physical factor on miracidia were the infection rates produced in susceptible snails. In some cases the factor was applied to the miracidia alone for a period of time before exposing the snails to them. Miracidia were examined under a dissecting microscope for mortality or degree of activity of survivors, where the factor (such as temperature or ultra-violet radiation) affected their metabolic rates. The degree of activity of miracidia which indicated the extent to which a physical factor affected them was described as "ACTIVE", "SLOW" or "LETHARGIC" and was quantified by calculating the mean rates of movement of miracidia in these categories. For factors which acted as stimuli, such as light and gravity, the responses of the miracidia were recorded as a positive or a negative taxis.

The use of carefully designed apparatus and simplified methods made it possible to reproduce the experiments several times, and statistical analysis of the results helped to assess the significance of differences obtained.

These studies have shown that the survival and infectivity of S. mansoni and S. haematobium miracidia were influenced in a very similar manner by the different physical factors and that both parasites have a remarkable capacity to locate, select and infect their snail hosts. It was found that they were not limited by depth, hydrostatic pressure or dispersion and that they

were capable of infecting snail hosts in water flowing at moderate rates. The different responses of S. mansoni and S. haematobium miracidia to light and gravity appear to increase their chances of meeting their particular snail hosts.

The use of the physical factors studied to interfere with the infective capacity of schistosome miracidia and thereby reduce transmission in endemic areas has been discussed.

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C O N T E N T S

I. GENERAL INTRODUCTION

The schistosome life-cycle involves the internal environments provided by a vertebrate definitive host and a molluscan intermediate host, and an external aquatic environment where the free-living larval stages are released. Infection of the definitive and intermediate hosts takes place in this common external environment.

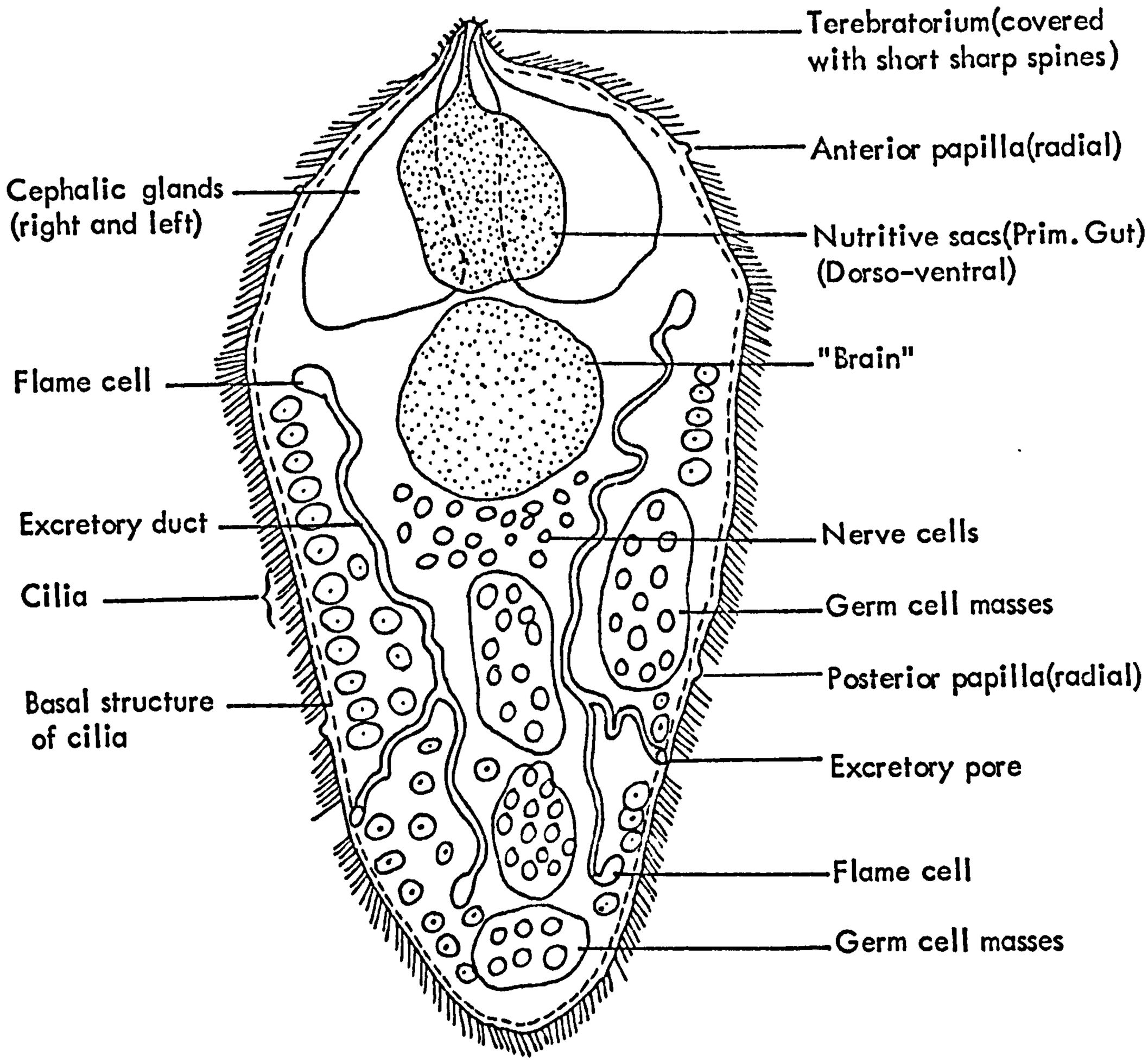
The adult female worms produce eggs which reach fresh water in the urine and faeces of infected persons. The free-swimming miracidia hatch from the eggs.

The miracidia of human schistosomes are morphologically indistinguishable and fairly similar in behaviour. They are typically pyriform or bullet-shaped ciliated organisms capable of swimming rapidly in straight lines at about 2.0 mm/sec at ordinary temperatures. An active miracidium can extend and contract the body but when relaxed measures on average 160 u x 64 u, with the widest diameter at one-sixth of the body length from the anterior end.

The body is covered by 21 ciliated epidermal cells arranged in 4 tiers (6, 8, 4, 3 antero-posteriorly) and lined by a thin subepithelium. The most anterior end, the retractable apical papilla or terebratorium, is however not ciliated. In the anterior third of the body is the primitive gut or apical gland with two flask-shaped cephalic penetration glands lying one on each side of the gut and each opening antero-laterally by a duct at the base of the terebratorium (Fig. 1). There is a neural mass, or brain, two pairs of flame cells and germinal cells (Gordon, Davy and Peaston, 1934; Ottolina, 1957; Lengy, 1962; Smyth, 1966; Maldonado, 1967; Kinoti, 1971).

Miracidia in general exhibit behaviour patterns somewhat similar to those of the molluscan host (Smyth, 1966), their phototactic and geotactic responses bringing them into the layers of the environment where a large proportion of their molluscan hosts live. In the immediate vicinity of

Fig. 1 S. mansoni - miracidium showing main anatomical features



Constructed from Maldonado 1967, Etges (1971-72 personal communication)

the snail, the miracidia are believed to be attracted along a chemical gradient set up by substances produced in the normal metabolism of the snail. Having located the snail host, the miracidia may attempt to penetrate the soft body of the snail, especially the headfoot, the base of the tentacles and the organs in the mantle cavity, or, as is often observed, fail temporarily to attempt penetration. During penetration of the snail the miracidium attaches its terebratorium to the snail tissue and bores by rotating on its longitudinal axis with intermittent rapid side to side lashing motions and occasional contractions and extensions of the body (Lengy, 1962). Within about thirty minutes penetration is completed. Some miracidia withdraw their terebratorium and cease penetration or try new sites. The miracidia which successfully penetrate develop and multiply asexually in the snail, producing numerous cercariae. Miracidia which fail to locate susceptible snails utilise their energy stores until the latter are exhausted and then die.

In endemic areas the snail-infested waters are used by the people for domestic and recreational purposes, for irrigation of parched agricultural land and for fishing. Contact with water is frequent and visits may be prolonged, with exposure to infection and contamination of the water taking place.

The snail has generally been regarded as the most vulnerable link in the schistosome life-cycle and, consequently, has been the main focus of attack in attempts to reduce transmission of infection. Accurate knowledge of the seeming and infective capacity of the miracidia under the influence of various external factors may, however, suggest other means of preventing infection of snail populations and thus controlling transmission.

The meagre data available in the literature on the influence of physical factors on the survival and infectivity of schistosome miracidia are mostly casual observations made during the course of other experiments; very few carefully controlled and reproducible studies have been carried out.

Maldonado and Acosta Matienzo (1948) in an introduction to studies on the miracidia of Schistosoma mansoni stated that the literature revealed a surprisingly small number of actual observations and proven facts on the behaviour of unhatched and free-swimming miracidia in the external environment. In 1966 Smyth wrote that little was accurately known of how miracidia found a snail host and pointed out that the metabolism of the miracidia has been particularly neglected. Wright (1971) remarked in the book "Flukes and Snails" that our knowledge of miracidial behaviour and responses to environmental stimuli is in an elementary state and that most of the recorded observations have been made under poorly controlled conditions. He added that....."miracidia-snail encounters are the result of complicated behaviour patterns which have been subjected to rigorous processes of natural selection."

Although Wen (1962) made an extensive study of the effects of external factors such as temperature, pH, osmotic pressure, oxygen, light, gravity, water currents and mechanical contact on host location and host penetration of miracidia and cercariae respectively, none of the data has been published.

Studies of the longevity of S. mansoni miracidia have been reported by several workers (Lampe, 1927; Faust and Hoffman, 1934; Porter, 1938; Maldonado and Acosta Matienzo, 1948; Schreiber and Schubert, 1949; Ottolina, 1957; Wen, 1962; Bönex and Deschiens, 1963; Chernin, 1968). The longevity of S. haematobium miracidia has been reported by Lampe (1927) quoting Christopherson, Porter (1938) and Wen (1962). In only two studies, Maldonado et al (1948) and Chernin (1968), was the relationship of ageing and infectivity tested. The effect of temperature on the penetration of Biomphalaria spp. by miracidia of S. mansoni has been studied by Standen (1952), Stirewalt (1954), DeWitt (1955), Wen (1962) and Purnell (1966a).

The phototactic responses of miracidia have been studied. Takahashi, Mori and Shigeta (1961) examined how the phototactic responses of S. japonicum

were affected by temperature and light intensity. The same authors studied the relationship between geotaxis and phototaxis. Chernin and Dunaven (1962) examined the vertical distribution of S. mansoni miracidia in response to light and gravity, and Wen (1962) studied the effects of intensity and direction of light on S. mansoni miracidia. No parallel study of the vertical distribution of S. haematobium miracidia has been reported. Recently Wright, Levigne and Ronald (1971) studied the responses of miracidia of Schistosomatium douthitii to monochromatic light (450-650 nm).

The tendency of some miracidia to travel towards the bottom of containers posed the question whether depth or hydrostatic pressure has any effect on miracidia. Chernin et al (1962) observed that depth did not seem to have any effect on the host-finding capacity of S. mansoni miracidia. Upatham (1971-1972, personal communication) has investigated the effect of depth on the capacity of S. mansoni miracidia to infect Biomphalaria glabrata. In a study of the behaviour of the miracidia of an Iraqi strain of S. haematobium, (Wajdi (1972) observed a strong positive geotaxis. But the actual effect of hydrostatic pressure on miracidia has not been investigated.

The ability of miracidia to locate snails contained in vessels of different capacity in which the host and parasite were initially separated to different extents has been studied by Chernin and Dunaven (1962). Chernin (1963) studied the effect of interfering with the host-finding process and the capacity of the miracidia of S. mansoni to infect confined target snails, B. glabrata. A parallel study was undertaken by Shiff (1968) with S. haematobium miracidia and Bulinus (Physopsis) globosus.

The influence of the rate of flow of water on the capacity of S. mansoni miracidia to infect B. sudanica tanganyicensis was examined by Webbe (1966). A similar study with S. haematobium and B. (P.) globosus was reported by Shiff (1969). Upatham (1971-1972, personal communication) also investigated the influence of different rates of flow of water on the capacity of

S. mansoni miracidia to infect *B. glabrata*.

No information is reported in the literature about the effect of ultra-violet radiation on the longevity and infectivity of schistosome miracidia, although a few papers give accounts of the effects of this factor on schistosome cercariae and some ciliates.

The present study was undertaken to compare the effects of physical factors which influence the integrity of miracidia of *S. mansoni* (Sambon, 1907) in *B. pfeifferi* (Krauss) from Arusha Chini, Tanzania, and of *S. haematobium* (Bilharz, 1852; Woinland, 1958) in *B. (P.) globosus* (Morelet) from Ibadan, Nigeria.

The following physical factors were studied: temperature; light; gravity; hydrostatic pressure; spatial dispersion of snails and miracidia; rate of flow of water and turbulence; ultra-violet radiation and length of contact time.

In investigating the influence of physical factors on the survival and infectivity of schistosome miracidia, certain points have been considered. Where a factor such as temperature and ultra-violet radiation affects the metabolic rate of the miracidia, the degree of activity (or rate of movement) is directly affected and consequently the longevity of the miracidia in the environment. It may be added that beyond a limited range of tolerance the metabolism of miracidia is disrupted, resulting in injury. Light and gravity, on the other hand, stimulate a "system" in the miracidia which then responds in a particular manner, resulting in a movement towards or away from the source of stimulus - a positive or a negative taxis respectively. The influence of spatial dispersion in still water, and of rate of flow of water and turbulence on the scanning capacity of schistosome miracidia, is not directly observable.

The infectivity of miracidia during or after the application of the above mentioned factors has been investigated. The criteria used were

the infection rates produced among surviving exposed snails. Changes in the degree of activity produced by different temperatures and different intensities of ultra-violet radiation have been compiled and these have been related to the infectivity of the miracidia.

In order to establish the precise effect of a particular factor either on miracidia alone or on both the miracidia and snail together, all other factors were kept constant. As far as possible all replicates were conducted under the same conditions. Particular attention was paid to the size and age of snails used, in order to minimize the variability among the snail samples.

II. MATERIALS AND METHODS

The materials and methods generally used in this study are presented here leaving the description of special materials and methods for the appropriate sections.

a. Maintenance of Snails

In maintaining aquatic organisms special attention should be given to the quality of the water used. In this study tap water supplied by the Colne Valley Water Company (Watford) was used after being stored in plastic dustbins and tubs for a minimum of 10 days at aquarium temperature (23-28°C). The usual age of water at use was 14 days, by which time all the chlorine had escaped and excess carbonate had settled out as a white scum on the top. Such "dechlorinated water" has no colour, is free from chlorine and ammonia and has no lead, zinc or copper. Dechlorinated water was used in the breeding and maintenance of exposed snails in the glass vessels and the flowing-water system.

The snails were maintained in rectangular all-glass tanks, 30 x 19 x 19 cm and 24 x 17 x 17 cm, with a capacity of 10 litres and 7 litres respectively. The bottom of each tank was covered to a depth of about 1 cm with fine gravel, previously boiled or sterilised. One to three tufts of clean aquatic plant were embedded in the gravel and the tank filled with dechlorinated water. The tanks were kept on shelves in constant temperature aquaria with the temperature regulated between 22 and 28°C. The tanks were illuminated by warm-white fluorescent tubes (fitted 30-35 cm (12-14") above each shelf) for twelve hours per day (9 am - 9 pm) controlled by automatic time switches.

Aeration was by "Hy-Flo" pumps which supplied air through a series of connecting tubes ending in airstones in each tank.

For breeding 10 to 15 and 8 to 12 adult snails were put into 10 and

7 litre tanks respectively. Snails were fed on oven-dried lettuce supplied in generous quantities three times a week. Usually algae grew on the walls of the tank and young snails fed on these until they were capable of eating the lettuce. Adult snails, also, browsed frequently on the algae. When snails reached the size and age adequate for infection they were transferred into fresh tanks set up as described above. When breeding tanks became dirty the snails were transferred into fresh tanks, together with any plant leaves supporting egg masses.

Infected snails were maintained in the same way as breeding ones, except that larger numbers of snails were put into the tanks. Apart from the 10 and 7 litre tanks which held 20 to 35 and 12 to 22 snails, smaller tanks of two different sizes were used, 4 litres (19 x 14 x 14 cm) holding 8 to 12 snails, and 2 litres (19 x 14 x 9 cm) which held up to 10 snails.

Tanks holding infected snails did not become sufficiently dirty to need cleaning during the 8-9 week period during which they were kept, but fresh dechlorinated water was added whenever needed.

b. Parasites

Two species of schistosomes were studied:

An East African strain of S. mansoni from Arusha Chini, Tanzania, was obtained for these experiments from Dr. Foster, Pfizer Drug Company, Sandwich. The parasite was maintained in a vervet monkey (Cercopithecus aethiops) from which faeces were taken and the parasite cycled through B. pfeifferi into Taylor's Original (T.O.) white mice and golden hamsters (Mesocricetus auratus).

S. haematobium was imported from Ibadan (Nigeria) in field-infected snails (B. (P.) globosus) and has been mainly maintained in baboons (Papio anubis) since 1969, but also passaged through golden hamsters.

S. haematobium miracidia used for this study were hatched from eggs which had been washed either from the urine or faeces of infected baboons

or from the livers of hamsters with a 7-10 week old infection.

S. mansoni miracidia were hatched from eggs washed from the faeces of the vervet monkey or from the livers of T.O. mice and golden hamsters with 6-9 and 7-10 week old infections respectively.

c. Preparation of Eggs

Faeces were emulsified in normal physiological saline (0.85%) and ground through No. 30 (500u) and No. 60 (250u) sieves and washed with more saline into a 700 ml urine glass. This suspension was allowed to sediment for 20 minutes. The supernatant fluid was decanted, fresh saline was added and allowed to sediment for a further 20 minutes. The washing process was repeated two or three times until the supernatant fluid was clear, using very cold distilled water (4-6°C) for the final wash.

Infected livers were macerated and ground through No. 60 mesh and washed in the same way as the faeces. Urine samples collected over a 24-hour period were sieved through No. 60 mesh and washed as the faeces.

d. Hatching of Eggs

The concentrated suspension of eggs was transferred into standard 9 cm petri dishes and distilled water at about 30°C was used to wash the urine glass into the dishes. Hatching occurred in the petri dishes which were placed in an incubator at 27-30°C under a bright light for thirty minutes to an hour. Miracidia which had emerged at the end of this period were picked up individually using a fine glass pipette, counted under a dissecting microscope and used as the experiment demanded.

e. Infection of Snails

Snails were infected either individually or en masse depending upon the physical factor being investigated. In these experiments 10 miracidia per snail were used except in the flowing-water system in which each snail

was exposed to 20 or more miracidia. The minimum period of exposure was 3 hours, with an average of 5 hours. When temperature was not the factor under investigation the exposure temperature was arranged to lie between 25 and 28°C.

Individual infections were performed in 3 x 1 inch (7.5 x 2.5 cm) tubes containing 5 ml dechlorinated water at 25-28°C and 10 miracidia. En masse infections for controls and other investigations were carried out in 9 cm (standard) petri dishes with 40 ml dechlorinated water and active miracidia in the ratio of 10 miracidia per snail.

Care was taken in the breeding of snails to achieve maximum uniformity in order that snails of known age and size, and without stunted growth, could be used for the infections. The age at infection of B. pfeifferi was 5-7 weeks and the size 3.5-7.0 mm maximum diameter, while B. (P.) globosus were used at 6-8 weeks old when the maximum height of shell was 5.5-7.7 cm.

f. Screening of Snails

Four weeks after exposure the snails were screened for cercarial emergence. Each snail was placed in a 3 x 1 inch tube containing 10 ml distilled water. The tubes were then put in a rack which was placed about 10 cm (4 inches) from a white-light fluorescent lamp, and the temperature was kept between 24 and 27°C. Those B. pfeifferi which were positive shed about 50% of the day's total cercarial output within the first two hours (9.00 am - 11.00 am) and over 90% by 2.00 pm (after 5 hours shedding). Cercarial shedding of B. (P.) globosus rises to a peak between 2.00 pm and 3.00 pm with only a few cercariae emerging after the peak. Screening was started at 9.00 am and terminated at about 4.30 pm, by which time all positive snails had shed cercariae. A record was kept of the number positive out of the total surviving and then the snails were returned to their tanks for screening the following week. On the third week of screening any B. pfeifferi that were still negative were declared uninfected

and discarded. A fourth week was allowed for B. (P.) globosus since this species had a longer prepatent period. The infection rates were recorded in the experiments as:

$$\frac{\text{Total number of snails positive}}{\text{Total number of snails surviving patency}} \times 100\%$$

Heavy mortalities occurred among B. (P.) globosus, with only 40% of exposed snails surviving to patency in some cases. Mortalities among B. pfeifferi, however, were very low.

g. Estimation of Cercariae

The suspension of cercariae shed by each snail was filtered onto No. 1 Whatman 7 cm (diameter) paper using the ninhydrin technique. The paper was placed in a Buchner funnel and the suspension poured onto it adding the washings of the tube. About 5-7 drops of 1% ninhydrin were put onto the cercariae which were retained on the paper. The paper was dried on a hot-plate and the cercariae, which stain purple, were counted under a dissecting microscope (McClelland, 1961; Webbe and James, 1969). The papers retain their characteristics for several months when kept in dry dark cupboards and, therefore, cercariae thus prepared provide a permanent record of the numbers produced by the snails.

h. Infection of Laboratory Mammals

To provide a large and constant supply of miracidia male hamsters (M. auratus) and T.O. white mice were infected with cercariae by the paddling method. S. mansoni was maintained in both hamsters and mice, while S. haematobium was maintained only in hamsters.

Cercariae were obtained by placing infected snails, known to have shed cercariae for at least a week, into a beaker of warm distilled water, 26°C (50 ml for B. pfeifferi and 25 ml for B. (P.) globosus). The snails were placed under strong illumination in the aquarium for 2-3 hours (S. mansoni) or 3-5 hours (S. haematobium). The total number of cercariae present in

the suspension was estimated from the number contained in three separate 0.5 ml aliquot samples which were taken with an automatic pipette and placed in discrete pools on a squared glass plate. A few drops of Lugol's iodine were added to each pool to kill and stain the cercariae and render them suitable for counting.

Mice and hamsters were placed in glass cylinders containing warm water bathing them to half-way up the abdomen and left for about 30 minutes in order to stimulate excretion. Each animal was then transferred into a jar of warm distilled water (24-26°C) with a suspension of cercariae and exposed for about an hour. Mice and hamsters were exposed to 200-220 and 300 cercariae, respectively. The animals were caged and used between the 6th and 10th week after exposure.

1. Estimation of Degree of Activity and Rate of Movement of Miracidia

In studying the influence of physical factors on the miracidia of S. mansoni and S. haematobium it became necessary to assess the direct effect of a factor on the miracidia and then to estimate their infective capacity in terms of the infection rates produced in susceptible snails to which they were exposed. It was observed that under normal laboratory conditions (22-23°C) the rate of rectilinear movement is directly proportional to the degree of activity, which in turn depends upon the age of the miracidia. Miracidia were therefore placed in three categories of activity - "ACTIVE", "SLOW" and "LETHARGIC". Newly emerged miracidia were active, and as they aged they became "slow" and then "lethargic" before they died. Many records of the rate of movement corresponding to the degree of activity were compiled and a table constructed from them.

TABLE I shows the degree of activity and the corresponding rate of movement of schistosome miracidia under normal temperature (22-28°C)

Degree of activity	Symbol	Mean Rates of Movement mm/sec, ± SD	Number of trials
"ACTIVE"	+++	2.06 ± 0.22	20
		1.80 ± 0.31	20
		2.04 ± 0.27	25
"SLOW"	++	0.94 ± 0.19	19
		0.92 ± 0.19	18
"LETHARGIC"	+	0.43 ± 0.10	20
		0.24 ± 0.12	10

To obtain estimates of the rate of movement of miracidia 40-60 newly emerged miracidia were pipetted into several 5 cm petri dishes containing 10 ml of dechlorinated water. Four or five dishes thus prepared were placed in an incubator of temperature 27-30°C, and at periodic intervals the degree of activity and the rate of movement of the miracidia were recorded. The degree of activity was estimated by inspection under a dissecting microscope, but the rate of movement was calculated by timing miracidia over known distances, using a stop watch. A microscope slide 3 x 1⅛ inches (7.5 x 3.8 cm) marked into 0.5 cm squares was placed on the stage of a dissecting microscope and the dish of miracidia was placed on it. The miracidia were timed as they traversed 0.5 cm or 1 cm in straight lines.

ii. Statistical Analysis of Results

Comparisons of the influence of different physical factors on the survival and behaviour of miracidia and on the infection rates produced in susceptible snails were carried out using the Student's t-test and the Normal Deviate, the former test being used when small numbers were involved. Infection rates produced in the snails have been compared either within

species to determine the effect of different levels of a physical factor or between species to assess the influence of a particular level of physical factor.

Differences were considered significant when the P value was less than or equal to 0.05 ($P < 0.05$) and highly significant when less than 0.01 ($P < 0.01$).

An Olivetti Computer machine was used to give Student's t-values, whichwwere then read from either the Statistical Tables for Students 1969 compiled by J.S. Fowlie (Oliver and Boyd, London) or Documenta Geigy, Scientific Tables, 7th Edition (1970), edited by K. Diem and C. Lentner, published by J.R. Geigy S.A., Basle, Switzerland. When the machine was not available the appropriate formulae in Statistical Methods in Biology (1959) by N.T.J. Bailey and Statistical Methods in Medical Research (1971) by P. Armitage were used to calculate the Normal Deviate and t-values.

III. TEMPERATURE

Introduction

The survival and infectivity of schistosome miracidia are markedly influenced by temperature (De Witt, 1955; Wen, 1962; Furnell, 1966; Smyth, 1966).

Faust and Meleney (1924) observed that S. japonicum miracidia lived for 60-72 hours and Faust later in the same year reported a longevity of 24-30 hours in tap water for the same parasite. The longevity of S. mansoni miracidia is the most studied. Lampe (1927) longevity of less than 40 hours at 33°C; Faust and Hoffman (1934) miracidia unconfined by debris lived for less than 24 hours; Porter (1938) longevity of 20 hours; Maldonado and Acosta-Maticenzo (1948) miracidia survived for less than 9 hours; Schreiber and Schubert (1949) survival was less than 22 hours in spring water at 24-26°C; Ottolina (1957) recorded longevity of 6-8 hours. At the maintenance temperature of 25-27°C Chernin (1963) observed S. mansoni miracidia to survive 12 hours. The longevity of S. haematobium miracidia has been reported by Porter (1938) to be 8-10 hours at about 30°C and less than 6 hours at 10-15°C.

In three studies the survival of schistosome miracidia was observed over a wide range of temperatures. Wen (1962) investigated the effect of different temperatures ranging from 14-41°C on the survival of S. mansoni and S. haematobium miracidia. He found that miracidia of both species survived for 9-12 hours at 21-26°C and for 26-30 hours at 14°C.

Bonax and Deschiens (1963) studied the effect of temperatures ranging from -25°C to +45°C on the longevity and vitality of S. mansoni miracidia and found that the miracidia survived for 24 hours at 4°C, 9 and 7 hours at 20°C and 25°C respectively, and only 1½ hours at 30°C. Furnell (1966a) investigated the effect of temperatures ranging from 12 to 33°C at 3° intervals on S. mansoni miracidia over a period of 2 and 6 hours. He

found that mortalities were low at all temperatures at the end of the second hour, but all miracidia at 35°C were dead at the end of the sixth hour.

The infectivity of miracidia in relation to their age has been studied by few authors. Maldonado et al (1948) studied the infectivity to B. glabrata of S. mansoni miracidia right from hatching and at hourly intervals to the eighth hour. Chernin (1963) investigated the infectivity of S. mansoni miracidia of increasing age to B. glabrata at 25-27°C. Upatham (1971-72 personal communication) observed the infectivity of S. mansoni miracidia from St. Lucia from the first to the tenth hour at room temperature 25-30°C.

Temperature has profound influence on the penetration of miracidia into snail hosts. DeWitt (1955) studied the influence of temperature ranging from 10-40°C on the penetration of four strains of B. glabrata by S. mansoni (Puerto Rican strain) miracidia. Wen (1962) studied the penetration of B. sudanica sudanica by S. mansoni miracidia at temperatures ranging from 0-46°C. Purnell (1966a) studied the effect of temperatures ranging from 9-39°C at 3° intervals on the infection of B. sudanica tanganyicensis by S. mansoni miracidia. The results of the three authors agree in that above 10°C infectivity increased with increase in exposure temperature.

Experiments and Results

The influence of temperature on the survival and infectivity of S. mansoni and S. haematobium miracidia was investigated in two ways.

(i) Survival studies: the mortalities and the degree of activity of the miracidia under different ranges of temperature were observed under the dissecting microscope and hence the effects of temperature were directly assessed.

(ii) Infectivity studies: susceptible snails were exposed either to miracidia of increasing age maintained at aquarium temperature (23-28°C)

or to young miracidia at different temperatures. The infectivity was assessed by the proportion of surviving snails that shed cercariae at patency.

a. <u>The effect of different temperatures on the survival and degree of activity of S. mansoni and S. haematobium miracidia</u>

The survival of miracidia was studied at the following ranges of temperature using the equipment listed:

5 - 10°C	(7°C)	In a refrigerator.
18 - 22°C	(20.5°C)	On the bench.
25 - 30°C	(27°C)	In an incubator (also used for hatching of eggs).
35 - 38°C	(37°C)	In an incubator (normal body temperature).
42 - 45°C	(43°C)	In a water bath.

One haemagglutination tray was allocated for each range of temperature and one parallel row of 10 cavities was chosen for each species of parasite (Fig. 2). 2-5 active miracidia about one hour old were pipetted into each cavity and 1 ml of dechlorinated water was added. A tray thus prepared was placed in the appropriate apparatus and miracidia were periodically examined for mortalities and degree of activity. The cavities were always examined in the same order so that records for each cavity were maintained over the whole period of examination. Miracidia were examined at least 4 or 5 times during the total period of 12-15 hours. The few observations made on miracidia at 43°C were at 30 minute intervals.

Tables 2 - 5 and Figs. 3 and 4 show the survival and degree of activity of <u>S. mansoni</u> miracidia. There was a progressive decline in survival with increasing age at all temperatures. All miracidia in the temperatures studied survived the first 3 hours, but in the 3-6 hour period survival ranged from 90.9% at 18-22°C to 61.7% at 35-38°C. In the 6-9 hour period only 8.4% of miracidia in 35-38°C range were alive, while 44% of those at 5-10°C and over 70% of those at 18-22°C and 25-30°C

Fig. 2. Miracidia being pipetted into the cavities of a haemagglutination tray.

TABLE 2

Longevity and degree of activity of S. mansoni miracidia at 5 - 10°C.

Experiment	Longevity of miracidia			
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.
c	17	17	-	-
e	23	21	-	-
f	21	-	11	-
g	24	18	9	8
% Alive	100	87.5	44.4	33.3
Degree of Activity in %				
+++	100	37.5	33.3	8.3
++	0	43.8	4.4	8.3
+	0	6.2	6.7	16.7

TABLE 3

Longevity and degree of activity of S. mansoni miracidia at 18 - 22°C.

Experiment	Longevity of miracidia				
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.	12 - 15 hrs.
c	66	63	-	44	0
d	21	20	18	17	0
e	24	24	22	-	0
f	21	19	17	12	0
g	22	14	12	12	10
% Alive	100	90.9	78.4	65.4	45.4
Degree of Activity in %					
+++	100	83.8	34.1	0	0
++	0	3.2	23.9	10.0	0
+	0	3.9	20.4	55.4	45.4

TABLE 4

Longevity and degree of activity of S. mansoni miracidia at 25 - 30°C.

Experiment	Longevity of miracidia				
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.	12 - 15 hrs.
b	48	40	36	-	
c	42	42	-	22	
d	35	33	26	16	
e	24	21	17	-	
f	20	17	15	7	
g	23	15	14	8	1
% Alive	100	87.5	72.0	44.2	4.3
Degree of Activity in %					
+++	100	81.8	9.3	0	
++	0	1.0	41.4	14.2	
+	0	4.7	21.3	30.0	4.3

TABLE 5

Longevity and degree of activity of S. mansoni miracidia at 35 - 38°C.

Experiment	Longevity of miracidia		
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.
b	44	38	9
e	21	12	0
f	22	9	0
g	20	7	0
% Alive	100	61.7	8.4
Degree of Activity in %			
+++	99.1	43.0	0
++	0	7.5	4.7
+	0.9	11.2	3.7

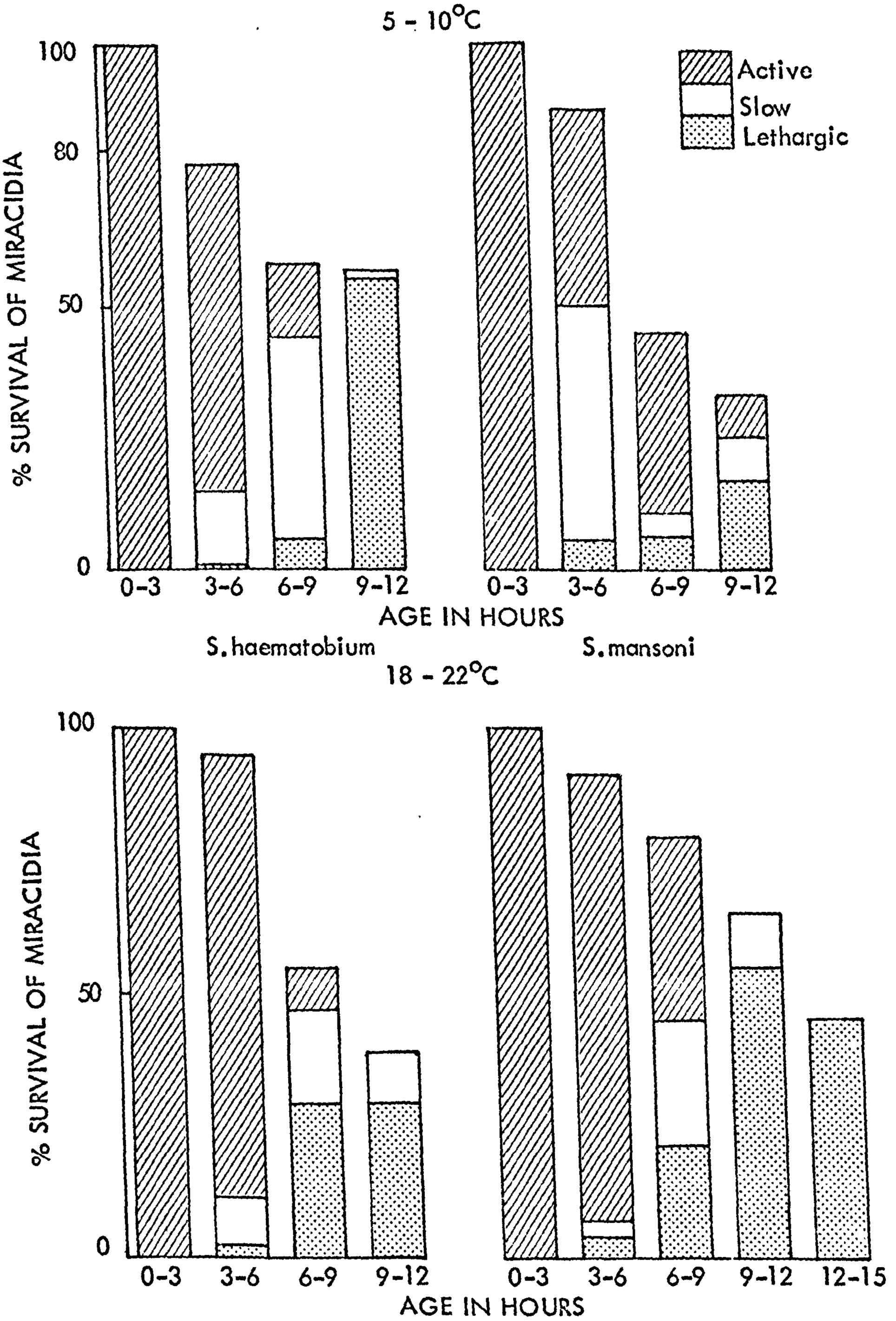


Fig. 3. The survival and degree of activity of *S. haematobium* and *S. mansoni* miracidia at different temperatures.

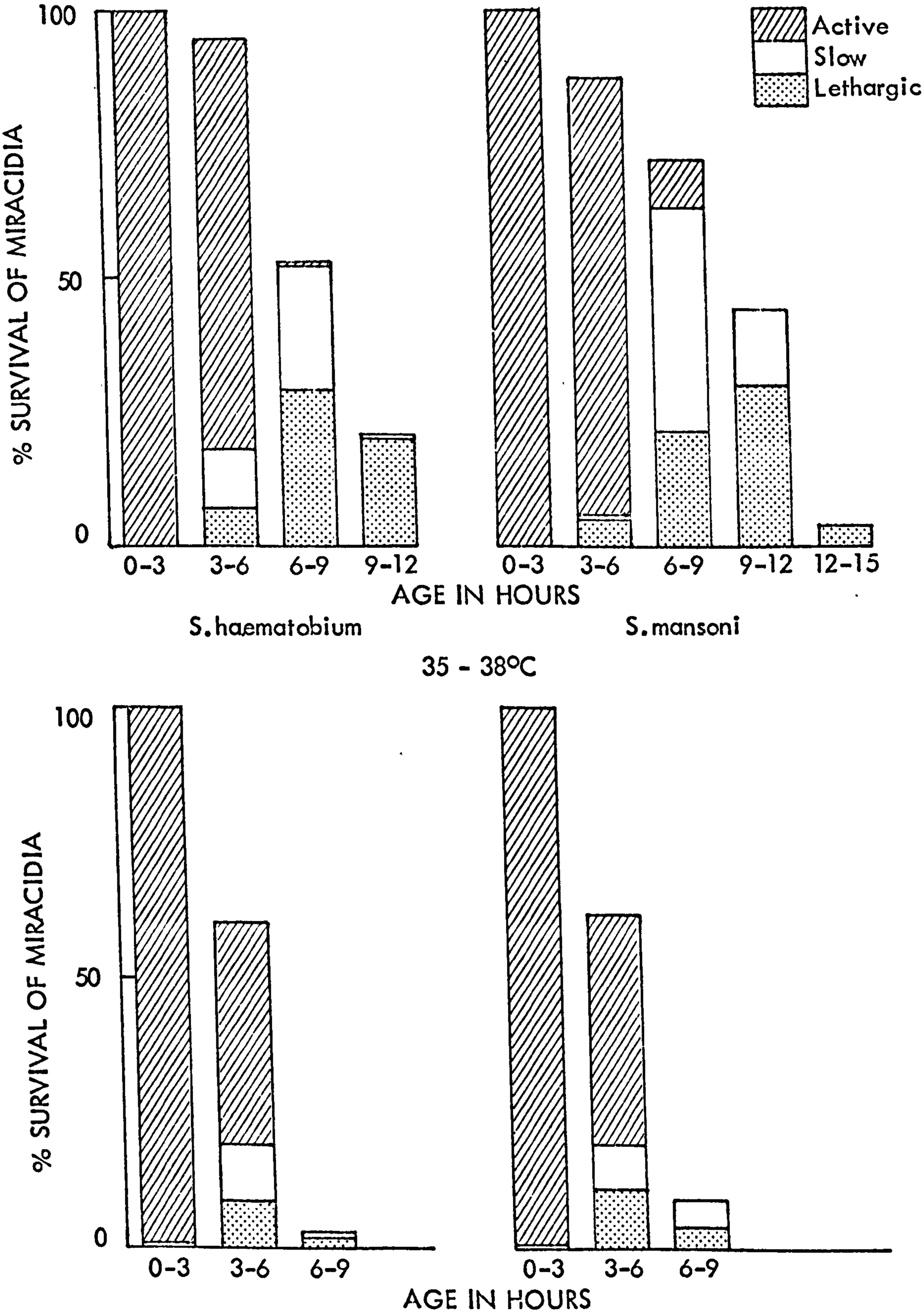


Fig. 4. The survival and degree of activity of S. haematobium and S. mansoni at different temperatures.

survived. All miracidia in the highest temperature were dead by the 9-12 hour period, while as much as 65.4, 44.2 and 33.3% of those at 18-22°C, 25-30°C and 5-10°C respectively were alive. Some of the miracidia in the moderate temperatures survived beyond 12 hours.

The degree of activity declined with increasing age. All miracidia were "active" during the first 3 hours at all the temperatures, but in the 3-6 hour period the proportion of "active" ones dropped from about 80% at 18-22°C and 25-30°C to 34.1% and 9.3% respectively. With increasing age more miracidia became "slow" and later "lethargic" until the 9-12 hour period when almost all the surviving miracidia were "lethargic".

Tables 6-9 and Figs. 3 and 4 show that a very similar survival pattern occurs with S. haematobium miracidia as does with S. mansoni miracidia. All miracidia in the different temperatures survived the first 3 hour period. Between 77-95% of miracidia survived in the 3-6 hour period at all temperatures except at 35-38°C where only 60% survived. Survival declined with age at all temperatures but the drop was steeper at higher temperatures. While over 50% of the miracidia survived the 6-9 hour period in temperatures below 30°C, only 3.3% of miracidia in 35-38°C were alive in this period. In the 9-12 hour period 21.3% of miracidia in 25-30°C survived while 57% and 40% survived at 5-10°C and 18-22°C respectively.

The degree of activity also declined with age in all temperatures. Miracidia in all temperatures were active in the first three hours but progressively slowed down with age. While 41-83% of the miracidia were active in the 3-6 hour period in all temperatures, there were only 1-14% active miracidia in the 6-9 hour period in temperatures under 30°C and no active ones at 35-38°C. There was an increase in the proportion of slow miracidia from 9.1-14.2% in the 3-6 hour period to 17.7-46.6% in the 6-9 hour period at temperatures under 30°C. The proportion of lethargic miracidia was very high among surviving miracidia during the 9-12 hour period; this ranged from 20.0 at 25-30°C to 55.3 at 5-10°C.

TABLE 6

Longevity and degree of activity of S. haematobium miracidia at 5 - 10°C.

Experiment	Longevity of miracidia			
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.
a	53	50	44	43
c	25	23	-	-*
e	24	17	-	-
f	32	-	13	-
g	32	13	10	5
% Alive	100	76.9	57.8	56.5
Degree of Activity in %				
+++	100	61.9	13.7	0
++	0	14.2	46.6	1.2
+	0	0.7	6.0	55.3

* Dash indicates that no observation was made.

TABLE 7

Longevity and degree of activity of S. haematobium miracidia at 18 - 22°C.

Experiment	Longevity of miracidia			
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.
a	65	65	14	-
d	31	30	30	24
e	20	19	17	-
f	24	24	20	6
g	24	17	9	1
% Alive	100	94.5	54.9	39.2
Degree of Activity in %				
+++	100	82.9	7.9	0
++	0	9.1	17.7	10.1
+	0	2.4	29.3	29.1

TABLE 8

Longevity and degree of activity of S. haematobium miracidia at 25 - 30°C.

Experiment	Longevity of miracidia			
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.	9 - 12 hrs.
a	52	50	28	8
b	55	54	29	-
c	40	40	-	11
d	35	32	26	16
e	20	19	16	-
f	26	25	7	3
g	25	19	7	0
% Alive	100	94.5	53.1	21.3
Degree of Activity in %				
+++	100	77.1	0.5	0
++	0	10.7	23.5	0.5
+	0	6.7	29.1	20.8

TABLE 9

Longevity and degree of activity of S. haematobium miracidia at 35 - 38°C.

Experiment	Longevity of miracidia		
	0 - 3 hrs.	3 - 6 hrs.	6 - 9 hrs.
a	53	27	0
b	50	49	6
c	24	16	0
f	27	12	0
g	28	6	0
% Alive	100	60.4	3.3
Degree of Activity in %			
+++	99.4	41.2	0
++	0.6	10.4	1.1
+	0	8.8	2.2

b. <u>The effect of different temperatures and increasing age on the rate of movement of S. mansoni and S. haematobium miracidia</u>

Freshly hatched miracidia in batches of 40-60 were pipetted into 5 cm petri dishes containing 10 ml of dechlorinated water. Two or three dishes of miracidia were placed in different temperatures. At specific intervals the miracidia were timed over known distances as already described under Materials and Methods. The rates of movement of the miracidia were calculated for the different temperature ranges and increasing age. The results are set out in Tables 10 and 11.

The rates of rectilinear movement of <u>S. mansoni</u> and <u>S. haematobium</u> miracidia at different temperatures and increasing age were calculated and set out in Tables 10 and 11. Many readings were taken at temperatures of 18-22°C and 23-30°C as these lie within the normal range of temperatures encountered in the field. Readings were also taken at lower and higher temperatures to provide some comparison. Figure 5 shows the mean and range of the rate of movement of <u>S. mansoni</u> and <u>S. haematobium</u> miracidia of increasing age at 18-22°C and 23-30°C.

<u>S. mansoni</u> miracidia: (Table 10 and Fig. 5).

The rate of movement of <u>S. mansoni</u> miracidia increased with rise of temperature. At the age of 0-2 hours the rate of movement at a temperature of 5-10°C was 0.50 mm/sec (range 0.78-0.22 mm/sec), while at the same age but at a temperature of 35-38°C the rate of movement was 2.52 mm/sec (range 2.80-2.22 mm/sec. At the higher temperature of 35-38°C the mean rate of movement declined from 2.52 mm/sec during the first two hours of life to 1.19 mm/sec in the 4-6 hour period, the rate of movement being almost halved in the intervening 4 hours. The decline in rate of movement at this high temperature was sharp and in the 4-6 hour period there were active, slow and lethargic miracidia, with a greater proportion of them being slow.

At temperatures of 18-22°C and 23-30°C the decline of the mean rate of movement was gradual and all miracidia were active in the 6-8 hour period

TABLE 10

The rate of movement of S. mansoni miracidia at different temperatures and increasing age

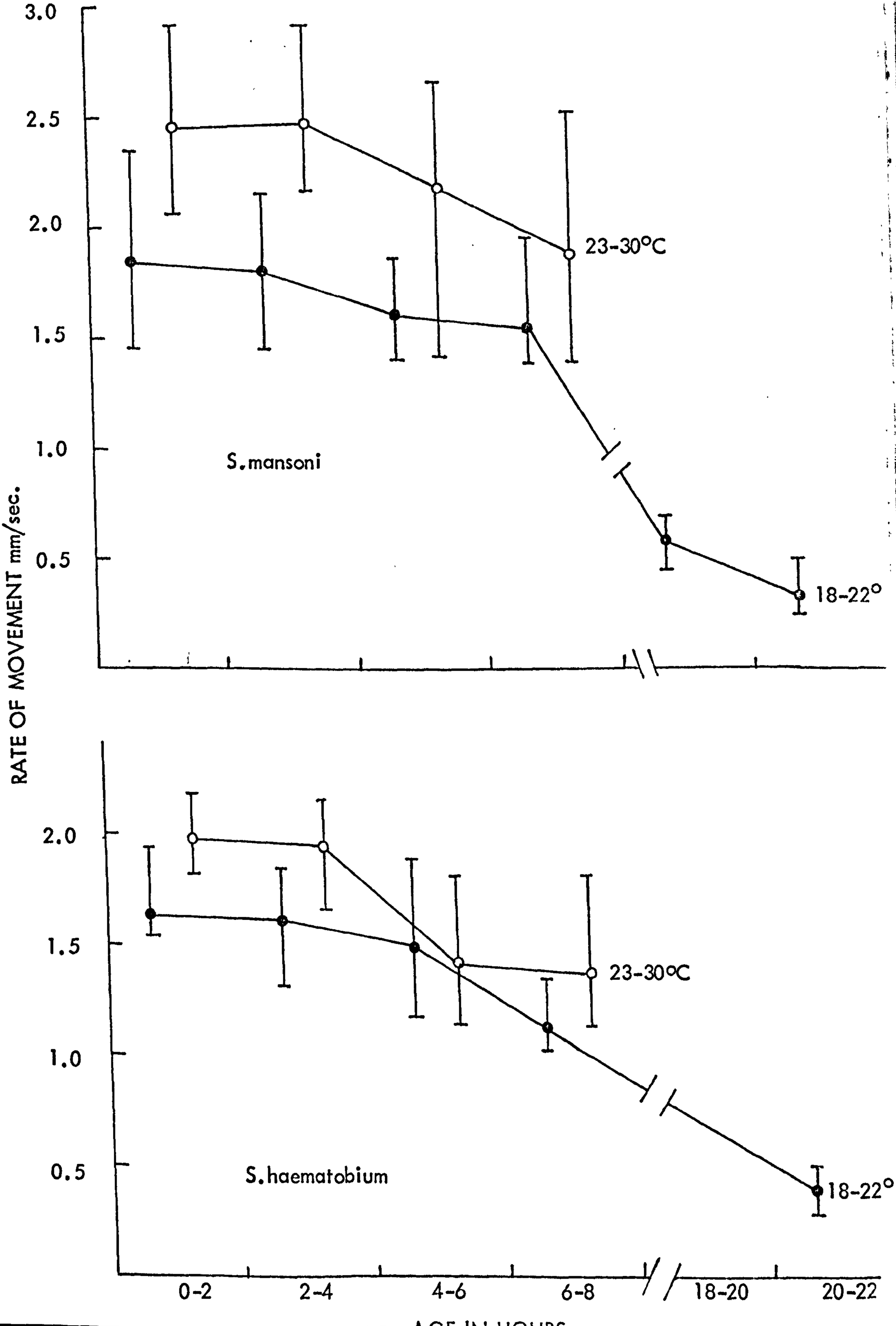
Age in hours	Mean rate of movement mm/sec and range (no. of readings)				
	5 - 10°C	12 - 17°C	18 - 22°C	23 - 30°C	35 - 38°C
0 - 2	0.50 0.78-0.22 (17)	1.05 1.27-0.93 (20)	1.86 2.35-1.46 (79)	2.47 2.92-2.06 (92)	2.52 2.80-2.22 (17)
2 - 4			1.81 2.15-1.45 (55)	2.47 2.91-2.17 (51)	2.04 2.40-1.06 (20)
4 - 6	1.01 1.30-0.80 (25)	1.61 1.86-1.41 (20)	2.18 2.65-1.41 (73)	1.19 1.91-0.47 (20)	
6 - 8			1.54 1.95-1.39 (31)	1.89 2.52-1.39 (48)	
18 - 20			0.58 0.71-0.45 (8)		
20 - 22			0.33 0.50-0.25 (9)		

TABLE 11

The rate of movement of S. haematobium miracidia at different temperatures and increasing age

Age in hours	Mean rate of movement mm/sec and range (no. of readings)			
	5 - 10°C	18 - 22°C	23 - 30°C	35 - 38°C
0 - 2	0.36 0.49-0.23 (12)	1.63 1.90-1.54 (32)	1.98 2.18-1.81 (28)	
2 - 4		1.60 1.84-1.30 (25)	1.94 2.15-1.65 (31)	1.48 1.73-1.20 (15)
4 - 6		1.48 1.88-1.16 (20)	1.40 1.79-1.13 (41)	
6 - 8		1.12 1.33-1.01 (18)	1.36 1.80-1.12 (47)	
20 - 22		0.39 0.50-0.28 (13)		

Fig. 5. The rate of movement of S. mansoni and S. haematobium miracidia at different temperatures and with increasing age.



(range 1.95-1.39 mm/sec and 2.52-1.39 mm/sec for 18-22°C and 23-30°C respectively). Although miracidia at temperatures of 23-30°C were by calculation more active than those at 19-22°C, this was not easily noticeable by inspection. S. mansoni miracidia survived for 20-22 hours at a temperature of 18-22°C but at this age they were very lethargic and moved at a mean rate of 0.33 mm/sec within the range 0.50-0.25 mm/sec.

S. haematobium miracidia: (Table 11 and Fig. 5).

Records were kept for temperatures 18-22°C and 23-30°C, and a set of readings each for 5-10°C and 35-38°C.

A rise in temperature resulted in increased rate of movement. In the first two hours miracidia at a temperature of 5-10°C moved lethargically at a mean rate of 0.36 mm/sec (range 0.49-0.23 mm/sec), while at temperatures 18-22°C and 23-30°C miracidia were active and moved at the mean rates of 1.63 and 1.93 mm/sec respectively.

The mean rates of movement declined with age, the decline being more gradual at 18-22°C than at 23-30°C. Some S. haematobium miracidia at a temperature of 18-22°C survived for 20-22 hours and moved lethargically at a mean rate of 0.39 mm/sec within the range of 0.50-0.20 mm/sec.

The only set of readings taken for 35-38°C showed that the miracidia at the 2-4 hour age were all active.

c. The effect of different temperatures on the penetration of B.pfeifferi and B.(P.)globosus by the miracidia of S.mansoni and S.haematobium respectively

In this investigation snails were exposed to miracidia at the following temperatures: 5-10°C, 10-15°C, 18-22°C, 23-28°C and 35-38°C. A "Hot Box", Fig. 6, provided the three lower temperature ranges and two incubators provided the 23-28°C and the 35-38°C ranges. The "Hot Box" consists of a wooden frame in which were fitted aluminium cabinets. In use the top shelf was filled with a large quantity of ice blocks and, as the aluminium walls quickly conducted the heat downwards, a temperature gradient was produced with the

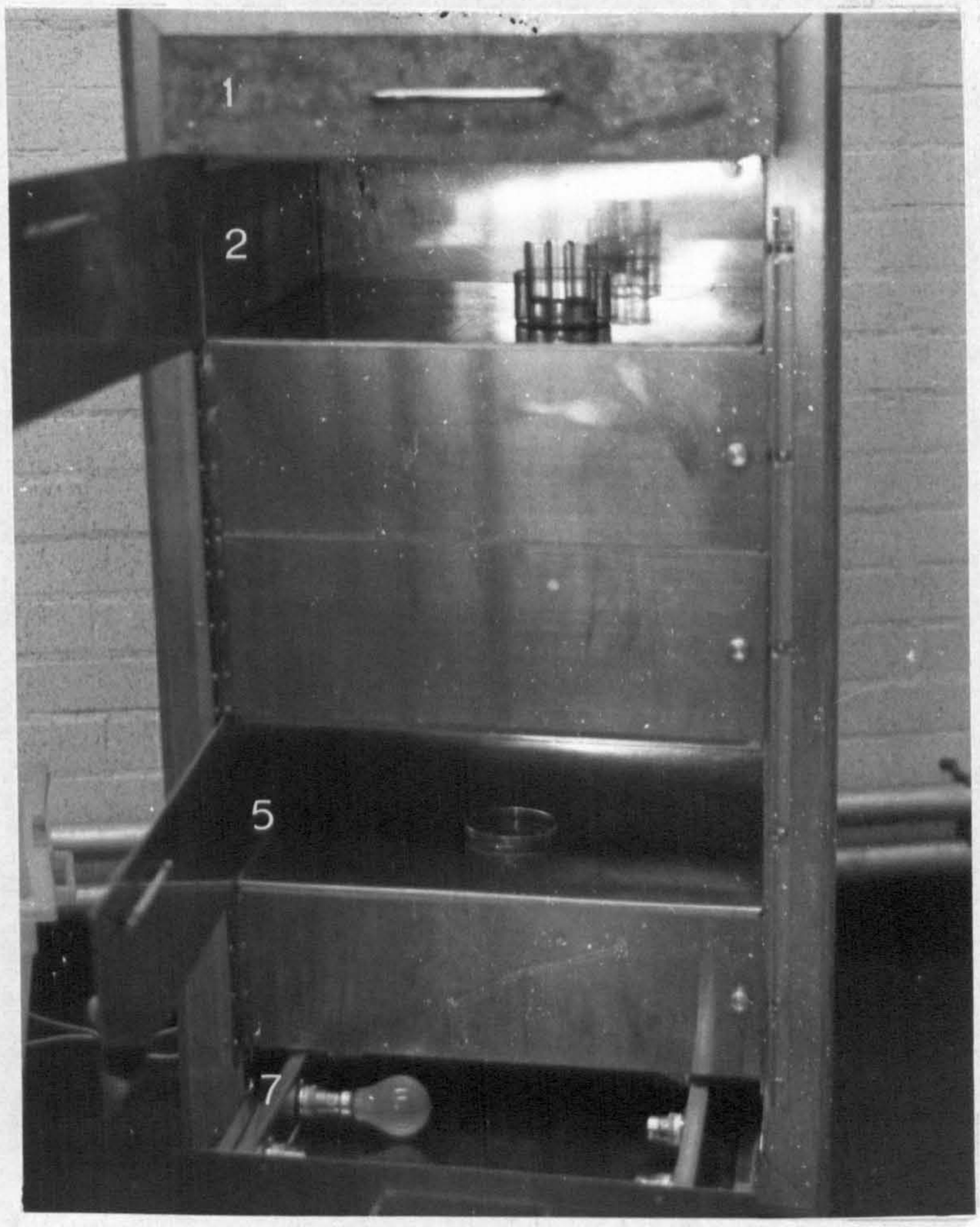


Fig. 6. "Hot Box" with cabinets designed to give different temperatures.

Cabinet 1 containing blocks of ice.

" 2 opened showing snails being infected in 3x1 specimen tubes.

" 5 opened showing snails being infected in petri dish.

" 7 opened showing bulb for heating box from below.

lowest temperature at the top and moderate temperatures towards the bottom. Although the bottom shelf had 25 watt bulbs fitted in it, originally intended to heat the cabinets from below, this was used only once because it was very difficult to keep the required temperatures at the top for a considerable period of time.

In initial experiments snails were exposed individually, but later en masse exposures were carried out. When snails were exposed individually each was placed in a 3 x 1 inch (7.5 x 2.5 cm) tube containing 5 ml of dechlorinated water within the particular temperature range, together with 10 active miracidia about one hour old. The tubes were placed in crystallizing dishes also containing water at the required temperature. In en masse exposures 4-8 snails were introduced into a 9 cm petri dish or crystallizing dish containing 40 ml of dechlorinated water at the appropriate temperature together with active miracidia in the ratio of 10/snail. The temperature of the water in each cabinet was checked at hourly intervals within the 3-6 hour period of exposure to keep it within the required range. The individual exposures needed constant attention as snails frequently crawled out of the infecting chamber.

The exposed snails were removed and put into aquarium tanks and maintained as already described.

Tables 12 and 13 show the infections produced in B. pfeifferi and B. (P.) globosus exposed at different temperatures to the miracidia of S. mansoni and S. haematobium respectively.

B. pfeifferi/S. mansoni: There were no individual exposures at 5-10°C. Infection rate increased with rise in temperature; 25.9% at 10-15°C increasing to 88.9% at 25-30°C. Infections at 10-15°C were very variable among the replicates but fairly uniform in the other temperatures. There was no significant difference between infection rates at 18-22°C and 23-30°C ($P > 0.40$) but there was a highly significant difference between infections at 10-15°C and 18-22°C ($P < 0.0001$). In en masse exposures no infections

TABLE 12

The proportion of surviving B. pfeifferi that were infected at the different exposure temperatures, and, in brackets, the number of snails exposed.

	5-10°C	10-15°C	18-22°C	23-30°C	35-38°C
Individual		2/11 (12)	6/10 (10)	13/15 (15)	
		7/12 (12)		12/13 (15)	24/27 (30)
		1/12 (12)	9/9 (10)	21/24 (24)	
		1/13 (15)	3/11 (12)	6/15 (15)	
		4/10 (10)	9/10 (10)	21/25 (26)	
Total:		15/53	27/40	73/92	24/27
Inf. rate:		25.9	67.5	79.3	88.9
In masse		12/12 (12)	4/4 (4)	11/12 (12)	
		20/23 (24)	21/23 (24)	22/22 (24)	
		0/20 (20)		10/10 (10)	28/30 (32)
		0/18 (20)			14/14 (15)
Total:		0/38	32/35	25/27	43/44	42/44
Inf. rate:		0.0	91.4	92.6	97.7	95.5
Combined infection rate of the two methods of exposure		0.0	39.8	77.6	85.3	93.0

TABLE 13

The proportion of surviving B. (P.) globosus that were infected at the different exposure temperatures and, in brackets, the number of snails exposed.

	5-10°C	10-15°C	18-22°C	23-30°C
Individual		1/5 (8)	4/5 (8)	6/8 (10)
		1/8 (10)	2/8 (10)	5/5 (10)
		3/7 (10)	2/8 (10)	5/8 (10)
		3/8 (12)	5/9 (12)	8/9 (12)
		0/5 (10)	5/8 (10)	6/10 (10)
Total:		8/33	24/28	39/54
Infection rate:		24.2	50.0	72.2
In Masse		8/12 (12)	11/12 (12)	5/5 (6)
	0/9 (10)			8/8 (10)
	0/11 (15)		6/8 (10)	7/8 (10)
		5/11 (15)	9/10 (10)	10/13 (15)
Total:	0/20	13/23	26/30	30/34
Infection rate:	0.0	56.5	86.7	88.2
Combined infection rate of the two methods of exposure	0.0	37.5	64.1	78.4

occurred at 5-10°C among the 30 surviving snails, while very high infection rates occurred at all temperatures above 10°C. Infection rates ranged from 91.4% at 10-15°C to 97.7% at 23-30°C.

B. (P.) globosus/S haematobium: No individual exposures were carried out at 5-10°C and no exposures either individual or en masse were carried out at 35-30°C. In these experiments, too, increase in infection rate corresponded to increase in exposure temperature. Infections at 10-15°C and 18-22°C were variable, while those at 23-30°C were fairly uniform. There was no significant difference between infections at 18-22°C and 23-30°C ($P = 0.05$), while there was a clear difference between infection rates at 10-15°C and 18-22°C (24.7% and 50.0% respectively). In en masse exposures no infections occurred among snails exposed at 5-10°C, but a fairly high infection rate of 56.5% occurred at 10-15°C while infection rates of over 85% occurred at 18-22°C and 23-30°C.

In general, therefore, individual exposures gave much lower infection rates than en masse exposures. A combination of the infections from the two methods of exposure gives infection rates that are very similar in the two species studied (Fig. 7). It is significant that in both species no infections occurred among snails exposed at temperatures below 10°C.

d. The influence of ageing on the penetration of B. pfeifferi and B. (P.) globosus by S. mansoni and S. haematobium miracidia respectively maintained at aquarium temperature 27-28°C.

Active miracidia in batches of 50, 60 or less (in the ratio of 10/snail) were pipetted into 9 cm petri dishes containing 40 ml of dechlorinated water. Several such prepared dishes were placed in the aquarium and at specific times the required number of snails were dropped into some of the dishes. It was customary to inspect the dishes before putting in the snails for survival and degree of activity of the miracidia. Exposure was over 3-6 hours, after which the snails were maintained in aquarium tanks as

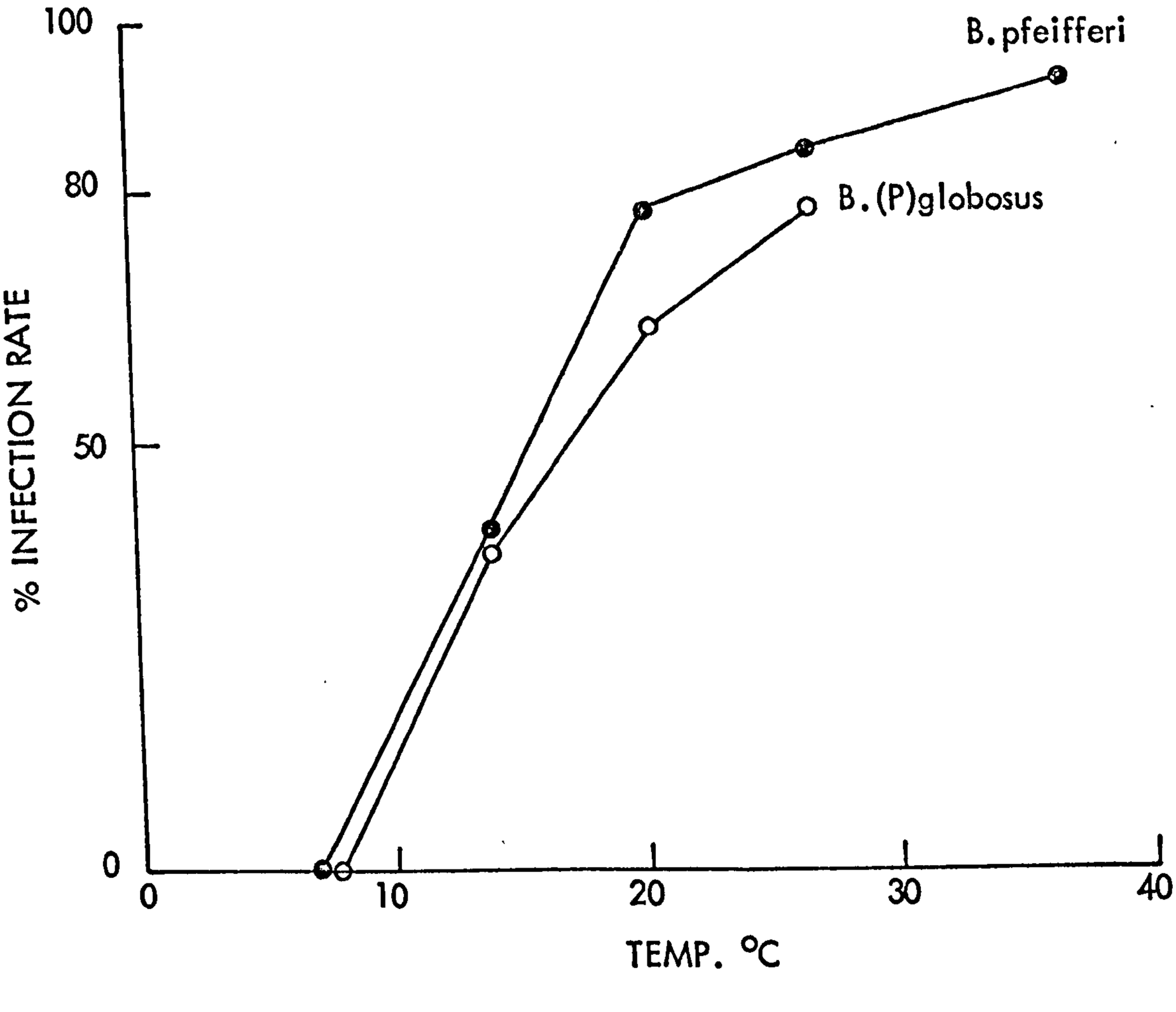


Fig. 7. Infection rates of B. pfeifferi and B.(P.) globosus exposed at different temperatures to S. mansoni and S. haematobium miracidia respectively.

already described.

Results: The results of the infections produced in surviving snails are shown in Table 14.

B. pfeifferi/S. mansoni: Infection rates ranged from 70-100% among snails exposed to miracidia 1-10 hours old except in one instance where at 8 hours a very low infection rate of 13.3% was obtained from 15 surviving snails. Infection rate dropped with increasing age of miracidia and in one series covering 10-18 hours infection rates dropped from 73.3% at 10 hours through 46.7% at 13 hours, 26.7% at 15 hours to 0% at 18 hours. At the moderately low temperature 19°C of the laboratory bench miracidia remained active over 17 hours and produced an infection rate of about 40% in snails.

In some supporting experiments miracidia maintained at 5-10°C and 35-38°C for varying periods of time were brought to room temperature and supplied with snails. It was found that high infection rates occurred throughout the first 4 hours at 35-38°C (1st hour 6/6, 100%; 2nd hour 15/15, 100%; 3rd hour 9/12, 75.0%; 4th hour 12/14, 85.8%). Those maintained at 5-10°C for 5 hours produced 45.5% (5/11) and 86.7% (13/15) in two trials, but at this temperature for 7 hours only 9.1% infection rate occurred.

B. (P.) globosus/S. haematobium: S. haematobium miracidia 1-8 hours old produced infection rates ranging from 80-100% in B. (P.) globosus. The drop in infection rate with increasing age of miracidia seemed to have started at about age 10 hours. The occurrence of infections at 17 hours is here too due to the moderately low temperature, 18-20°C, of the laboratory bench on which the petri dishes were left after 6 hours in the aquarium. Only 6 snails were exposed to the miracidia of this age. The significance of this is that miracidia retain their infective capacity over a much longer period at moderately low temperatures than at very low or high temperatures.

TABLE 14

The proportion of surviving snails infected when B. pfeifferi and B. (P.) globosus were respectively exposed to S. mansoni and S. haematobium miracidia of increasing age at aquarium temperature (24°C)

Age of miracidia in hours	B. pfeifferi/S. mansoni				B. (P.) globosus/S. haematobium		
	a	b	c	d	a	b	c
1	8/10 (80.0)		11/12 (91.6)		4/4 (100)		
2		23/29 (79.3)					
3						8/8 (100)	3/3 (100)
4							8/9 (88.9)
5	10/14 (71.4)		15/15 (100)		11/11 (100)		
6		9/12 (75.0)					9/9 (100)
7							
8		2/15 (13.3)				4/5 (80.0)	
9	20/21 (95.2)		20/22 (90.9)				
10				11/15 (73.3)		2/7 (28.6)	
11							
12							
13				7/15 (46.7)		2/5 (40.0)	
14							
15				4/15 (26.7)			
16							
17			*6/15 (39.9)				*2/3 (60.0)
18				0/11 (0.0)			
19							
20							

* Petri dishes were placed on laboratory bench of temperature 19°C.

a - d = equal batches of miracidia.

Conclusions

The survival and degree of activity of S. mansoni and S. haematobium miracidia at different temperatures were very similar, although S. mansoni miracidia appeared to survive slightly longer. Both species of miracidia survived longer at moderate temperatures than at high or low ones. At high temperatures they were more active but activity fell quickly resulting in high mortalities, and at very low temperatures miracidia were "lethargic" even though they retained their activity by virtue of their age. Prolonged maintenance at low temperatures was harmful to the miracidia as survival was considerably shortened.

The degree of activity of the miracidia, expressed as the mean rate of their rectilinear movement, was directly influenced by temperature and increasing age. Both S. mansoni and S. haematobium miracidia were active during the first 6-8 hours of free life in temperatures of 18-22 and 23-30°C when the mean rates of movement ranged between 1.5 and 2.5 mm/sec, and 1.1 and 2.0 mm/sec for the two species respectively (Fig. 5). The mean rates of movement for S. mansoni agree with those obtained by other workers: Chernin et al (1962) at 25-27°C obtained 2.1 mm/sec; Davenport, Wright and Causley (1962) at room temperatures obtained 1.8 mm/sec; and Wen (1962) at 25°C obtained 2.2 mm/sec.

The infectivity of miracidia was directly influenced by the exposure temperature and their age. The overall infection rates obtained in the present study show that infectivity increased with the rise in exposure temperature but snails became infected equally well at moderately low temperatures as long as the miracidia remained active. It was significant that no infections occurred at exposure temperatures below 10°C, which agrees with the results obtained by De Witt (1955), Wen (1962), Lengy (1962) and Purnell (1965a) While at the normal aquarium temperature (23-28°C) miracidia were infective up to the age of 15 hours, at the moderately low temperature of 19°C infectivity was prolonged to 17 hours. Chernin (1968)

found that at 25-27°C, 12 hours was the maximum age at which S. mansoni miracidia were infective, whereas Maldonado et al (1948) put this at 8 hours at normal laboratory temperatures.

IV. LIGHT

Introduction

Visible light is an electromagnetic wave radiation of the same nature as the radio waves, the infra-red ("heat") waves and the ultra-violet radiation but differ by their wavelengths. (Giese 1962), Dobson 1968). Table 15 shows visible light in relation to the other electromagnetic wave radiation in the sun's spectrum (from Dobson 1968).

TABLE 15

Radiation	Wave Length in A^{o}
Radio waves	10^{6} - 3×10^{14}
Infra-red	8,000 - 4×10^{6}
Visible (light)	4,000 - 8,000
Ultra-violet	500 - 4,000

In nature the sun is the source of visible light and however intense if not absorbed by an aquatic animal it is not harmful (Giese 1962). Rather, reactions to light and other factors guide the animals into places of ecological importance to the particular species (Fraenkel and Gunn, 1960). The intensity of light decreases from the surface of water towards the bottom, The extent of this decrease depending upon the turbidity of the water (i.e. the amount of suspended matter, either sediment or plankton) (Hardy, 1971). Where the waterbody is overhung by trees sunlight may be almost completely cut off, with further reduction taking place towards the bottom of the water.

Unlike the miracidia of Fasciola hepatica and Philophthalmus megalurus

studied by Kümmel (1960) and Isseroff (1964) respectively, which have "eyespots", the schistosome miracidia have no morphologically recognizable photoreceptors that are known to respond to light.

The phototactic response of S. japonicum was reported by Faust and Meleney (1924) as being positive but S. haematobium miracidia were observed to distribute themselves equally throughout a column of water (Faust, 1924). Porter (1938) observed that S. mansoni miracidia moved towards light and away from darkened areas. These authors actually observed the effect of both light and gravity on the miracidia but in later studies by Takahashi, Mori and Shigeta (1961), Chernin and Dunavan (1962), Wen (1962) and Shiff (1969) the effect of gravity was eliminated.

Takahashi et al (1961) studied the phototactic response of S. japonicum miracidia at different temperatures and different light intensities and found that the miracidia were positively phototactic in all light intensities at 15°C, but at light intensities above 2000 lux and at temperatures above 18°C the miracidia were negatively photactic. Miracidia were positively phototactic in light intensities of 100 lux and below, and at all temperatures up to 28°C. Chernin and Dunavan (1962) found that 70-100% of S. mansoni miracidia congregated at the illuminated half of horizontally placed capillary tubes.

Wen (1962) studied the influence of light on S. mansoni miracidia in a rectangular perspex box divided into two compartments, one illuminated, the other blackened. He found that 66.1% of the miracidia congregated in the lighted compartment. Shiff (1969) investigating the phototactic response of a Rhodesian strain of S. haematobium, found that the miracidia congregated at the shaded (88 lux) half of a brightly illuminated (2400 lux) rectangular dish and produced a high infection rate of 91.3% among Bulinus (P.) globosus confined in the shade. These experiments show that S. japonicum and S. mansoni miracidia studied were positively phototactic while the Rhodesian strain of S. haematobium was negatively phototactic.

There have been other studies to indicate the phototactic behaviour of miracidia but these have been conducted in relation to gravity. Gravity, unlike light, is invariable both in intensity and in direction (Fraenkel and Gunn, 1961). Phototaxis and geotaxis have often been considered to augment one another and the importance of each factor per se has been examined. Takahashi et al (1961) found that S. japonicum miracidia were negatively geotactic at all temperatures in the dark but at 20°C and at light intensity 5000 lux the miracidia began to show negative phototaxis and positive geotaxis. Above 5000 lux miracidia showed positive geotaxis and moved to the bottom. Chernin and Dunavan (1962) used a blackened flask carrying a brightly illuminated side arm (McMullen and Beaver type 1945), and found higher concentrations of S. mansoni miracidia in the lighted side arm than at the top and bottom of the flask together but there were four times as many miracidia at the top as there were at the bottom in the blackened flask. In another experiment they studied the phototactic and geotactic responses of S. mansoni miracidia in 100 ml graduated cylinders illuminated in four different ways. The first cylinder was covered leaving 2 cm of the bottom, the second cylinder was completely covered, the third was covered except for 2 cm near the bottom and the fourth was not covered. Samples taken from top and bottom of each cylinder at 15 minute intervals showed that miracidia concentrated at the top in all the cylinders with a slight increase in number at the illuminated bottom of the first cylinder.

Wen (1962) used a glass column about 3 feet long with detachable collecting tubes along its length. There was no significant difference between the proportion of S. mansoni miracidia contained in samples from the top half and those from the bottom half.

Wright (1962) observed a remarkable negative phototaxis and positive geotaxis of S. haematobium miracidia obtained from the urine of an Iraqi student. The miracidia retained the behaviour of remaining near the bottom

of the urine glass in which they hatched even after the first passage through hamsters. Kinoti (1967) quotes McClelland that a Tanzanian strain of S. haematobium was indifferent to light.

Chernin and Perlstein (1969) in their further studies on the interference with host-finding found that although S. mansoni miracidia scanned preferentially the lighted half of parallel channels producing high infections among target snails there, they effectively scanned the shaded end if the bright zone contained no snails.

Experiments and results

a. The horizontal movement of miracidia in response to light of different intensities

In this experiment the effect of gravity was eliminated and so the responses were to the stimulus of the particular light intensity. The apparatus used was a tap-device, shown in Fig. 8, which consists of an 8 mm glass tap with two horizontal arms turned up at their ends. The arms can be connected and disconnected by turning the key of the tap through a right angle. A suspension of about 30 miracidia pipetted individually into 10 ml of dechlorinated water was poured down one arm when the two arms were connected. Two other pieces of this tap-device were provided with miracidia suspensions and the three were placed in sockets in a box, half of which could be blackened with a black cloth and the other half illuminated Fig. 9. After ten minutes the arms were disconnected and the suspension in each arm was poured into a 5 cm petri dish. The miracidia contained in each dish were counted after adding a few drops of Lugol's iodine to kill and stain them.

Light intensities ranging from 0 lumens/sq.ft (darkness) to 300 lumens/sq.ft were read from a light meter; light intensities beyond 300 lumens/sq.ft were marked 300^{++} as the value was unknown.

Fig. 8. Tap device for studying horizontal distribution of miracidia under uniform illumination.

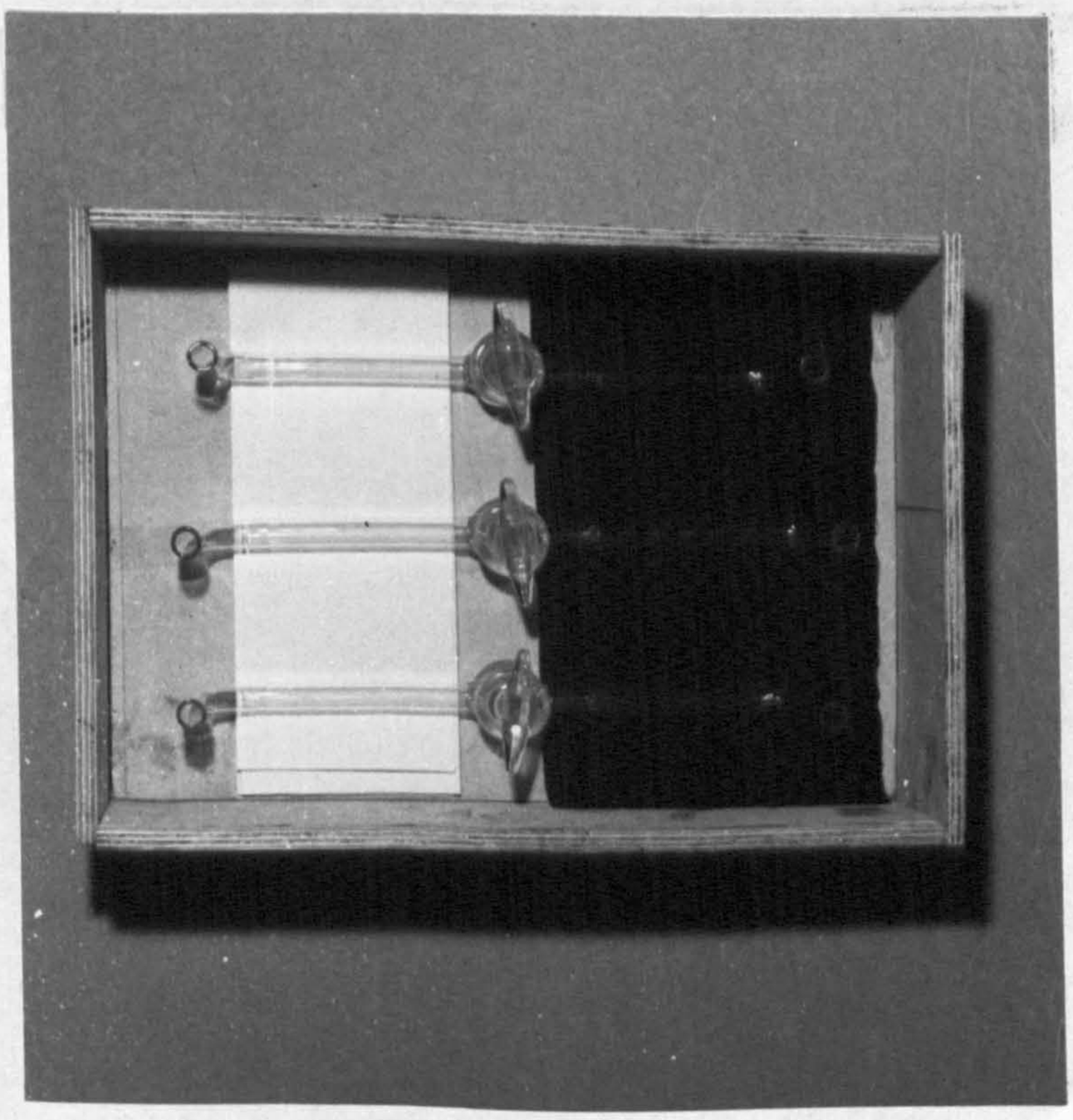


Fig. 9. Three replicas of the tap device for studying distribution of miracidia under different intensities of light.

Tables 16 and 17 show the horizontal distribution of miracidia under uniform and different light intensities respectively.

S. mansoni: Under uniform illumination S. mansoni miracidia distributed themselves equally between the two arms. Under different light intensities against darkness the miracidia congregated in the illuminated arm, the difference being highly significant ($P < 0.001$). Even where the difference between light and darkness was only 10 lumens/sq.ft S. mansoni miracidia detected this difference and congregated in the illuminated half. In experiment set 9 of Table 17 I. where one arm was slightly brighter than the other arm (arm A = 22 lumens/sq.ft, arm B = 8 lumens/sq.ft). S. mansoni miracidia were able to differentiate between the light intensities and congregated in the relatively brighter arm.

S. haematobium: In uniform illumination of 25-60 lumens/sq.ft S. haematobium miracidia distributed themselves equally between the two arms (P ranged from 0.14 to 0.91). There was no significant difference between the number of miracidia recovered from the two arms ($P > 0.65$) when one was under light intensity 25 lumens/sq.ft and the other in darkness. This suggests that S. haematobium miracidia were unable to distinguish a low light intensity of 25 lumens/sq.ft from darkness. At light intensities above 50 lumens/sq.ft versus darkness, however, S. haematobium miracidia congregated in the darkened arm. Table 16 II. shows the light intensities at which experiments were carried out, the number of miracidia recovered from each arm and the significance of the difference between the distributions.

In these experiments gravity had been eliminated and therefore response was purely for light and its intensity.

S. mansoni miracidia were positively phototactic and responded to light intensities as low as 10 lumens/sq.ft while S. haematobium miracidia were indifferent to weak light intensity of 25 lumens/sq.ft but were negatively phototactic to light intensities above 50-60 lumens/sq.ft.

TABLE 16

Horizontal distribution of (I) S. mansoni and (II) S. haematobium miracidia in a tap device under uniform illumination

Exp. set*		Arm	Light Intensity lumens/sq.ft	No. of miracidia recovered from arms	Distribution of miracidia in arms %	P	Temperature and Age of miracidia
I	1	a	20-50	39	46.4)	0.51	23°C
		b		45	53.6)	N.S.	1½ hours old
	2	a	20-50	45	51.1)	0.83	23.5°C
		b		43	48.9)	N.S.	1½ hours old
	3	a	20-50	35	40.9)	0.08	23°C
		b		51	59.3)	N.S.	1 hour old
II	1	a	25-60	45	50.6	0.91	23°C
		b		44	49.4	N.S.	1½ hours old
	2	a	25-60	52	57.8	0.14	23.5°C
		b		38	42.2	N.S.	1 hour old
	3	a	25-60	49	52.1	0.69	23.5°C
		b		45	47.9	N.S.	1 hour old

* Each set consists of 3 replicates

TABLE 17

Horizontal distribution of (I) S. mansoni and (II) S. haematobium miracidia in a tap device under different light intensities

Exp. set*	Arm	Light Intensity lumens/sq.ft	No. of miracidia recovered from arms	% Distribution of miracidia in arms	P	Temperature and Age of miracidia
I	1 a	10	107	91.4	0.001	23°C
	1 b	0	13	8.6	S	½ hours
	2 a	25	89	98.9	0.001	23°C
	2 b	0	1	1.1	S	1 hour
	3 a	28	88	96.7	0.001	23°C
	3 b	0	3	3.3	S	1 hour
	4 a	130	57	83.8	0.001	23°C
	4 b	0	11	16.2	S	1½ hours
	5 a	200	37	78.7	0.001	23.5°C
	5 b	0	10	21.3	S	1-2 hours
	6 a	200	54	81.8	0.001	23.5°C
	6 b	0	12	18.2	S	1-2 hours
	7 a	300++	64	80.0	0.001	23.5°C
	7 b	0	16	20.0	S	1½ hours
	8 a	300++	66	77.6	0.001	23.5°C
	8 b	0	19	22.3	S	1½ hours
	9 a	22	65	69.1	0.001	23°C
	9 b	8	29	30.9	S	1½ hours
II	1 a	25	42	52.5	0.65	23°C
	1 b	0	38	47.5	N.S.	1 hour
	2 a	50	19	25.7	0.001	23°C
	2 b	0	55	74.3	S	1 hour
	3 a	60	21	31.3	0.01	23°C
	3 b	0	46	68.7	S	1½ hours
	4 a	200	30	36.1	0.02	23°C
	4 b	0	53	63.9	S	1 hour
	5 a	200	30	33.7	0.01	24°C
	5 b	0	59	66.3	S	1 hour
	6 a	200	17	28.3	0.001	24°C
	6 b	0	43	71.7	S	1 hour

* Each set consists of 3 replicates

b. <u>The vertical distribution of miracidia demonstrating their phototactic and geotactic responses</u>

The method of Chernin and Dunavan (1962) was used with slight modifications.

Four 100 ml graduated measuring cylinders were illuminated in four different ways (Fig. 10).

Cylinder 1 was completely illuminated

Cylinder 2 had top 3 cm illuminated

Cylinder 3 had bottom 3 cm illuminated

Cylinder 4 was completely blackened.

About 280 or 140 active miracidia (usually about 1 hour old) were pipetted into each cylinder containing 90 ml of dechlorinated water. More water was added to the 140 ml mark on the cylinder. (Miracidial density was therefore 2 or 1/cc). The pieces of cloth covering the cylinders were secured with sellotape and a 60 watt lamp was placed about 1 foot away producing an average illumination of 120 lumens/sq.ft. on the cylinders. By means of a rubber teat on a 10 ml graduated pipette, 6 ml samples were taken from the top and bottom 3 cm of each cylinder. The miracidia in the samples were killed and stained with a few drops of Lugol's iodine and counted under the dissecting microscope. Three samples were taken over a period of about 6 hours for each set of experiments.

In preliminary studies samples were taken from the top and bottom of completely illuminated cylinders containing <u>S. mansoni</u> and <u>S. haematobium</u> miracidia. Table 18 shows that in the sampling covering 6 hours about 60-70% of the <u>S. mansoni</u> miracidia were at the top while about 30-40% were at the bottom. This is an indication of positive phototaxis and negative geotaxis with a significantly higher proportion at the top ($P < 0.0001$). The situation was reversed in the case of <u>S. haematobium</u> where over 96% were at the bottom with only 4% at the top, indicating a negative phototaxis and

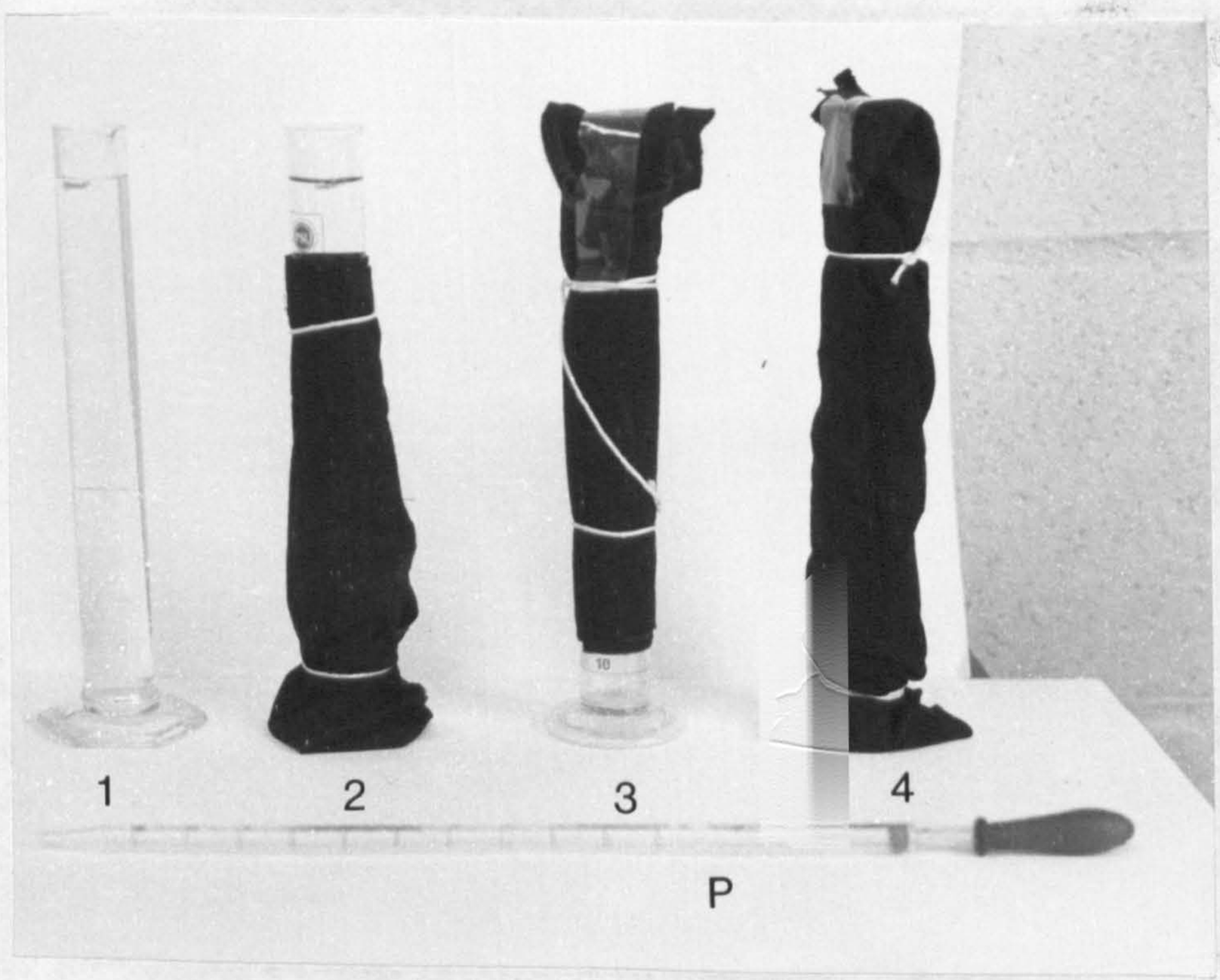


Fig. 10. Four 100 ml measuring cylinders illuminated in different ways.

1. Completely illuminated.

2. Top illuminated.

3. Bottom illuminated.

4. Completely dark.

P = sampling pipette.

a positive geotaxis. To find out the extent to which each of the taxes (phototaxis and geotaxis) was influencing the behaviour of the miracidia, a further investigation was carried out by selectively playing one factor against the other. Tables 19 and 20 show the samples of S. mansoni and S. haematobium miracidia from the top and bottom of cylinders illuminated in four different ways.

TABLE 18

Samples of S. mansoni and S. haematobium miracidia from the top and bottom of completely illuminated cylinders

Exp. No.	Sampling time in hours	S. mansoni Completely illuminated Top	S. mansoni Completely illuminated Bottom	S. haematobium Completely illuminated Top	S. haematobium Completely illuminated Bottom
I	1½	19	4	0	26
	3	13	9	1	45
	Total	32	13	1	71
	% Distribution	71.1	28.9	1.4	98.6
2	1	16	9	1	30
	3	17	4	1	27
	6	12	13	1	22
	Total	45	26	3	79
	% Distribution	63.4	36.6	3.7	96.3
3	1	10	3	0	28
	4	12	5	0	23
	6	4	3	2	8
	Total	26	11	2	59
	% Distribution	70.3	29.7	3.3	96.7

S. mansoni: In cylinders 1 and 2 which had light at the top 57 to 73% of S. mansoni miracidia stayed at the top while 27 to 43% stayed at the bottom. In cylinder 3 where the bottom was illuminated the distribution of miracidia was reversed; 31-43% stayed in the darkness at the top while 57 to 69% concentrated in the light at the bottom. In the completely darkened cylinder the distribution was uniform (almost 50 to 50). S. mansoni miracidia therefore showed positive phototaxis but an indifference towards gravity. They dived to the bottom of the cylinder along with gravity in order to be in light. In nature the negative geotaxis is concomitant with positive phototaxis and S. mansoni miracidia in responding positively to light were at the same time responding negatively to gravity. There was no significant difference between the distribution of S. mansoni miracidia in cylinders 1 and 2 (i.e. those with illumination at the top) ($P = 0.56$). The distribution of miracidia in cylinders 2 and 3 (lighted at the top and lighted at the bottom respectively) is remarkable since one seems to be an exact reversal of the other; 62% at the illuminated top and 38% at the darkened bottom as against 61% at the illuminated bottom and 39% at the darkened top. Within the 6½ hour period of sampling there did not seem to be any observable effect of age on the distribution of miracidia in the cylinders. It was observed during the course of the experiments that miracidia moved constantly up and down the middle of the completely illuminated cylinder. S. haematobium miracidia behaved differently from S. mansoni miracidia. In cylinders 1 and 2 an average of 5% stayed at the top while about 95% concentrated at the bottom. In the cylinder illuminated at the bottom about 60% of the miracidia concentrated in the relative darkness at the top leaving about 40% in the bottom light. It was observed that when the black cloth covering the top of the 3rd cylinder was temporarily drawn down S. haematobium miracidia streamed into the relative shade in the middle of the cylinder.

In the completely blackened cylinder, 4, over 80% of the miracidia stayed at the bottom while under 20% stayed at the top.

There was no significant difference between the distribution of miracidia in the first, second and fourth cylinders but a very highly significant difference existed between the distribution of miracidia in cylinders 2 and 3 ($P < 0.0001$).

TABLE 19

Samples of S. mansoni miracidia from the top and bottom of cylinders illuminated in four different ways

Experiment	Time in hours	Completely illuminated		Top illuminated		Bottom illuminated		Completely dark	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
1	1	14	5	24	5	1	0	24	2
	3	8	11	15	9	4	13	13	15
	5½	10	8	6	13	6	12	5	32
	Total	32	24	45	27	11	25	42	49
	%	57.1	42.9	62.5	37.5	30.6	69.4	36.2	53.8
2	2	18	10	26	13	8	19	16	6
	4	18	15	12	11	15	14	8	8
	6	30	7	15	12	17	20	20	17
	Total	66	32	53	36	40	53	44	31
	%	67.3	32.7	59.5	40.5	43.0	57.0	56.7	41.3
3	2	9	0	13	3	7	17	5	3
	4	3	4	10	6	2	3	6	8
	6½	4	2	3	4	4	2	5	5
	Total	16	6	26	13	13	22	16	16
	%	72.7	27.3	66.7	33.3	37.1	62.9	50.0	50.0
Total % of series		64.8	35.2	62.0	38.0	39.0	61.0	51.0	49.0

TABLE 20

Samples of *S. haematobium* miracidia from the top and bottom of cylinders illuminated in four different ways.

Experiment	Time in hours	Completely illuminated		Top illuminated		Bottom illuminated		Completely dark	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
1	1	2	48	3	110	16	17	4	52
	3	1	41	4	10	20	10	2	24
	5½	2	4	0	0	13	5	6	14
	Total	5	93	7	120	49	32	12	90
	%	5.1	94.9	5.5	94.5	60.5	39.5	11.8	88.2
2	1	2	14	7	6	9	2	3	9
	3	2	12	1	16	2	6	2	14
	6½	0	14	0	34	11	8	0	0
	Total	4	40	8	56	22	16	5	23
	%	9.1	90.9	12.5	87.5	57.9	42.1	17.9	82.1
3	2	0	11	1	47	7	2	0	0
	4	0	22	0	4	9	4	0	7
	6½	0	12	0	3	2	6	0	0
	Total	0	45	1	54	18	12	0	7
	%	0.0	100	1.8	98.2	60.0	40.0	0.0	100
Total % of series		4.8	95.2	6.5	93.5	59.7	40.3	12.4	87.6

c. The effect of light and shade (darkness) on the scanning capacity of S. mansoni and S. haematobium miracidia

In this study two intensities of light (about 130 lumens/sq.ft and 52 lumens/sq.ft) and darkness (or deep shade) were employed. A rectangular plastic vessel 55 x 40 x 30 cm was filled with dechlorinated water. One half of the vessel was covered with a black cloth leaving the other half illuminated with a 100 watt lamp such that the illumination at the surface of the water was about 130 or 52 lumens/sq.ft. A snail bag containing 10 snails was placed in the vessel immediately below the light and another bag of snails placed on the opposite side in the darkness (shade). Active miracidia in the ratio of 10/snail were released at the junction of the light and darkness (shade). Exposure was over 3-6 hours.

Table 21 shows infections produced in B. pfeifferi and B.(P.) globosus by S. mansoni and S. haematobium miracidia respectively.

B. pfeifferi/S. mansoni: High infection rates ranging from 70-100% occurred among snails in both the illuminated and the blackened halves of the vessel. There was no significant difference between infections in the illuminated (130 and 52 lumens/sq.ft) and blackened (0 lumens/sq.ft) halves ($P > 0.79$). Using different intensities of illumination in the lighted half did not influence the scanning capacity of the miracidia. S. mansoni miracidia therefore scanned both the lighted and the blackened halves of the vessel effectively.

B.(P.) globosus/S. haematobium: The numbers of snails involved here were small but there was a tendency for more miracidia to go into the shade at the bottom of the vessel to infect target snails; 69.2% (9/13) and 26.7% (4/15) infections occurred in the blackened and illuminated halves respectively.

TABLE 21

The infections in (I) B. pfeifferi and (II) B.(P.) globosus snails confined at the bottom of a vessel with one-half illuminated and the other half blackened. Miracidia were released at junction of the light and darkness

	Exp.	Light intensity lumens/sq.ft	No. of snails exposed	No. infected No. surviving	Infection rate	Temperature and age of miracidia in hours
I.	1	130	10	7/10	70.0	23°C
		0	10	6/9	75.0	1½
	2	130	10	8/9	88.9	25°C
		0	10	6/6	100.0	1½
	3	130	10	8/9	88.9	25°C
		0	10	9/9	100.0	1
	4	52	10	9/10	90.0	23°C
		0	10	6/8	75.0	1½
	5	55	10	10/10	100;0	26°C
		0	10	9/10	90.0	1½
II.	1	52	10	1/6	16.7	24°C
		0	10	3/6	50.0	1
	2	50	10	3/9	33.3	25°C
		0	10	6/7	85.7	1½

Conclusions

In the "tap device" which allowed distribution in the horizontal plane only, both S. mansoni and S. haematobium distributed themselves equally between the two arms under uniform illumination. When the intensity of light was varied between the two arms S. mansoni miracidia showed a strong positive phototaxis, a significantly higher proportion detecting and congregating in light intensities as low as 10 lumens/sq. ft. as against darkness. This behaviour is in contrast to that shown by S. haematobium miracidia which could not distinguish between light intensity of 25 lumens/sq. ft. and darkness; they however showed a significant negative phototaxis at higher intensities of light against darkness. Chernin and Dunavan (1962) and Wen (1962) found that over 66% of S. mansoni miracidia congregated in the illuminated portion of a test apparatus. S. japonicum (Takahashi et al, 1961) also showed positive phototaxis which was not affected by light intensities of 100 lux at temperatures up to 28°C but at light intensities above 2000 lux and at temperatures above 18°C negative phototaxis occurred. In the present studies the point at which a reversal of positive response to light by S. mansoni might have occurred was not reached at the highest intensity used (300+ lumens/sq.ft).

In the vertical distribution of miracidia carried out in the 100 ml graduated cylinders illuminated in four different ways, the influence of light was tested against that of gravity. S. mansoni miracidia showed positive phototaxis and were indifferent towards gravity; 60-70% of them congregated in the illuminated area whether this was at the top or bottom of the cylinder. This indifference to gravity was clearly demonstrated in the completely darkened cylinder where the miracidia distributed themselves equally between the top and bottom. Wen (1962) using S. mansoni miracidia from East Africa came to the same conclusion. Chernin et al (1962) reported that the Puerto Rican strain of S. mansoni congregated at the top in all

cylinders with a slight increase in number at the illuminated bottom of one cylinder. S. haematobium miracidia however showed a strong positive geotaxis, over 80% of them congregating at the bottom of the completely darkened cylinder. They showed a marked negative response to light which was demonstrated in the cylinder illuminated at the bottom; about 55% of the miracidia congregated in the darkened top. Negative phototaxis and positive geotaxis have been reported among a number of S. haematobium strains. Wright (1962a) reported the remarkable behaviour of S. haematobium miracidia of Iraqi origin which remained at the bottom of the urine glass in which they were hatched even after the first passage through hamsters. Recently Wajdi (1972) also reported the strong positive geotaxis of a strain of S. haematobium from the Summer region of Iraq which produced high infections among B. truncatus at the bottom of water columns 1 m tall. Shiff (1969) found a Rhodesian strain of S. haematobium to be negatively phototactic.

Chernin and Perstein (1969) found that S. mansoni miracidia scanned preferentially the illuminated half of parallel channels producing high infection rates but if the bright zone contained no snails the miracidia scanned the shaded end. Shiff (1969) found that the S. haematobium miracidia preferred the shade at the bottom of an outside pond producing high infection rates among confined B.(P.) globosus. The results obtained in the rectangular vessel in the present study showed that the S. mansoni scanned equally effectively both the illuminated and darkened halves but that the S. haematobium miracidia scanned more effectively the shaded bottom of the vessel. It may be inferred therefore that B. pfeifferi at different depths have an equal chance of becoming infected since S. mansoni miracidia tend to distribute themselves uniformly along the vertical plane:

V. DEPTH OF WATER

Introduction

The observed positive geotaxis of some schistosome miracidia and the recovery of naturally infected snails from considerable depths poses the question of the effect of depth on the scanning capacity of schistosome miracidia.

Webbe (1962) dredged infected B. choanomphala from depths of over 2 metres (6 ft) from the southern shore of Lake Victoria. McClelland and Jordan (1962) in their study of schistosomiasis in the Bukoba Province on Lake Victoria concluded that the transmission taking place in the body of the lake was through infected B. choanomphala. Prentice, Coles and Panesar (1970) dredged naturally infected B. choanomphala from depths of 2-3 metres (about 10 ft) from the northern shore of Lake Victoria. Although it is not known at what levels the snails get infected Magendantz (1972 personal communication) believes they were infected in the immediate area where they were collected because she observed that there is no appreciable movement of B. chonaomphala in the lake.

In the laboratory, experimental evidence indicates that snails become infected at varying depths and that depth does not seem to be any barrier to host location and infection by schistosome miracidia. Chernin and Dunavan (1962) confined B. glabrata individually at 2, 10 and 20 cm depths in 100 ml cylinders and provided them with a single miracidium each. They obtained infection rates which did not differ significantly among the three depths indicating that water columns up to 20 cm in height did not constitute a barrier to host location and infection by S. mansoni miracidia. In another set of experiments in a large tank S. mansoni miracidia dived to a depth of 33 cm to reach and infect confined snails. Upatham (1971 and 1972, personal communication) in his studies on the influence of water depth on the infection

of B. glabrata by S. mansoni miracidia obtained 81.4% and 3.5% infection rates at the surface and at 1.21 metres respectively at a 20 miracidia/snail level. In his studies infections in snails decreased gradually from the surface towards the bottom of the column when caged snails were placed either at all levels together or at one level at a time. Wajdi (1972) studied the behaviour of the miracidia of an Iraqi strain of S. haematobium in a glass column 1 m tall and 15 cm in diameter. She found that infection rates of 24%, 56% and 78% occurred among snails placed at the top, middle and bottom respectively. She observed that the miracidia rarely rose from the bottom of the container in which they hatched and obtained a very high infection rate (93.7%) at the bottom and no infections at the surface when miracidia were released at the bottom of the cylinder. It was concluded therefore that the Iraqi strain of S. haematobium was strongly positively geotactic.

The present experiments were designed to study whether miracidia will locate and infect susceptible snails confined at the bottom of water columns 1 m (3 feet) and 2 m (6 feet) tall.

Experiments and Results

a. Miracidia released at the surface of a column of water

Glass cylinders with a diameter of 5 cm and heights of 1 m and 2 m provided 3 ft and 6 ft depths of water respectively. Figure 11 shows two columns 1 m tall and one column 2 m tall; the former have a capacity of 2.2 litres each and the latter 4.3 litres. Target snails were confined to the bottom of the water column in cylindrical bags measuring 7.5 x 3 cm made from 14 gauge nylon gauze. Up to 10 snails were put into a bag which was then sealed by sewing up the rim. After the temperature of the water had been checked to ensure it was between 23 and 28°C a prepared bag of snails was dropped to the bottom of the column, usually 20 to 30 minutes

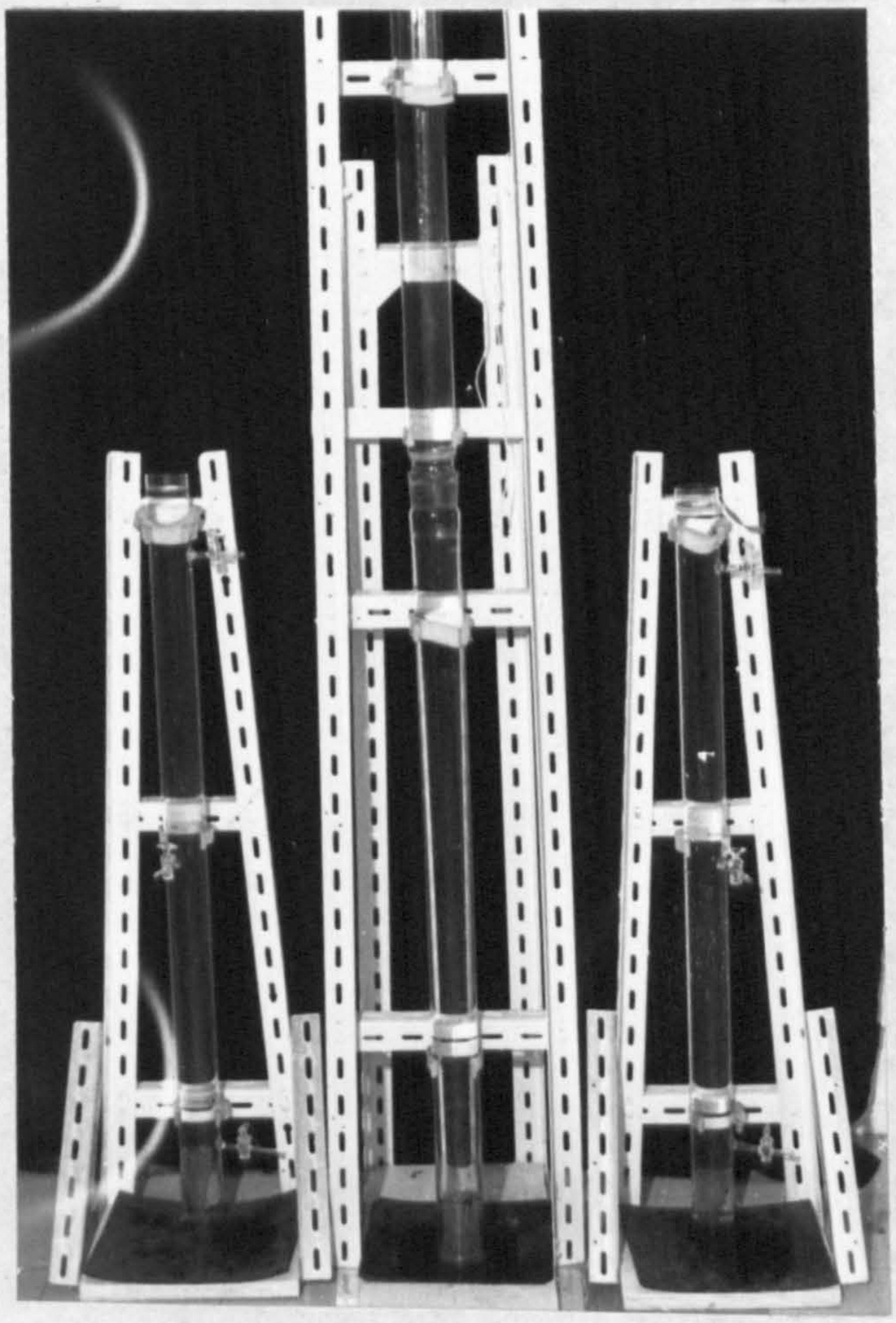


Fig. 11. Glass cylinders used to test the influence of depth on the infection capacity of schistosome miracidia.

A and C 1 m. tall.

B 2 m. tall.

At bottom of each cylinder is a snail bag containing snails.

before the introduction of miracidia. Active miracidia about 1 hour old were pipetted in the ratio of 10/snail into a beaker of dechlorinated water and the suspension was gently poured on to the surface of the water column. After an exposure period of 3-5 hours the snails were removed and maintained as described under "Materials and Methods". After each experiment the columns were emptied, rinsed and filled with dechlorinated water and left for at least a day before being used again.

B. pfeifferi/S. mansoni: Table 22 shows the infections produced in B. pfeifferi confined at depths of 1 m and 2 m of water. Infection rates ranged from 70-100% in the 1 m column and 60-100% in the 2 m column. There was no significant difference between infections at the two depths ($P > 0.36$).

B.(P.) globosus/S. haematobium: Infections produced in B.(P.) globosus are shown in Table 23. Infection rates ranged from 62.5-100% in the 1 m column and 25-100% in the 2 m column. The overall infection rates at the two depths were almost the same: 84% and 83% for 1 m and 2 m respectively.

When B. glabrata and S. mansoni (Puerto Rican strain) were tested in these columns 72.2% (13/18) and 80.0% (8/10) infections occurred at 1 m and 2 m depths respectively. In preliminary experiments using B. choanomphala and S. mansoni (Arusha Chini strain) infection rates of 57.5% (50/87)and 60.9% (28/46) were produced at depths of 1 m and 2 m respectively.

b. Miracidia released in the middle of a column of water

A second series of experiments was designed to test whether miracidia, when released in the middle of a column, would swim up to the surface or dive down to the bottom to infect confined snails. One bag of snails was dropped to the bottom of a 1 m column of water and another bag of snails hung up just under the surface. Active miracidia about one hour old were gently released in the middle of the column by means of a narrow glass tube about 60 cm long. After 3-5 hours of exposure the snails were put into aquarium tanks and maintained as already described.

TABLE 22

The infections produced in B. pfeifferi confined at the bottom of water columns of 1 m and 2 m by S. mansoni miracidia introduced at the top of the column.

Experiment No.	No. of snails exposed	No. infected / No. surviving	% Infection rate	Temperature at exposure
1 m Depth				
1	20	16/20	80.0	27
2	20	16/19	84.2	27
3	20	18/20	90.0	22 - 28
4	10	7/10	70.0	28
5	15	13/13	100.0	26
Totals	85	70/82	85.4	-
2 m Depth				
1	10	7/10	70.0	27
2	10	5/6	83.3	27
3	10	8/8	100.0	22 - 28
4	10	6/10	60.0	28
5	10	9/10	90.0	26
6	10	5/8	62.5	23
7	10	8/9	88.9	23
Totals	70	48/61	78.7	-

TABLE 23

The infections produced in B. (P.) globosus confined at the bottom of water columns of 1 m and 2 m by S. haematobium miracidia introduced at the top of the column.

Experiment No.	No. of snails exposed	No. infected / No. surviving	% Infection rate	Temperature at exposure °C
1 m Depth				
1	10	5/8	62.5	26
2	8	3/4	75.0	22 - 28
3	8	5/5	100.0	22 - 27
4	12	6/8	75.0	27
5	12	9/9	100.0	25
6	10	6/7	85.7	25
7	10	7/8	87.5	23
Totals	70	41/49	83.7	-
2 m Depth				
1	10	6/6	100.0	26
2	8	1/4	25.0	22 - 28
3	10	7/9	77.8	22 - 27
4	8	4/5	80.0	27
5	10	7/8	87.5	25
6	10	6/6	100.0	25
7	6	4/4	100.0	27
8	10	3/5	60.0	28
Totals	72	38/46	82.6	-

The results of the infections in snails placed at the surface and at the bottom of a 1 m column of water are shown in Table 24.

B. pfeifferi/S. mansoni: The overall infection rates of 88.5% and 80.4% in snails confined at the top and bottom of the column respectively were high and the difference between them is not statistically significant ($P > 0.27$). Miracidia of S. mansoni had therefore distributed themselves equally between the top and bottom of the 1 m column.

B.(P.) globosus/S. haematobium: Although the numbers involved in the experiment were small a significantly high infection rate occurred at the bottom than at the top ($P < 0.001$). It may therefore be inferred that most of the S. haematobium miracidia dived to the bottom to produce higher infections among confined snails. Therefore, while a somewhat uniform distribution of S. mansoni miracidia occurred in the column, S. haematobium miracidia clearly showed a preference for the bottom of the column.

Conclusions

The results of the experiments show conclusively that the maximum of 2 m was no barrier to host location by S. mansoni and S. haematobium miracidia. The high infection rates, ranging from 79-85%, that occurred at 1 m and 2 m were not significantly different in either B. pfeifferi ($P > 0.36$) or B.(P.) globosus ($P > 0.88$). A similar trend was obtained with B. glabrata/S. mansoni (P.R. strain) and B. choanomphala/S. mansoni (Arusha Chini strain), the infection rates reflecting the susceptibility of the snail host rather than the scanning capacity of the miracidia.

When miracidia were released in the middle of the 1 m column, thus having the choice of diving to the bottom or swimming up to reach target snails, the infection rates obtained in B. pfeifferi and B. (P.) globosus indicated a striking difference in the behaviour of S. mansoni and S. haematobium; the former distributed themselves evenly while the latter preferentially moved to the bottom.

TABLE 24

Infections among (I) B. pfeifferi and (II) B. (P.) globosus confined at the surface and bottom of 1 m water columns with the S. mansoni and the S. haematobium miracidia respectively released in the middle of the column

Experiment No.		No. of snails infected / No. of snails surviving (No. exposed) Surface	Bottom	Infection Rate Surface	Bottom	Temperature of Exposure
S. mansoni						
I	1	17/18 (20)	13/13 (16)	94.4	100.0	25.5
	2	19/19 (20)	17/20 (20)	100.0	85.0	26.0
	3	10/15 (16)	7/13 (16)	66.7	53.8	23.0
Totals:		46/52 (56)	37/46 (52)	88.5	80.4	
S. haematobium						
II	1	2/5 (8)	5/5 (8)	40.0	100.0	25.0
	2	5/10 (10)	8/9 (10)	50.0	88.9	25.0
	3	2/9 (10)	7/7 (10)	22.2	100.0	26.0
Totals:		9/24 (28)	20/21 (28)	37.5	95.2	

Upatham (1971-72, personal communication) obtained results that seemed to set a limit to the depth to which S. mansoni (St. Lucian strain) may dive; infection rates of 81.4% and 3.5% were produced in B. glabrata at the surface and at a depth of 1.21 m respectively. The present studies do not bear this out. Wajdi (1972) however, using S. haematobium miracidia in 1 m columns, obtained higher infection rates in B. truncatus at the bottom than at the top, which agrees with the present findings.

VI. HYDROSTATIC PRESSURE

Introduction

There is no information in the literature about the effect of hydrostatic pressure on the infective capacity of schistosome miracidia except a preliminary report (Prah, 1972). There are, however, a few reports on the effect of pressure on marine plankton animals in connection with the phenomenon of vertical migration, a habit of rising to the surface at night and swimming away in the daytime (Harris, 1953; Knight-Jones and Qasim, 1955; Hardy, 1971). Some of the animals are known to cover 200-300 feet or more in 24 hours in response to a particular light intensity. Although laboratory experiments (Hardy and Bainbridge, 1954; Knight-Jones and Qasim, 1955) have shown that some planktonic animals are able to detect slight changes in hydrostatic pressure Hardy (1971) is of the opinion that an aquatic animal should feel no ill effects of pressure provided it has no spaces or bubbles filled with air or gas inside it. He adds that since all liquids are only slightly compressible, the semi-fluid protoplasm of the body covered by a flexible elastic skin contracts only very slightly under the greatest pressure, thus the animal can have a most delicate structure and make the finest movements just as well in great depths as near the surface.

Experiments and Results

Apparatus used: In the laboratory it was not practicable to produce water columns as tall as 5 metres; neither was it possible to confine miracidia to any particular depth in a column, so a glass apparatus was designed in which pressure equivalent to a depth of 5 metres (16½ ft) of water was produced. The apparatus depends upon the principle that water is almost incompressible and that any pressure exerted on it is transmitted equally in all directions (Smith, 1961) and the fact that water and mercury are

immiscible. An added advantage is the large specific gravity of mercury which makes it a convenient substance to work with.

Fig. 12 shows the apparatus which consists of a main cylindrical tube, A, capacity 170 ccs with two side arms, B and C, attached to the base. The side arm B is connected to a tap (b) which is used to equalize the pressure in the system with that of the atmosphere. The other arm C with a plastic tube attached carries a funnel; the pressure in the system was altered by raising or lowering the level of mercury in it. With the key, b, opened to the atmosphere, dechlorinated water was poured into cylinder A to fill the system. Mercury, previously washed with dechlorinated water, was poured down the funnel so that the base of the cylinder was filled up to about 2.5 cms (1 inch). Water was displaced from A and B and the system was checked for air bubbles by tapping at the junctions, especially the base of the main cylinder.

The apparatus was that prepared to test the influence of hydrostatic pressure on the infective capacity of miracidia. This study was carried out in two ways: in the first series pressure was applied on the miracidia and snail together for a period of time, but in the second set pressure was applied on the miracidia alone for 3 and 5-hour periods and then snails were exposed to the miracidia to test their infectivity. Table 25 shows the relationship between height of mercury and depth of water.

a. <u>The influence of hydrostatic pressure on miracidia and snail together</u>

A circular disc, a, cut from the 14-gauge nylon gauze, was fitted into cylinder A to prevent them from falling on to the mercury. About 20 ml of water was withdrawn from A and 10 or less snails were dropped into it. A suspension of about 1-hour-old miracidia pipetted in the ratio of 10/snail was poured on to the snails and more dechlorinated water was added, filling A to the brim. Tap b was then disconnected from the atmosphere and the rubber stopper was carefully pushed into A and then

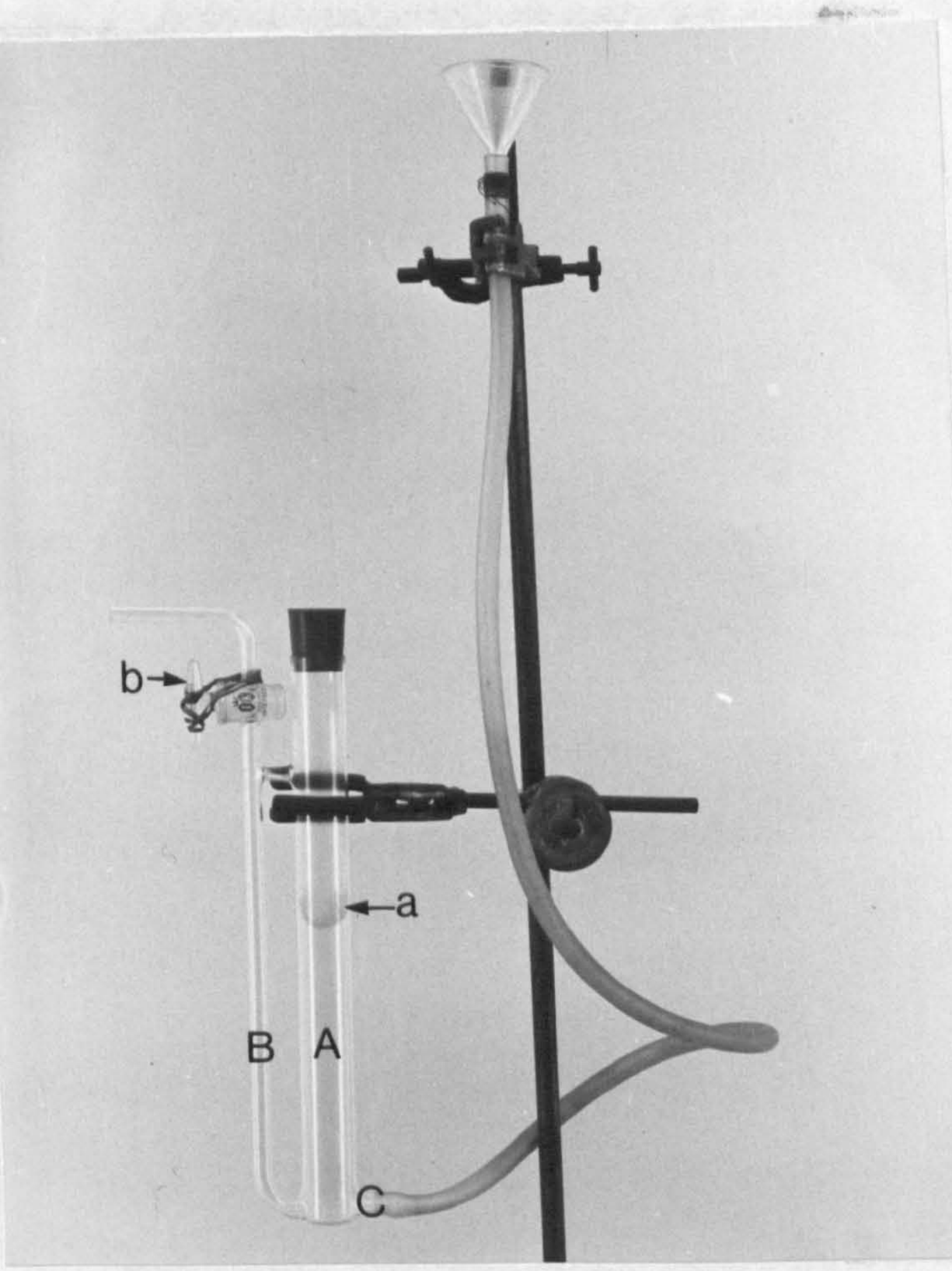


Fig. 12. Pressure apparatus.

A. main cylindrical tube.

a. disc of 14 gauge nylon gauze.

B. side arm.

b. tap for equalizing pressure in the system with that of the atmosphere.

C. side arm with plastic tube attachment containing mercury.

tightly secured so that no air bubbles were trapped under it. The pressure in the system was then again equalized to that of the atmosphere and the level of mercury in arm C was noted on the graph sheet placed behind it. This was pressure zero, equivalent to that at the surface of water. The pressure in the system was disconnected again from the atmosphere and arm C was raised until the mercury level indicated the required pressure when it was clamped up tightly. The pressure exerted by the difference in height of mercury was then transmitted equally in all directions in the water due to the incompressibility of the latter. There was always a control set of apparatus in which pressure was equal to that of the atmosphere, i.e. pressure as at the surface of water. Fig.13.

TABLE 25 Showing the relationship between the height of mercury and depth of water

Height of mercury (in cms)	Depth of water (in metres)
0	Surface
7	1.0
14	2.0
21	2.9
27	3.6
36	5.0

The exposure was over a period of 3-5 hours after which snails were put into aquarium tanks and maintained as already described.

Tables 26 and 27 show the infections that occurred among B. pfeifferi and B.(P.) globosus under varying levels of hydrostatic pressure with S. mansoni and S. haematobium miracidia respectively.

B.pfeifferi/S. mansoni: There were high infection rates ranging from 84-96% under water pressures as at surface to depth 2.9 m. but infection rates dropped significantly at depths 3.6 m. and below ($P<0.001$).

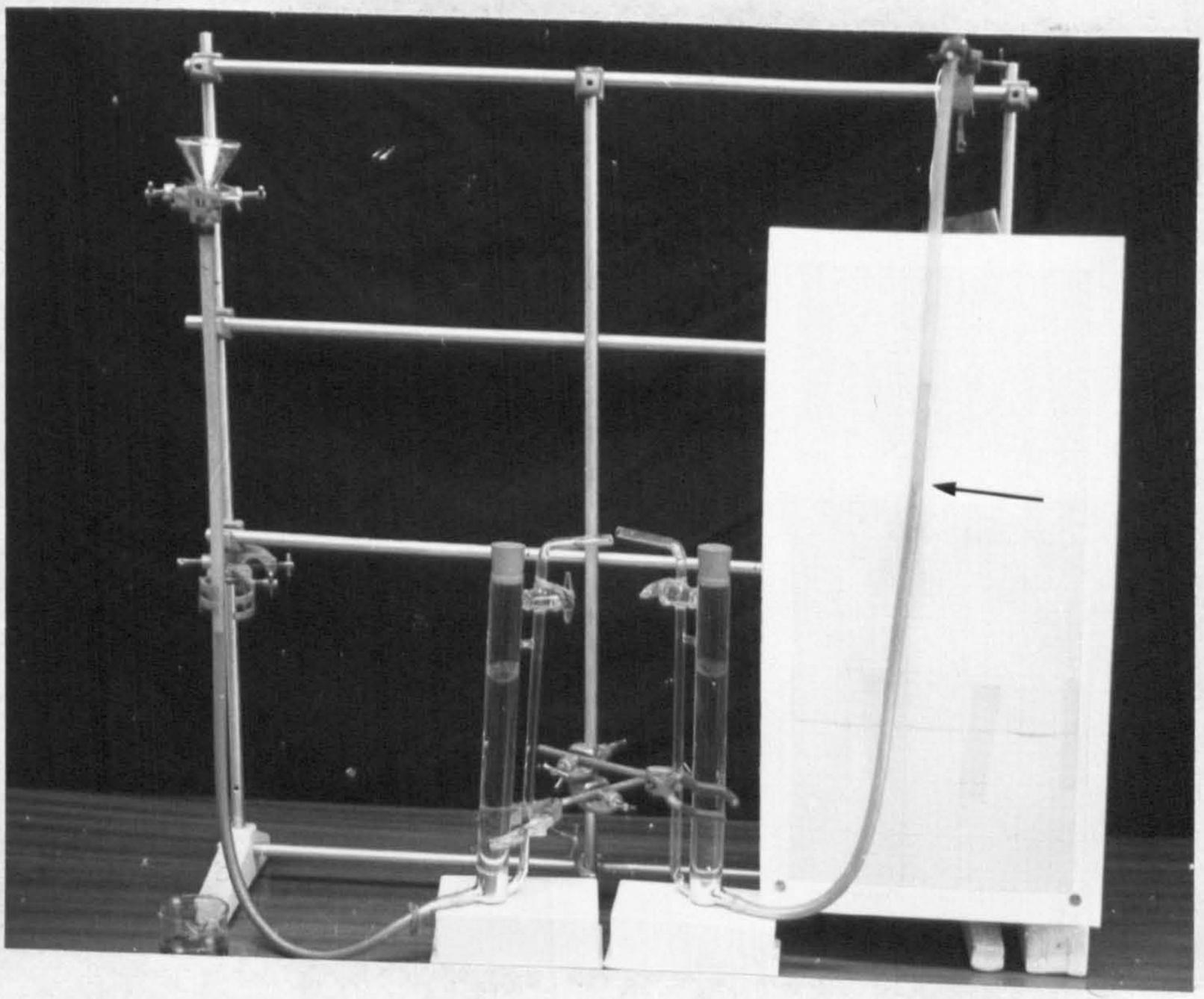


Fig. 13. Pressure apparatus in use.

Control set, Left, under atmospheric pressure.

Test set, Right, under pressure equal to that at 5.0 m. depth.

TABLE 26

Infections produced in B. pfeifferi by S. mansoni miracidia under hydrostatic pressures equivalent to the depths indicated

Exp. No.	Depth of water (in metres) 0	2.0	2.9	3.6	5.0
1		15/18 (20)			
2	9/9 (10)	8/9 (10)			
3	10/10(10)		9/10 (10)		
4	9/10(10)		10/10 (10)		
5	9/10(10)		9/9 (10)		
6	7/10(10)			3/9 (10)	
7	8/10(10)	9/10 (10)		5/10 (10)	
8	9/10(10)			4/10 (10)	4/10 (10)
9				8/8 (10)	9/10 (10)
10		10/10 (10)			6/10 (10)
11	0/9 (10)				6/9 (10)
Totals:	69/78(80)	42/47 (50)	28/29 (30)	20/37 (40)	23/39 (40)
Mean Infection Rate	88.5	89.4	96.6	54.1	59.0

TABLE 27

Infections produced in B.(B) globosus by S. haematobium miracidia under hydrostatic pressures equivalent to the depths indicated

Exp. No.	Depth of water (in metres) 0	2.0	2.9	3.6
1	5/6 (6)	2/4 (6)	3/4 (6)	
2		4/4 (10)	4/7 (10)	
3	5/5 (10)		6/9 (10)	2/7 (10)
4	3/4 (6)	4/6 (6)		
5		5/6 (10)		2/9 (10)
6	7/9 (10)	7/8 (10)		6/10 (10)
7	6/7 (10)		8/10 (10)	4/8 (10)
Totals:	26/31 (42)	22/28 (42)	21/30 (35)	14/34 (40)
Mean Infection Rate	83.9	78.6	70.0	41.2

The overall infection rate at the surface was significantly different from those at depths 3.6 m. ($P = 0.0001$) and 5.0 m. ($P<0.01$). The reduction in infection rate at depths 3.6 m and below may be attributed to an observed retraction of most of the soft body of the snail into its shell during the application of such high pressures.

B.(P.) globosus/S. haematobium: Infection rates of 70-83% occurred among snails exposed to pressures as at the surface to a depth of 2.9 m. While there was no significant difference between infections at the surface and at a depth of 2.9 m ($0.19<P<0.20$) there was a highly significant difference between infections at the surface and at a depth of 3.6 m. ($P<0.001$). Here too, as occurred in B. pfeifferi, it was observed that the snails had withdrawn most of their soft bodies into their shells, particularly at a depth of 3.6 m.

b. The influence of hydrostatic pressure on miracidia alone

In this series smaller replicas of the apparatus were used. The volume of tube A was only 14.5 ml. The procedure was the same as that described for the first series except that here there were no snails in tube A; the pressure was exerted on miracidia alone. Active miracidia were put into cylinder A, more water was added and the stopper pushed in firmly. The required pressure was exerted for a period of 3 or 5 hours after which the miracidia were transferred into petri dishes. The miracidia were examined casually under a dissecting microscope for the degree of activity and then supplied with snails at a ratio of 1/10 miracidia. Exposure was for an average period of 3 hours after which snails were put into aquarium and maintained as described under 'Materials and Methods'.

It was observed during the application of pressure that miracidia in cylinder A behaved in the same way under different hydrostatic

pressures as they did in the 100 ml measuring cylinders. S. mansoni miracidia were mostly at the top with an even distribution down to the bottom. Most of the S. haematobium miracidia on the other hand concentrated at the bottom with a sharp decline in numbers above, and only a few miracidia at the top. When examined under dissecting microscope miracidia did not exhibit any ill effects from the applied pressure even at a depth of 5.0 m.

The results of infections in snails exposed to 'pressure-treated' miracidia are shown in Tables 28 and 29.

B. pfeifferi/S. mansoni: High infection rates ranging from 73.3-100% occurred at the three depths studied. The hydrostatic pressures at 3.6 and 5.0 depths for 3 hours did not affect the infectivity of the miracidia and even after 5 hours at 5.0 m depth S. mansoni miracidia were capable of producing high infection in snails.

B.(P.) globosus/S. haematobium: Infections produced by S. haematobium miracidia in B.(P.) globosus under different hydrostatic pressures were also high and there was no significant difference between the infectivity of miracidia maintained at pressures equivalent to that at the surface and at depths of 3.6 m and more ($P = 0.31$). When pressure equivalent to a depth of 5.0 m was exerted on S. haematobium miracidia for 5 hours their infectivity was unaffected, the infection rate of 73.7% being of the same order as that at the surface, 91.7% ($t_2 = 1.373$; $P > 0.30$). The numbers involved, though small, give a true indication of the fact that hydrostatic pressure has no influence on the infective capacity of schistosome miracidia.

Conclusions

The results of the experiments show clearly that pressure has no effect on the miracidia which were observed to behave in a manner similar to their behaviour in the 100 ml cylinders for vertical distribution (cf light).

TABLE 28

Infections produced in B. pfeifferi by S. mansoni miracidia after applying on the latter hydrostatic pressure equivalent to the depths indicated

Period of application	Depth of water in metres 0	3.6	5.0	Temperature in °C
3 hours	19/20 (20)	19/19 (20)	16/20 (20)	25.0
	11/15 (15)	12/15 (15)	13/15 (15)	24.5
	14/15 (15)	14/15 (15)	13/15 (15)	26.0
Totals	44/50	44/40	42/50	
% Infection rate	88.0	93.7	84.0	
5 hours	17/19 (20)	-	18/20 (20)	25.0
	13/15 (15)	-	8/9 (10)	26.0
Totals	30/34		26/29	
% Infection rate	88.2		89.7	

TABLE 29

Infections produced in B.(P.) globosus by S. haematobium miracidia after applying on the latter hydrostatic pressure equivalent to the depths indicated

Period of application	Depth of water in metres 0	3.6	5.0	Temperature in °C
3 hours	14/15 (20)	-	11/13 (20)	26.0
	0/0 (12)	10/11 (12)	-	26.5
	6/7 (10)	5/6 (10)	7/9 (12)	25.0
	6/7 (10)	-	5/5 (10)	26.0
Totals	34/37	15/17	23/27	
% Infection rate	91.9	88.2	85.2	
5 hours	5/6 (10)	-	5/7 (10)	26.0
	6/6 (10)	-	9/12 (15)	26.5
Totals	11/12		14/19	
% Infection rate	91.7		73.7	

S. mansoni miracidia were positevely phototactic, most of them being at the top and S. haematobium positively geotactic with most congregated at the bottom. Infections using miracidia previously exposed to varying pressures for 3 and 5 hours did not appear to be significantly affected by pressure; infections were as high as with miracidia not treated under pressure.

The behaviour of snails under high pressures resulted in an overall reduction in infection rate. The infections at 3.6 m and 5.0 m were more variable, being apparently dependent on the availability of the soft body of the snail for penetration by the miracidia.

The opinion expressed by Hardy (1971) regarding the effect of hydrostatic pressure on marine planktonic animals also appears to apply to miracidia which have flexible and elastic outer coverings and have no airspaces or pockets in them; the stresses due to high pressure at great depths are counterbalanced in the miracidia which thus suffer no ill effects. Miracidia should therefore be able to scan the environment unaffected by pressure.

VII. HOST-PARASITE DISPERSION

Introduction

In the laboratory miracidia and snails are confined in small volumes of water to ensure the highest possible infections among the snails. But even under the conditions that offer maximum opportunity for snail-miracidia interaction 100% successes do not always occur. Here close confinement, therefore, does not always result in high infections. In nature snails become infected in varying expanses of water ranging from small water holes, ponds, borrow pits and streams to large water bodies such as Lake Victoria, Lake Volta and the River Nile. Although the concentrations of miracidia at which infections occur in such large bodies of water are not known, laboratory infections in increasing volumes of water give indications of the remarkable scanning capacity of the schistosome miracidia. In small circumscribed habitats where there are little or no waves the water may be still and miracidia may have to traverse considerable distances by their own swimming activity to the submerged margins including even the bottom where snail-miracidial interactions occur. In large water bodies and in rivers and streams, however, water currents and flow carry miracidia long distances away from the point of entry.

Laboratory studies have been carried out to test the scanning capacity of miracidia under varying conditions of mutual dispersion in an attempt to elucidate the mechanisms of host location. Some authors have investigated the effect of different concentrations of miracidia in small volumes of water on the infection of snails, and others have investigated the effect of dispersal of snail and miracidia in increasingly larger volumes of water on the ability of the miracidia to locate and infect snails. The vessels used ranged from 3" x 1" specimen tubes to large aquarium tanks and small outdoor ponds.

Schreiber and Schubert (1949) exposed B. glabrata individually to 1, 3, 7 and 12 S. mansoni miracidia in 3 ml of water and obtained respectively 14%, 55%, 70% and 85% infection rates, a progressive increase in infection rate with increase in number of miracidia. Standen (1952) studied the effectiveness of individual and en masse exposures of B. glabrata to S. mansoni by the infection rates among the snails. He exposed snails individually to 6 miracidia in 2 ml of water and en masse to 6 miracidia per 10 ml and 500 ml of water. The results he obtained in two years indicated that individual infections were more effective than en masse infections. In 1950 he obtained 82% for individual exposures and 72% and 67% for en masse 10 ml and 500 ml of water respectively. A similar trend of 49%, 39% and 34% was obtained for 1949 individual and en masse exposures respectively.

Chernin and Dunavan (1962) exposed B. glabrata individually to 1 and 5 miracidia in 1.5 ml of water and obtained 42% and 87% infection rate respectively. They also found that single S. mansoni miracidia were able to infect B. glabrata individually placed in vessels ranging from 2.5 - 1526 cm^2 in surface area and containing 1.5-700 ml of water. Infection rates were similar up to 100 ml of water (33-43%) but thereafter declined in larger volumes (22-6%). They found that S. mansoni miracidia traversed at least 86 cm in a large aquarium to infect target snails.

Kinoti (1964a) observed that increase in number of S. bovis miracidia from 4-6 to 8-9 per snail produced 67% and 100% infections among B.(P.) africanus ovoideus.

McClelland (1965) exposed B.(P.)nasutus to varying concentrations of S. haematobium miracidia individually and en masse. Individual exposures were carried out in 3" x 1" specimen tubes containing 1, 5 and 10 miracidia in 4 ml of water. He found that while no infections

occurred at 1 miracidium/snail level the infection rate increased from 30% at 5 miracidia/snail level to 40% at 10 miracidia/snail level. In en masse infections 25 snails were exposed to varying concentrations of miracidia in 5 l. and 20 l. of water. It was found that infection rate increased with increase in density of miracidia. Infections in 20 l. of water differed slightly from those carried out in 5 l. - hence even a fourfold increase in volume did not result in a pronounced reduction in infection at all levels.

Shiff (1968) also studied the ability of single S. haematobium (Rhodesian strain) miracidia to locate and infect single free-moving B.(P.) globosus in cylindrical vessels ranging from 2.3-29 cm in diameter with 0.5 cm depth of water. He found that in spite of the disparity in vessel size there was no significant change in the proportion of snails infected. (Infection rates ranged from 37.5 - 46.6%).

The ability of miracidia to traverse long horizontal distances to infect target snails in linear channels has been investigated by two authors. Chernin (1968) found that S. mansoni miracidia released 150 cm away from confined target B. glabrata produced a high infection rate of 84% two hours after release of miracidia. Shiff (1960) also found that S. haematobium miracidia ranged over 395 cm in still water to infect B.(P.) globosus producing 11.1% infection rate. He found that over shorter distances infection rates were higher.

In this study the scanning capacity of S. mansoni and S. haematobium miracidia under conditions of increasing mutual dispersion was tested in vessels of different sizes. Two types of dispersion were considered:

(a) dispersion in increasing volumes of water

(b) dispersion over increasing horizontal distances.

Experiments and Results

a. The effect of host-parasite dispersion in increasing volumes of water on the scanning capacity of S. mansoni and S. haematobium miracidia

The different vessels used ranged from 3" x 1" specimen tubes containing 5 ml of water to a fibre glass storage tank 80 x 64 x 48 cm which held over 240 litres of water. Tables 30 and 31 show the vessels used, their dimensions, the volumes of water they contained, the resultant miracidial density and the infections that occurred in them. The depth of water in the 3" x 1" tubes, the petri dishes and the aquarium tanks was about 1 cm. Active miracidia in the ratio of 10/snail were pipetted into the specimen tubes, petri dishes and aquarium tanks and the appropriate number of snails were dropped into them. The snails were thus free to move around. In the plastic and fibre glass tanks snails were put into bags and placed at the bottom at one end and the miracidia were released on to the surface of the water at the opposite end. Exposure was over a period of 3-5 hours after which the snails were removed and maintained as already described.

The infections that occurred among B. pfeifferi and B.(P.) globosus in the various vessels are set out in Tables 30 and 31.

B. pfeifferi/S. mansoni: Infection rates were high and ranged from 86.8-97.6% in the smaller vessels while in the plastic and fibre glass tanks infection rates were 72.1 and 62.5 respectively. Infections in the smaller vessels were consistently high, the lowest being 84.0% while infections in the fibre glass tank for example ranged from 26.7-94.1%. In the plastic tank the highest infection rate was 100% (17/17) but in another experiment only 43.0% infections occurred.

B.(P.) globosus/S. haematobium: The highest overall infection rate of 84% occurred in the petri dishes while infections in the specimen tubes,

TABLE 30

Infections produced in B. pfeifferi by S. mansoni miracidia in increasingly larger volumes of water

Vessel	Specimen tubes	Petri-dishes	Aquarium tank	Plastic tank	Fibre glass storage tank
Dimensions	3" x 1" Dia.	9 cm dia	24 cm dia	55x40x30 cm	80x64x48 cm
Volume of water	5 ml	40 ml	400 ml	70 l	240 l
Number of snails/ vessel	1	6 (or less)	6-10	10-20	10-20
Miracidial density/ml* or /l.	2.0 per ml	1.5 per ml	0.15-0.25 per ml	1.4-2.8 per l.	0.42-0.84 per l.
Exp. 1	7/8 (10)	5/5 (6)	7/7 (8)	7/16 (18)	-
2	21/25 (25)	23/24 (24)	9/9 (10)	-	-
3	12/13 (15)	-	-	25/35** (40)	-
4	-	12/12 (12)	-	-	16/17 (18)
5	6/7 (10)	-	11/13*** (14)	17/17 (20)	4/15 (18)
Totals:	46/53 (60)	40/41 (42)	27/29 (32)	49/68 (78)	20/32 (36)
%Infection rate	86.8	97.6	93.1	72.1	62.5

* Miracidial density = No. of miracidia per unit volume; ml or litre.

** 2 plastic tanks were used, each with 20 snails.

*** 2 aquarium tanks were used, each with 7 snails.

TABLE 31

Infections produced in B.(P.) globosus by S. haematobium miracidia in increasingly large volumes of water

Vessel	Specimen tubes	Petri-dishes	Aquarium tank	Plastic tank	Fibre glass storage tank
Dimensions	3" x 1" dia.	9 cm dia	24 cm dia	55x40x30 cm	60x64x48 cm
Volume of water	5 ml	40 ml	400 ml	70 l.	240 l.
Number of snails/ vessel	1	6 (or less)	6-10	10-20	10-20
Miracidial density * /ml or /l.	2.0/ml	1.5/ml	0.15-0.25 per ml	1.4-2.8 per l.	0.42-0.84 per l.
Exp. 1	5/6 (8)	-	-	6/6 (10)	3/7 (10)
2	3/3 (5)	-	-	3/5 (10)	-
3	9/14 (15)	-	-	5/6 (10)	5/7 (10)
4	-	9/11 (12)	-	7/12 (15)	5/10 (10)
5	-	8/8 (12)	7/10 (12)	-	-
6	8/9 (12)	4/6 (6)	-	-	8/15 (20)
7	6/10 (12)	-	9/12 (12)	-	-
Totals	31/42 (52)	21/25 (30)	16/22 (24)	21/29 (45)	21/39 (50)
%Infection rate	73.8	84.0	72.7	72.4	53.8

* Miracidial density = No. of miracidia per unit volume; millilitre or litre.

aquarium tank and the plastic tank were very similar (73.0, 72.7 and 72.4 respectively). Here too, as in B. pfeifferi, the infections were uniformly high in the smaller vessels. The difference between infections in the plastic tank and those in the fibre glass tank was not significant at 5% level ($P > 0.10$) although the miracidial densities differed about threefold.

Infections among B. pfeifferi and B.(P.) globosus in the plastic and fibre glass tanks were in the same order 53.0 - 72.1% which indicates that S. mansoni and S. haematobium miracidia were equally capable of scanning such large volumes of water for their hosts.

b. The effect of host-parasite dispersion in increasing linear horizontal distance on the scanning capacity of S. mansoni and S. haematobium miracidia

Short lengths of plastic guttering trapezoidal in cross-section (measuring 8.5 and 7.0 x 4.0 cm depth) were joined together to give the required length of trough. The maximum of 5.1 m (18 ft) used was made up of three 1.7 m (or 6 ft) cutterings joined end to end. Dechlorinated water of temperature 24-28°C was poured into the trough up to about 2 cm depth (about 17 litres). Snails were confined within 30-40 cm at one end of the trough by means of a 14 gauge nylon gauze screen and active miracidia pipetted in the ratio of 10/snail were released at the other end. The room was illuminated with a ceiling white fluorescent lamp and hence illumination on the trough was uniform. Exposure was over a period of 5 hours after which the snails were removed and maintained as already described under Materials and Methods.

Table 32 shows infections that occurred in B. pfeifferi and B.(P.) globosus at different horizontal distances from the point of release of S. mansoni and S. haematobium mircoidia respectively.

TABLE 32

Infections among B. pfeifferi and B.(P.) globosus confined at different distances away from the point of introduction of S. mansoni and S. haematobium miracidia respectively in plastic channels

	Linear horizontal distances in metres			
	1.1 - 1.4	2.0 - 2.3	3.6 - 4.0	4.9 - 5.1
B.pfeifferi/ S. mansoni	10/16 (20)	20/25 (25)	5/9 (10)	1/14 (15)
	13/15 (15)	19/23 (25)	14/22 (25)	9/32 (32)
	10/10 (10)	13/20 (20)	20/27 (30)	16/26 (30)
	16/19 (20)	-	-	-
Totals:	49/60 (65)	52/68 (70)	39/58 (65)	26/72 (77)
% Infection Rate	81.7	76.5	67.2	36.1
B.(P.) globosus/ S. haematobium	9/13 (15)	5/14 (18)	7/8 (10)	8/17 (20)
	12/13 (20)	7/11 (15)	7/14 (20)	3/8 (10)
	7/10 (10)	-	-	-
Totals:	28/36 (45)	12/25 (33)	14/22 (30)	11/25 (30)
% Infection Rate	77.8	48.0	63.6	44.0

Replicates at the different distances did not run concurrently.

B. pfeifferi/S. mansoni: Infection rates declined with the increase in linear distance covered by miracidia to reach confined snails. An infection rate of 81.6% was produced in snails 1.1-1.4 m away, while 36.1% infection occurred among snails 5.1 m away. There was little variation in the infections at 1.1-2.3 m which ranged from 62.5% (10/16) to 100% 10/10), while infections at 5.1 m varied from 7.1-61.5%

B.(P.) globosus/S. haematobium: Infection rates declined with increase in linear distance covered by the miracidia to reach snails. The drop in infection rate at a distance of 2.0-2.3 m is not easily explained, but this may be due to the small numbers involved.

Although not much importance can be attached to the similarity of infection rates in B. pfeifferi and B.(P.) globosus, the results show clearly that both S. mansoni and S. haematobium miracidia by their own swimming activity in still water reachcand infect their hosts placed as far away as 5.1 m (18 ft). Other factors that are not immediately obvious may be in operation to produce the marked variations in infections among snails at a considerable distance from the scource of miracidia.

Conclusions

The dispersion of 10 miracidia/snail in vessels of increasing volumes of water produced miracidial densities (miracidia/unit volume, no/ml or no/l) ranging from 2.0/ml to 0.15-0.25/ml in the smaller vessels and 1.4-2.8/litre to 0.42-0.84/litre in the larger vessels. The results of the infections showed that for both B. pfeifferi and B.(P.) globosus the petri dishes provided the most favourable snail-miracidial interaction resulting in very high infection rates. On the whole infections in the smaller vessels were consistently high while those in the larger vessels fluctuated a great deal. The larger vessels involved covering horizontal distances of 50-75 cm and vertical distances

of 30-45 cm but the infection rates produced in B. pfeifferi and B.(P.) globosus were not significantly different indicating that S. mansoni and S. haematobium miracidia at those miracidial densities were equally capable of reaching and infecting their hosts.

There was a gradual fall in infection rate with increase of horizontal distance between target snails and point of miracidial introduction in both B. pfeifferi and B.(P.) globosus. The longest distance studied (5.1 m) was traversed by miracidia in infective condition and produced infection rates close to those at shorter distances. Chernin (1968) and Shiff (1968) reported that S. mansoni and S. haematobium miracidia covered 1.5 m and 4.0 m respectively to infect target snails. Upatham (1971-1972, personal communication) found that infection rate decreased with increase in distance and that S. mansoni miracidia located and infected snails situated 7.5 m away in a field site. Miracidia are known to swim at about 2.0 cm/sec (Chernin et al, 1962; Davenport et al, 1962; and the present studies) and at this rate can theoretically cover 7.2 m in an hour. It is therefore not surprising that miracidia cover 5.1 m to reach and infect target snails within the exposure period of 5 hours.

VIII. RATE OF FLOW OF WATER AND TURBULENCE

Introduction

Intermediate snail hosts of schistosomes are rarely found in rapidly flowing water with average velocities in excess of 30-35 cm/sec (approximately 1.1 ft/sec) since the snails are unable to maintain their hold even on firm smooth surfaces such as concrete or stone. At such high flow rates clinging to surfaces is difficult and more so when surfaces are loose and shifting such as silt and loam. Under such conditions the snails are first immobilized and then dislodged (McJunkin, 1970). Field observations from various endemic areas confirm this claim.

In West Africa Bulinus species usually live in stationary water (lakes, ponds, swamp pools, borrow pits etc.) but those that live in streams have been observed to prefer streams with sluggish current and muddy bottom, and seasonal streams that break into residual pools during the dry season (Blacklock and Thompson, 1924; Gordon, Davy and Foaston, 1934; Gerber, 1952; McCullough and Duke, 1954; Smithers, 1956; McCullough, 1957, 1962; Onabamiro, 1971). In East Africa both Bulinus and Biomphalaria species are found in permanent and seasonal watercourses and irrigation systems where flow is sluggish and are absent from fast-flowing streams such as those from hills (Ransford, 1940; McClelland, 1957; Maclean, Webbe and Msangi, 1950; Webbe and Msangi, 1950; Webbe, 1962b; Teesdale, 1962; Webbe and Jordan, 1966). William and Hunter (1960) found that Bulinus and Biomphalaria species in Khartoum and Blue Nile Provinces in the Sudan preferred slower running water and that B. truncatus was better able to withstand moderately faster running water than the other species.

In the primary canals of Egypt where water velocities are high snails are absent; in one main canal where the average rate of flow was 36.9 cm/sec with a range of 12.0 cm/sec to 77.8 cm/sec there were no B. truncatus but

some snails were collected from a canal where the flow rate was gentle (6.5 cm/sec) (Danso, Hairston and Dawood, 1966). They found that snails were particularly abundant in smaller canals and drains where the flow was gentle. Watson (1957) noted that Bulinus species in Iraq could not stand violent water movements including rapid flow and changes in water level.

In North Eastern Brazil B. glabrata, the intermediate host snail of S. mansoni, lives in natural watercourses of moderate flow rate (Barbosa, 1962).

In all these habitats a fast flow rate is known to be inimical and especially during periods of heavy rains the floods carry with them downstream large numbers of snails leading to a definite decline in snail populations. In fact this factor alone may account for wide seasonal fluctuations in the density of snail populations (McCullough and Duke, 1954). Strong lashing waves in lakes especially where the shores are steep and devoid of vegetation and to a lesser extent turbulence are also detrimental to snails but aquatic vegetation may shelter snails from the current and back water areas such as coves and holes form favourable living areas in fast-flowing streams (Maldonado, 1967; McJunkin, 1970).

The rate of flow from the edge to the centre of the bottom, i.e. the peripheral velocity, is an important factor for this is what dislodges the snails. De Araoz (1962) calculated the peripheral velocity in a channel of parabolic cross-section and found this to be equal to 0.40 of the velocity on the water surface in the centre line.

Naturally infected snails have been collected from many watercourses and irrigation canals where the flow rate was moderately low. Maclean et al (1958) collected infected Bulinus species from rivers and streams in Tanga district of Tanganyika (Tanzania). Teesdale (1962) found that 1.1-1.7% of over 10,000 B. pfeifferi collected from a river and two streams in Kenya were infected with S. mansoni and that 5.4% of the B.(P.) africanus ovoideus were infected with S. haematobium. Kinoti (1964b) also reported

natural infections of S. haematobium, S. bovis and S. mattheei in B. (P.) africanus ovoideus collected from very slowly flowing streams made up of pools connected by very gently flowing water. He considered this snail to be an essentially riverine species. Hira and Muller (1966) recorded a maximum cercarial infection rate of 13% from B.(P.) globosus collected from slow flowing streams in Western Nigeria.

In the laboratory flowing water systems have been devised to study the mechanisms involved in host location by schistosome miracidia in different flow rates of water. Webbe (1966) devised a flowing water system in which he exposed caged B. sudanica tanganyicensis to different concentrations of S. mansoni miracidia in water flowing at velocities of between 0.5 and 3.5 ft/sec (15 and 105 cm/sec). When the miracidial level was 25/snail and above, high infection rates were obtained at all water velocities and high infection rates were still obtained at low miracidial concentrations between 0.5 and 1.5 ft/sec (15 and 45 cm/sec); even at exposures of about 1 miracidium/snail there was only a slight reduction in infection rates.

Shiff (1968) exposed B.(P.) globosus in an asbestos trough to S. haematobium miracidia at 10/snail level at water velocities 7.5 and 15 cm/sec. The snails were free to move within two 14 gauge nylon screens 1 metre apart. A very low infection rate of 6.7% was obtained at water velocity of 7.5 cm/sec and no infections occurred among snails exposed to miracidia in water flowing at 15 cm/sec. When a wooden weir was placed in the trough this held back the water, produced turbulence and resulted in a marked reduction in velocity; and in this condition 39.6% infection rate was produced in the exposed snails.

Webbe and James (1969, personal communication) exposed B.(P.) globosus to S. haematobium miracidia at 20/snail level at a water flow rate of 10cm/sec (0.33 ft/sec) in a plastic trough of diameter 7.5 cm (3 inches). The snails moved freely in the trough and a high infection rate of 39.2% (31/79) was obtained.

Upathan (1971-1972, personal communications) caged and suspended laboratory-bred B. glabrata in streams (field sites) for 24 hours and with a flow rate of 10 cm/sec (0.34 ft/sec) obtained infection rate of 3.94% among 1699 surviving snails. In one experiment an infection rate of 9.82% was produced in 448 snails with an estimated miracidia inoculation level of 1-4/snail. No infections occurred at water velocities above 12.5 cm/sec (0.42 ft/sec).

Turbulence can be considered to be a disturbance in a waterbody, an obstruction in the uniform flow of water or movement by wave action in still water. A weir or stone placed along the course of a stream produces turbulence, when water falls from a height into a container below the disturbance created is turbulence, the air bubbles produced by an airstone in an aquarium tank sets up turbulence, a snail placed in a trough of slowly flowing water creates turbulence around it. The effect of turbulence on the scanning capacity of schistosome miracidia has not been tested and there are no units for turbulence. Shiff (1960) however attributed infections among B.(P.) globosus to the presence of a weir in a trough with water at a flow rate of 15 cm/sec which produced eddy flow and turbulence.

Experiments and Results

a. B. pfeifferi and B.(P.) globosus exposed to S. mansoni and S. haematobium miracidia respectively in water flowing at varying velocities.

The flowing water system: The flowing water system, similar to the one described by Webbe(1966), consisted of four components. (Fig. 14).

A large cylindrical storage tank	-	St.
A small head tank	-	Ht.
The trough	-	T.
The aspirator bottle	-	A.

Fig. 14. The flowing water device ("Mile Run") showing

St. - storage tank.

Ht. - head tank.

T. - S-shaped trough made up of P.V.C. guttering.

A. - aspirator bottle holding 5 litres dechlorinated water containing miracidia.

P. - protractor fixed on tap of head tank.

The large cylindrical storage tank (70 x 45 cm dia) is a plastic barrel of 180 litres (40 gallons) capacity. It was filled with tap water which was allowed to dechlorinate over a period of at least 10 days. (It provided enough water for the one hour period of exposure).

The small head tank (25 x 25 x 25 cm) of approximately 13.5 litres (3 gallons) capacity was placed immediately below the storage tank. A constant head of water was maintained by regulating the amount of water entering from the storage tank and the amount leaving into the trough. The top of the head tank carried a protractor which helped to maintain a set volume of discharge.

The trough was constructed of 3 pieces of Marley P.V.C. guttering of 7.5 cm (3 inches) diameter joined to form an S-shaped trough with a total length of 880 cm (29 feet). Water entered into the proximal end of the trough from the small head tank and ran into a sink at the distal end. The glass aspirator bottle, A, placed 50 cm away from the proximal end of the trough, contained about 5 litres of dechlorinated water and miracidia pipetted at 20 or more/snail level. It was fitted with a bottom tap and an air-inlet, air being introduced through an outer rubber connection with air bubbles passing up from the base to agitate the miracidial suspension, thus maintaining a uniform distribution. In addition, the aspirator was frequently given a gentle shaking to help distribute the miracidia evenly throughout the water. The aspirator was regulated to dispense miracidia over a period of about one hour.

The rate of flow of the water was calculated by floating tiny balls of polystyrene on the water and timing them over known distances in all three channels of the trough. The rate of flow was varied by altering the angle of inclination of each length and by the amount of water entering the trough.

Twenty-four hours before an experiment the room was heated so that the water temperature was between 21 and 26°C. Water was run into the

trough to wet it sufficiently, then the snails were distributed in the channels. When the snails had attached themselves to the surface of the trough the water was turned on, at first gradually and then the pressure was increased until the required rate of flow was attained. In order to prevent dislodged snails being washed into the overflow sink 14-gauge nylon screens were placed in the middle of the second channel and towards the end of the third channel. (It was noticed that the presence of the nylon screens resulted in a negligible reduction in the rate of flow of water).

Exposure was continued for a period of about one hour during which a constant head of water was maintained in the head tank and the miracidia in the aspirator bottle were constantly checked for even distribution. Snails were left in the trough for a further period of about 2 hours with only a trickle of water running through the trough. After the two hours snails were removed and maintained as already described.

During the experiments it was observed that B.(P.) globosus were more capable of maintaining themselves in the troughs than B. pfeifferi. Consequently at the low flow-rates of 4-5 cm/sec and 7-10 cm/sec only the screen towards the end of the third channel was used for B.(P.) globosus while for B. pfeifferi the two screens were used for all flow rates (i.e. 4-5 cm to 25 cm/sec).

The results of infections produced in B. pfeifferi and B.(P.) globosus by S. mansoni and S. haematobium miracidia in water of varying flow rates are shown in Table 33.

B. pfeifferi/S. mansoni: High infection rates of 65.9% and 60.0% were produced in B. pfeifferi at flow rates of 4-5 cm/sec and 7-10 cm/sec respectively. There was an infection rate of 49.0% at the flow rate of 12-15 cm/sec but no infection at 20-25 cm/sec and above. There was no significant difference between infection rates at flow rates of 4-5 cm/sec and 7-10 cm/sec ($P > 0.61$) and those at 4-5 cm/sec and 12-15 cm/sec ($P > 0.10$).

TABLE 35

Infections produced in B. pfeifferi and B.(P.) globosus by S. mansoni and S. haematobium miracidia respectively in water of varying flow rates

	Rate of flow of water in cm/sec				
	4-5	7-10	12-15	20-25	25
B. pfeifferi/ S. mansoni	17/23(25)	19/26(30)	18/32(33)	0/26(30)	
	10/18(21)	12/25(27)	6/17(21)	0/33(33)	0/20(30)
Total infections	27/41	31/51	24/49	0/59	0/20
% Infection rate	65.8	60.9	49.0	0.0	0.0
B.(P.) globosus/ S. haematobium	5/13(20)	20/43(45)	0/15(10)	0/18(24)	
	8/15(20)	0/14(18)	20/30(39)	0/14(21)	
	7/20(24)	10/15(29)	0/8 (15)		
			9/21(30)		
Total infections	20/48	30/72	29/74	0/32	
% Infection rate	41.7	52.8	39.2	0.0	

N.B. Replicates at varying flow rates did not run concurrently.

B. (P.) globosus/B. haematobium: At the flow rates of 4-5 cm/sec and 7-10 cm/sec infection rates of 41.7% and 52.8% respectively occurred among B. (P.) globosus. At the flow rate of 12-15 cm/sec there was a total infection rate of 39.2% but there was a great deal of variability in infections that ranged from zero to 60%. There was no significant difference between infection rate at the flow rates of 4-5 cm/sec and 12-15 cm/sec ($P > 0.78$) and those of 7-10 cm/sec and 12-15 cm/sec ($P > 0.10$). Here too, as in B. pfeifferi, there were no infections at the flow rate of 20-25 cm/sec.

b. Exposure of B. pfeifferi to S. mansoni miracidia in turbulent and still water

Production of turbulence: Turbulence was produced by a battery powered hand stirrer which was dipped into a rectangular plastic tank (50 x 40 x 30 cm) containing dechlorinated water of temperature between 23 and 28°C (Fig. 15). The blades of the "stirrer" were about 10 cm from the bottom of the tank. A bag of snails was placed in the tank immediately below the stirrer, and another bag of snails was placed at the opposite end about 40 cm away. The stirrer (moving at 250 revs/min in air, but a little slower in water) was switched on and created turbulence at one end of the plastic tank. Freshly hatched miracidia pipetted in the ratio of 10/snail were released in the centre of the tank. Exposure was for a period of 2 hours, after which the snails were maintained as already described.

Only B. pfeifferi and S. mansoni were used in this experiment. The results of the infections produced are shown in Table 34.

While almost all the snails in the "no-turbulence" end of the tank were infected (infection rate 96.7%), only about 7% of those in the "turbulence" end of the tank became infected. In one experiment, the stirrer stopped for about 15 minutes after the first hour of exposure, and this explains the infection of two out of ten snails at this end. There was, therefore, a very highly significant difference between the effect

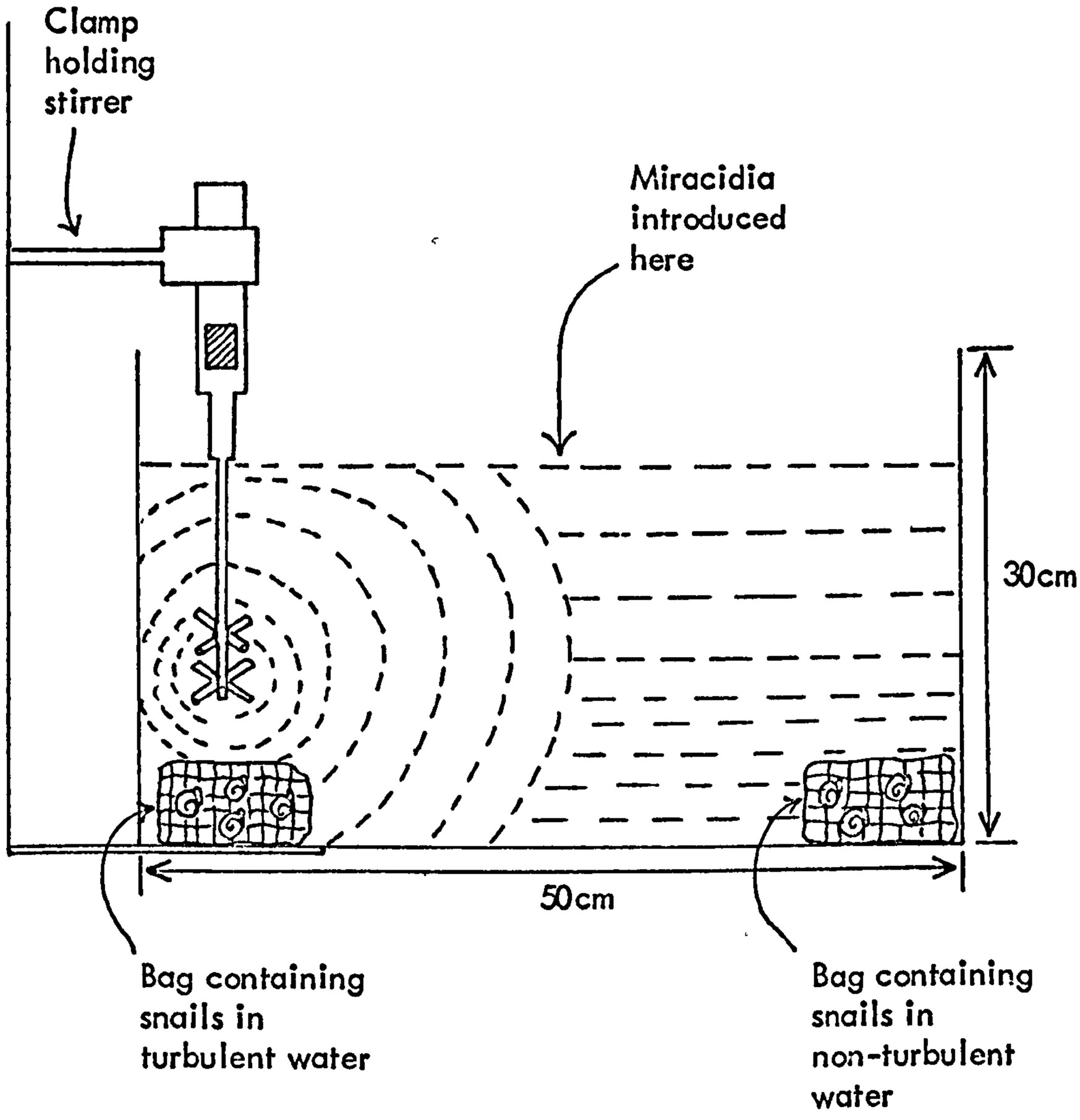


Fig. 15. Apparatus used for determining the influence of turbulence on the infective capacity of S. mansoni miracidia.

TABLE 34

Infections in B. pfeifferi by S. mansoni miracidia in a tank with turbulence and no-turbulence at opposite ends

Exp. No.	Turbulence	No Turbulence	Temperature
1	0/9	10/10	25.0
2	2/10*	9/10	25.0
3	0/10	10/10	27.0
Total Infections	2/29	29/30	
% Infection rate	6.9	96.7	

* Stirrer stopped for about 15 mins after the first hour

of "turbulence" and "no-turbulence" ($P<0.0001$) on the scanning capacity of S. mansoni miracidia.

Conclusions

While very high infection rates occurred at flow rates of 4-7 cm/sec and 12-15 cm/sec in both B. pfeifferi and B.(P.) globosus no infections occurred at the flow rates of 20-25 cm/sec and above in either snail. There was no significant difference between infection rates produced in the two snail species at 12-15 cm/sec ($P>0.20$). However at the flow rate of 12-15 cm/sec the presence of the screens may have reduced the effective flow in the troughs and therefore allowed snail-miracidia contact. At the flow rate of 20-25 cm/sec and above the presence of screens did not increase the chances of snail-miracidia interaction. Other workers have obtained results which agree quite closely with those in the present study. Shiff (1968) obtained a low infection rate at the flow rate of 7.0 cm/sec but no infections at 15 cm/sec. Webbe and James (1969, personal communication) obtained reasonably high infections in B.(P.) globosus at the flow rate of 10 cm/sec and Upatham (1971-72, personal communication) obtained infections in B. glabrata at the flow rate of 10 cm/sec but none at 12.5 cm/sec. It appears that at the flow rates beyond 12-15 cm/sec infections do not occur because miracidia are being swept along too fast for snail-miracidia interaction to be possible.

The rate of flow of water is however a very important factor in increasing the scanning capacity of schistosome miracidia, whereas the penetration and infection of the snail takes place in appreciably slower flowing water than the rates measured. The high infection rates obtained by Webbe (1966) were probably as described, due to the stable conditions in the snail cages which provided submerged sites favouring snail-miracidia interaction.

The turbulence produced in the present experiment swept the miracidia from this zone and thus physically prevented contact between miracidia and snails. Shiff (1968) however found that eddy flow and turbulence created favourable conditions for infections among B.(P.) globosus. Turbulence may therefore enhance infection by distributing miracidia in the environment but the level above which it becomes inimical to snail-miracidia interaction is not known.

IX. ULTRA-VIOLET RADIATION

Introduction

Sunlight is the natural source of ultra-violet radiation, together with other electromagnetic wave radiations. The relative position of ultra-violet radiation in the sun's spectrum is between the visible light and X-rays. Table 35 shows some of these radiations and their wavelengths (compiled from Giese, 1960, and Dobson, 1968).

TABLE 35

Radiation		Wave lengths in Å
X-rays		0.005 - 200
Ultra-violet	very short	150 - 2000
	short	2000 - 3000
	long	3000 - 3900
Visible light		3900 - 7000

Ultra-violet radiations of wavelengths shorter than about 3000 Å (especially wavelengths around 2500 Å) present in the sunlight are absorbed by ozone up in the earth's atmosphere before the radiations can reach the surface of the earth (Giese, 1960; Dobson, 1968). While the amount of ultra-violet radiation reaching the earth varies according to the amount of ozone and clouds up in the atmosphere (which may vary from day to day), the radiations in or near the visible part of the spectrum remains constant from day to day and from year to year (Dobson, 1968). Sunlight therefore contains ultra-violet radiations of long wavelengths from about 3000 Å to 3900 Å (the beginning of the visible range) and occasionally in clean clear atmosphere down to 2300 Å (Stiff, 1971). The most energetic and perhaps injurious wavelengths are the short ones which range between 2,000 and 3,000 Å, thus including the most energetic peak at 2537 Å. Ultra-violet radiation in the sunlight is, however, well known to have biological effects (Heather, 1955)

and indeed it has been observed that strong direct sunlight falling on the surface layers of lakes, streams and waterfalls produces varying degrees of disinfection by reducing bacterial populations (Stiff, 1971).

A common artificial source of ultra-violet radiation in recent times is the mercury vapour discharge lamp which emits monochromatic ultra-violet radiation predominantly (80-85%) at a wavelength of 2537 Å. There are other lamps which give ultra-violet radiations over much greater range of wavelengths including those between 2540 Å and 2970 Å (Heather, 1955; Stiff, 1971).

Many forms of microorganisms are vulnerable to ultra-violet radiation below about 3000 Å; bacteria, viruses and most moulds (Gates, 1929, 1930; Hollaender, 1955).

Exposure of Chlorophyceae, Daphnia and Euglena for a few minutes to ultra-violet radiation, mainly at a wavelength of 2540 Å, destroys them. Other organisms such as Coccomyxa which are more resistant are devitalized after much longer exposures (Heather, 1955). Paramecia irradiated for 55 minutes were killed, while sublethal dosages such as 33 minutes of irradiation depressed vital activities though normal locomotion and ability to divide were restored in two days (Aleksandrov,1948, quoted by Dogiel, 1965).

Several workers have investigated the effect of ultra-violet radiation on the eggs of helminths (Hollaender, Jones and Jacobs, 1940; Jones, Jacobs and Hollaender, 1940; Wolf, 1932; Spindler, 1940; and others) and found that eggs of all stages were quickly destroyed and that sublethal dosages retarded the rate of hatching. Hatched larvae of irradiated eggs were always less active than controls. The effect of ultra-violet radiations on helminth larvae has also been studied. Stevens (1942) irradiated Trichinella spiralis larvae and found that the reduction in recoverable adult worms in the intestine and of encysted larvae in the muscle was commensurate with the length of exposure. Keeling (1960) found that irradiation of the infective larvae of Nippostongylus muris for 16 seconds completely inhibited development to the adult stage. Two authors have

studied the effect of ultra-violet radiation on S. mansoni cercariae. Krakower (1940) observed that 20 minutes of irradiation permanently injured cercariae of S. mansoni and that a similar effect was produced by strong sunlight at 34°C for half an hour. Exposure to ultra-violet light for 45 minutes killed all cercariae outright, while exposure to strong sunlight for one hour also killed all cercariae. Standen and Fuller (1959), using an ultra-violet lamp emitting over 80% of its radiation at 2537 Å, found that cercariae were killed within 4-10 minutes from a distance of 1-2 cm. They found that cercariae irradiated with sublethal dosages penetrated the skin of mice but their development was inhibited to varying extents. Complete inhibition of development occurred when cercariae were irradiated for 10 seconds at 2.5 cm from source.

There has been no previous investigation into the effect of ultra-violet radiation on the survival and infectivity of schistosome miracidia. The behaviour of irradiated miracidia was therefore observed and their capacity to infect susceptible snails tested.

Experiments and Results

a. The effect of solar ultra-violet radiation on the survival and degree of activity of S. haematobium miracidia

Active S. haematobium miracidia were pipetted in batches of 4-8 into selected cavities of haemagglutination plates and about 1 ml of distilled water was added to each cavity. Two plates were prepared and placed side by side in a white enamel tray (40 x 30 x 5 cm). One plate was left exposed to the sun while the other was covered by supporting a smoked glass plate on rubber bungs placed at the corners of the plate. The space between the glass plate and the haemagglutination plate allowed free passage of air over the cavities. Three cavities, separate from those containing miracidia, were filled with distilled water and were periodically checked for changes in temperature. At hourly intervals the miracidia were

examined under the dissecting microscope for survival and degree of activity. As much as possible the experiments were carried out on bright, warm, sunny days when the skies were clear and the sun shone consistently. Exposures commenced from about 13.00-14.00 hours to about 17.00 hours. As the experiments depended on the condition of the weather, only a few were done.

Tables 36 and 37 show the survival and degree of activity respectively of S. haematobium miracidia covered and exposed to the ultra-violet radiation of the sun.

Over 90% of both covered and exposed miracidia survived the first hour, but in the second hour only 39.4% of the exposed ones survived while as many as 90% of the covered miracidia were alive. By the third hour only 8.4% of the exposed and 58.6% of the covered miracidia were alive. All miracidia in the exposed plate were dead by the 4th hour. While the decline in the survival of miracidia in the covered plate was gradual, that of the exposed miracidia was sharp and terminated in the death of all miracidia within 4 hours (Fig. 16).

The degree of activity of miracidia covered and exposed to solar ultra-violet radiation is shown in Fig. 16.

All the surviving miracidia in the covered plate were 'active' during the first hour; in the second hour over two-thirds of them were 'active', while even in the third hour about a quarter of them were 'active'. Some of the miracidia in the covered plate were 'slow' in the second hour, and in the third hour miracidia of all the three categories of activity ('active', 'slow' and 'lethargic') were present. In the first hour only about a quarter of the surviving miracidia in the exposed plate were 'active', over twice as many were 'slow' and the remainder were 'lethargic'. There was a sharp decline in degree of activity in the second and third hours of exposure to the sun. Of the 39.4% surviving miracidia in the second hour about a third (14.3%) were 'slow' and the remaining two-thirds (25%) were 'lethargic'. In the third hour the few remaining miracidia were 'slow' and 'lethargic' in about equal numbers.

TABLE 36

The survival of S. haematobium miracidia in solar ultra-violet radiation

Experiment No.	Number of miracidia alive after exposure to solar ultra-violet radiation: Age of miracidia in hours 0.5	1.0	2.0	3.0	4.0	Temperature °C
COVERED						
1	50	46	36	18	6	24
2	61	61	61	54	41	25
3	47	47	44	29	12	23
4	45	45	44	18	10	22.5
Proportion alive	203/203	199/203	185/203	119/203	69/203	
% alive	100	98.0	90.1	58.6	33.9	
EXPOSED						
1	56	50	23	0	0	24
2	43	46	36	17	0	26
3	42	33	5	0	0	23
4	57	56	16	0	0	22.5
Proportion alive	203/203	185/203	80/203	17/203	0/203	
% alive	100	91.1	39.4	8.4	0.0	

TABLE 37

The degree of activity of S. haematobium miracidia in solar ultra-violet radiation

Degree of activity	Age of miracidia in hours 0.5	1.0	2.0	3.0	4.0
COVERED					
% in					
+++ ('Active')	100	98.0	67.5	13.8	-
++ ('Slow')	0	0	23.6	21.2	26.6
+ ('Lethargic')	0	0	0	22.1	7.3
Total % alive	100	98.0	90.0	56.1	33.9
EXPOSED					
% in					
+++ ('Active')	100	24.6	0	0	0
++ ('Slow')	0	52.7	14.3	3.9	0
+ ('Lethargic')	0	13.8	25.0	4.4	0
Total % alive	100	91.1	39.4	8.4	0.0

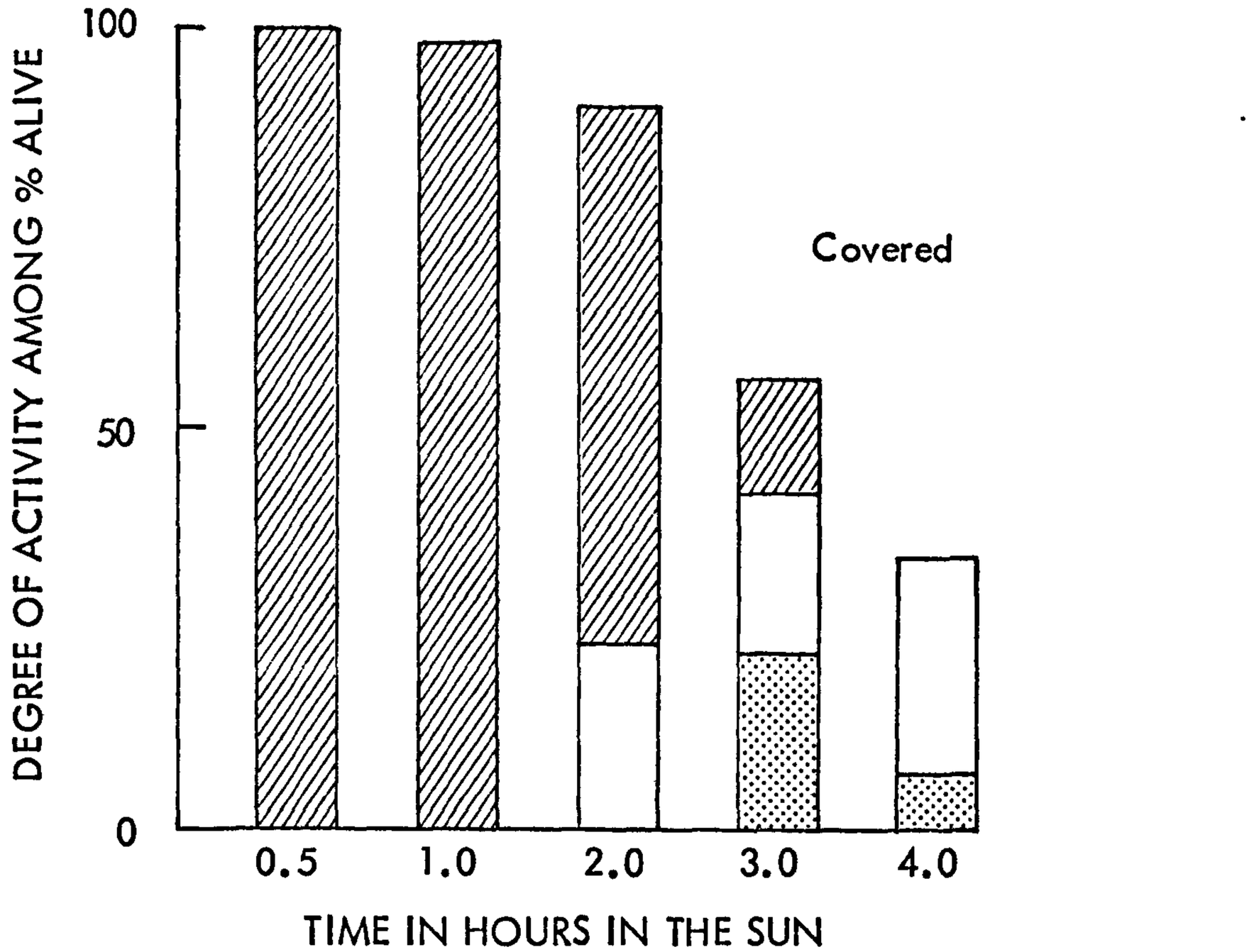


Fig. 16. The survival and degree of activity of *S. haematobium* miracidia in solar ultra-violet radiation.

b. <u>The effect of artificial ultra-violet radiation on the survival and rate of movement of S. mansoni and S. haematobium miracidia</u>

Apparatus used to produce ultra-violet radiation:

The ultra-violet radiation was produced by a "medium pressure" mercury discharge lamp, Hanovia Model 4, which emits numerous bands of germicidal value with useful peaks at 2540 Å, 2800 Å and 3130 Å. A full intensity of output was reached in 5-3 minutes (Stiff, 1971, personal communication). The lamp shown in Fig. 17 was placed on a platform to keep it at a constant distance of 25 cm from the laboratory bench. The miracidia were always placed in a petri dish at this distance from the bulb. A stop watch was used to time irradiations and non-flam actinotherapy goggles were used to shield the eyes from the radiation.

Active miracidia (50 or 60) were pipetted into a 5 cm petri dish containing 10 ml of dechlorinated water. The lamp was switched on for about 10 minutes to obtain maximum output of irradiation. The petri dish was then placed directly below the bulb. At the end of the period of irradiation the petri dish was removed. The miracidia were immediately examined under the dissecting microscope for degrees of activity and movement of miracidia was timed over the fixed distances of 0.5 cm and 1.0 cm in order to determine their rates of movement (cf. Materials and Methods). Miracidia were irradiated for different periods and records of the survival and rates of movement were compiled. Table 38 shows the rate of movement of <u>S. mansoni</u> miracidia irradiated over different periods of time and Fig. 10 shows the percentage reduction in the rate of movement calculated from the combined rates of 4 replicates.

In preliminary tests it was found that the effects of ultra-violet radiation on <u>S. mansoni</u> and <u>S. haematobium</u> miracidia were almost identical and so <u>S. mansoni</u> miracidia were frequently used in this study.

Irradiation for 15 and 25 seconds did not seem to have any observable effect on the rate of movement of the miracidia which were as active as

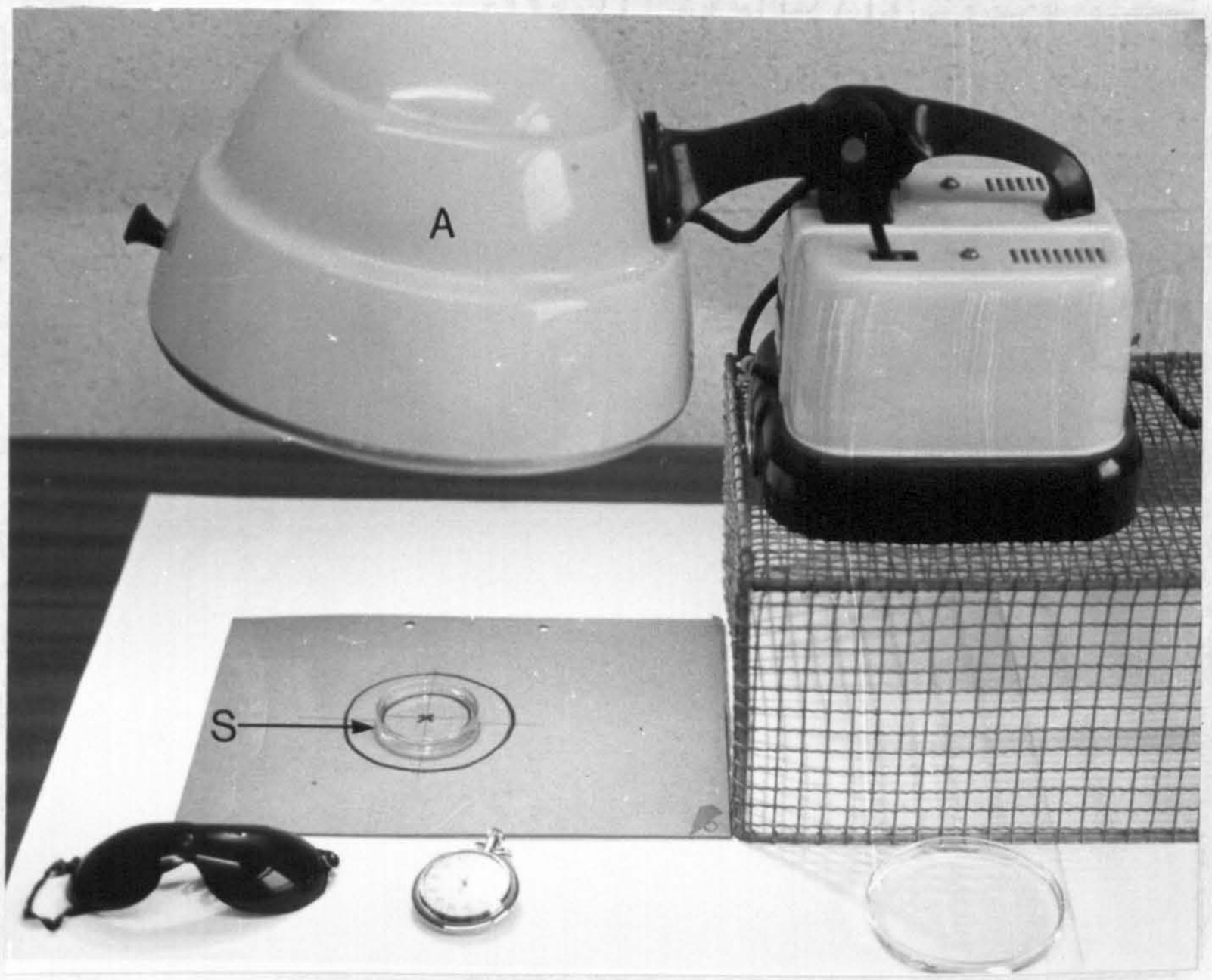


Fig. 17. Showing A. Hanovia medium pressure lamp model 4.
S. Petri dish in position.

TABLE 38

The rate of movement of S. mansoni miracidia after irradiation over different periods of time

Exposure time secs.	Mean rate of movement mm/sec ± S.D. (no. of records)					
	1	2	3	4	Combined rate	% Reduction in mean rate
0 Control	1.88±0.10 (8)	2.30±0.30 (28)	2.23±0.27 (13)	2.21±0.27 (15)	2.21±0.28 (64)	0
15	-	1.47±0.25 (25)	-	1.80±0.26 (15)	1.59±0.30 (40)	28.1
25	-	-	-	1.50±0.26 (9)	1.50±0.26 (9)	32.1
30	0.84±0.20 (5)	0.97±0.19 (18)	0.93±0.21 (8)	1.35±0.30 (30)	1.14±0.33 (61)	48.4
35	0.69±0.10 (5)			1.20±0.22 (13)	1.06±0.30 (18)	52.0
40	0.62±0.21 (8)	0.75±0.14 (25)	0.82±0.20 (6)	0.92±0.21 (16)	0.79±0.20 (55)	64.3
45	0.45±0.11 (7)	0.49±0.11 (15)	0.69±0.08 (7)	-	0.53±0.14 (29)	76.0
50	0.53±0.25 (3)	0.35±0.17 (14)	0.43±0.10 (6)	0.67±0.14 (12)	0.50±0.21 (40)	77.4
55	0.24±0.12 (4)	0.33±0.16 (14)	0.33±0.05 (5)	-	0.32±0.14 (22)	85.5
60	-	-	-	0.33-0.08 (7)	0.33-0.08 (7)	85.1

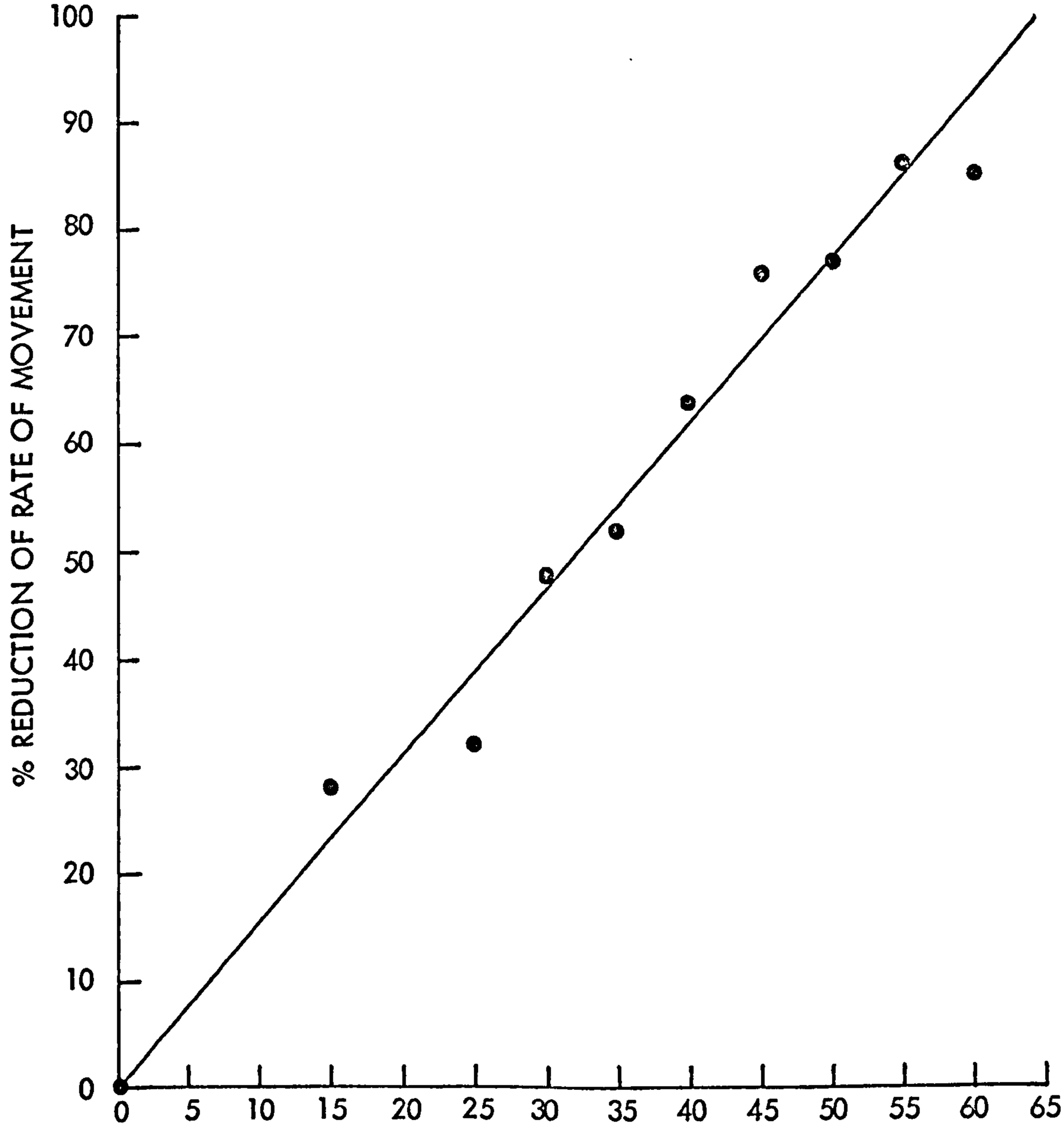


Fig. 18. Percentage reduction of rate of movement of S. mansoni miracidia exposed to sublethal dosages of artificial ultra-violet radiation.

non-irradiated controls. Irradiation for 30 seconds, however, resulted in a change from 'active' miracidia to 'slow' ones and with 40 seconds of irradiation there was a well marked change in the rate of movement, many of the miracidia being 'slow' and some 'lethargic'. Irradiation for 45 seconds and more rendered most miracidia 'lethargic' and some almost motionless. Irradiation for 60, 75 and 90 seconds showed progressively increased lethargy and at 105 seconds most miracidia were motionless and presumably dead, and at this point they assumed a characteristic tadpole shape.

It was observed that generally reduction of the rate of movement was accompanied by an apparent elongation of the body and that longer exposures resulted in the assumption of a characteristic tadpole shape. Fig. 19 shows the changes in size and shape resulting from irradiation for 1½ minutes and 5 minutes and immersion in 0.4% Nembutal (veterinary anaesthetic) for 10 and 30 minutes. Measurement of a large number of these specimens showed a highly significant elongation of the irradiated specimens ($P < 0.0001$). (Forty specimens irradiated for 1½ minutes had mean length and standard deviation 224.7μ ± 11.3μ in the range 255μ - 204μ. Sixty-two specimens placed in 0.4% Nembutal for 10 minutes had mean length and standard deviation 160.0μ ± 13.5μ in the range 184μ - 137μ. Degree of Freedom = 100, t-value = 25.7).

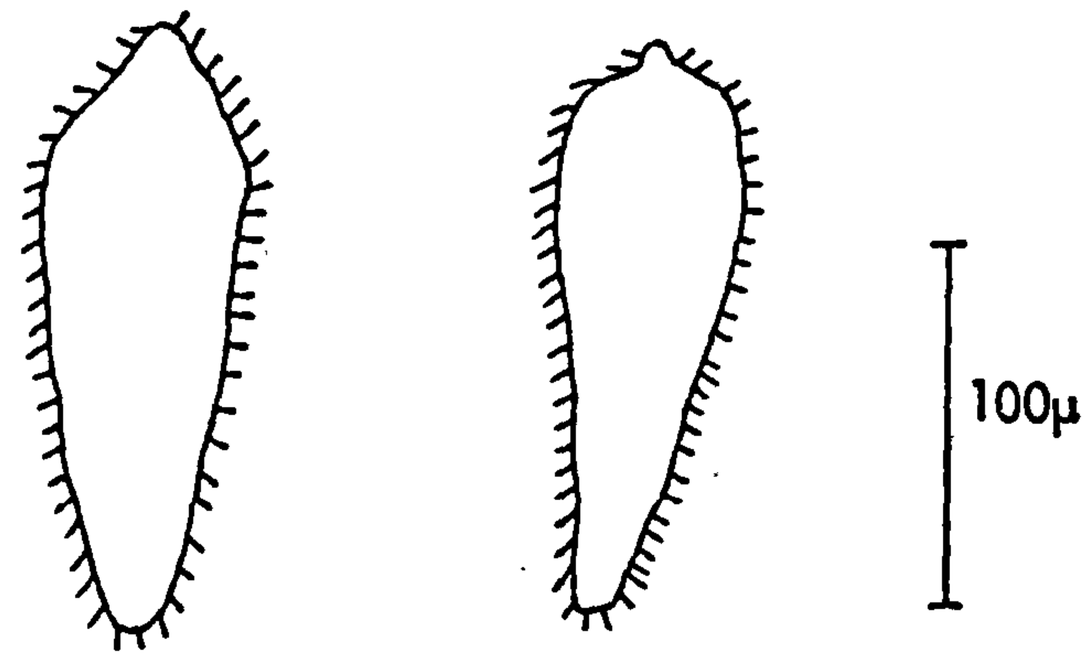
c. <u>The effect of sublethal dosages of ultra-violet radiation on the infective capacity of S. mansoni and S. haematobium miracidia</u>

This experiment was carried out in two series. The first series involved irradiating <u>S. mansoni</u> miracidia for periods of 15-40 seconds and exposing <u>B. pfeifferi</u> to them; the second series involved irradiating both <u>S. mansoni</u> and <u>S. haematobium</u> miracidia for longer periods of 60-105 seconds and exposing <u>B. pfeifferi</u> and <u>B. (P.) globosus</u> respectively to the irradiated miracidia.

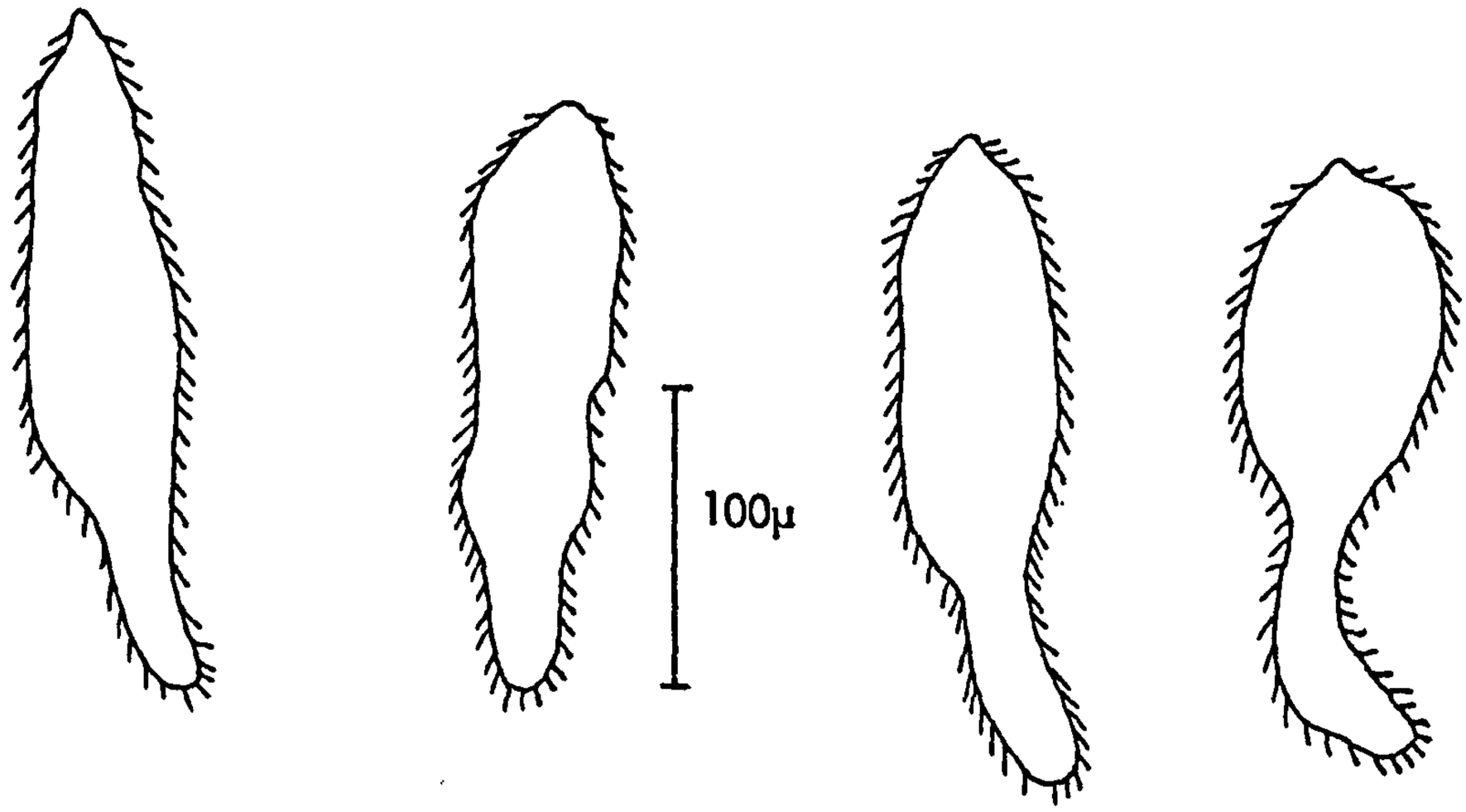
Active miracidia pipetted in the ratio of 10/snail (usually 60 miracidia)

Fig. 19. Changes in size and shape of *S. mansoni* miracidia under different conditions.

Magnification x 200

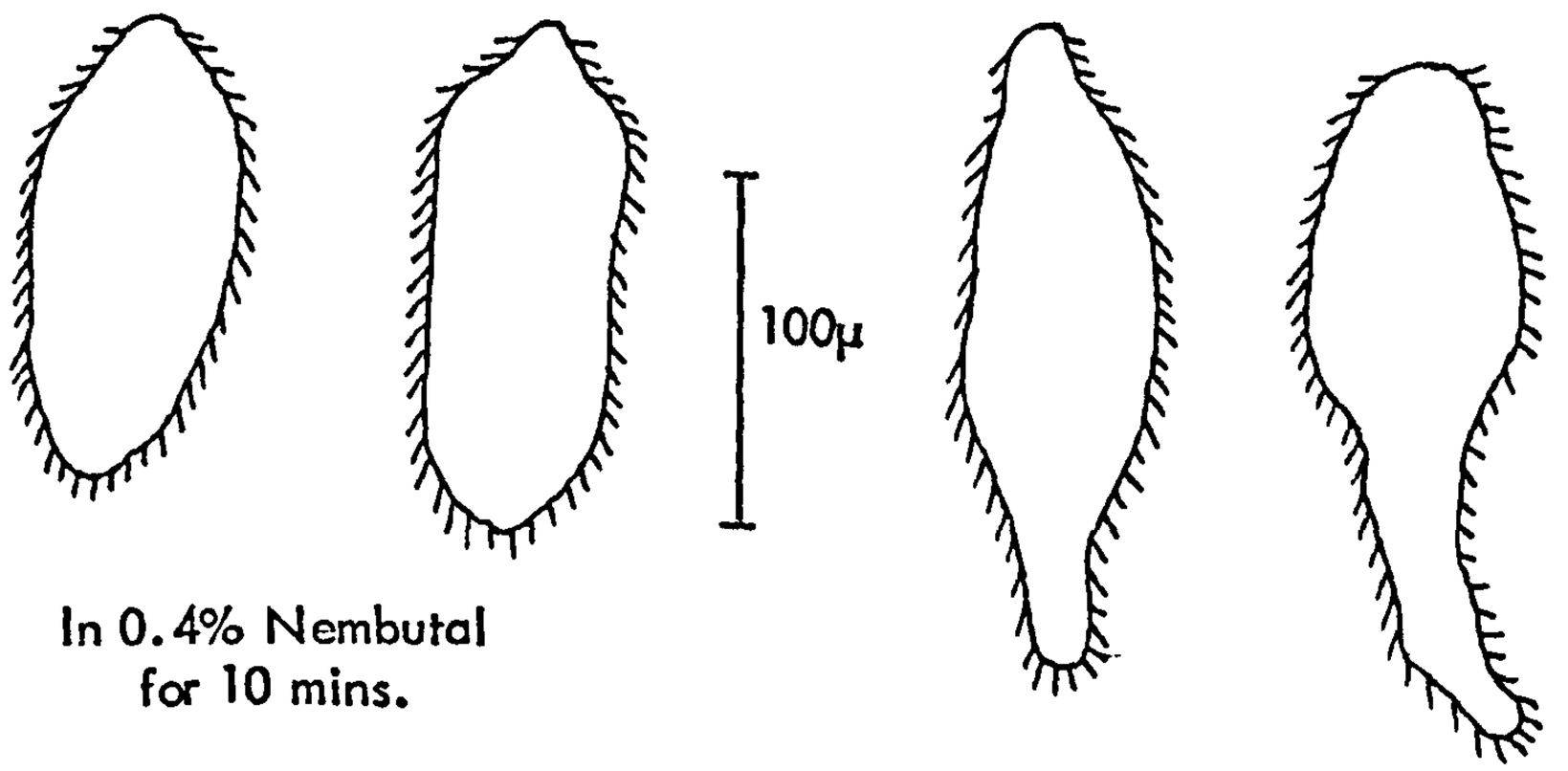


Normal miracidia (constructed)



Irradiated for 1½ mins.

Irradiated for 5 mins.



In 0.4% Nembutal for 10 mins.

In 0.4% Nembutal for 30 mins.

were put into 9 cm petri dishes containing 40 ml of dechlorinated water. Control dishes of miracidia were kept for comparison. The miracidia were inspected for degree of activity under these conditions and the required number of snails were put into the dishes. After 3-5 hours exposure the snails were removed and maintained in aquarium tanks as described under Materials and Methods. In the second series S. mansoni and S. haematobium miracidia were irradiated for longer periods ranging from 60 to 105 seconds and after the rate of movement of the miracidia had been calculated the appropriate number of snails were put into the dishes. Exposure was for 3-5 hours, after which snails were maintained in the usual manner.

Table 39 shows the infections that occurred among B. pfeifferi when exposed to S. mansoni miracidia irradiated for 15-40 seconds and the accompanying non-irradiated controls. Irradiation for 15-40 seconds did not seem to have any damaging effect on the infectivity of the miracidia, infection rates of 77.8 - 93.1% being of the same order. It was difficult to distinguish between the activity of miracidia irradiated for 15 to 30 seconds and the non-irradiated controls just by inspection; likewise, there was no significant difference between infections produced by non-irradiated controls and those irradiated for 30 seconds ($P > 0.46$). Although with 40 seconds irradiation a slight slowing down was observed, the miracidia were nevertheless quite active and an infection rate of 77.8% was obtained.

Irradiation over longer periods of time, 60-105 seconds, shown in Table 40, caused a highly significant damage to the miracidia resulting in no infections in all susceptible snails exposed. The controls produced high infection rates in both B. pfeifferi and B. (P.) globosus.

It is noteworthy that while miracidia that became 'slow' as a result of age at moderate temperatures were capable of infecting susceptible snails, those that became 'slow' as the result of ultra-violet irradiation were incapable of penetrating susceptible snails. Sixty seconds of irradiation rendered the miracidia sufficiently damaged to inhibit penetration. It

TABLE 39

The infections produced in B. pfeifferi by S. mansoni miracidia irradiated for varying periods of time (15 - 40 seconds)

Experiment No.	Control	Period of irradiation in seconds				
	0	15	20	25	30	40
1	23/24	22/24	-	-	19/20	-
2	17/17	14/16	-	-	-	-
3	8/11	-	13/18	11/13	-	-
4	-	-	13/17	14/18	-	-
5	6/6	-	11/12	11/12	9/12	7/9
Totals	54/58	36/40	37/47	36/43	28/32	7/9
% Infection rate	93.1	90.0	78.7	83.7	87.5	77.8

TABLE 40

Infections produced in B. pfeifferi and B. (P.) globosus by S. mansoni and S. haematobium miracidia respectively irradiated for 60 - 105 seconds and their mean rate of movement

B. pfeifferi/ S. mansoni		Period of irradiation in seconds				
	Mean Rate of movement ± S.D. (no. of trials)	0	60	75	90	105
		1.91±0.31 (28)	0.85±0.17 (16)	0.54±0.30 (15)	0.30±0.10 (10)	-
	1	12/12	0/12	0/10	0/9	0/10
	2	10/10	0/12	0/11	0/10	0/6
	3	12/12	0/7	0/4	0/4	-
	Totals	34/34	0/31	0/25	0/23	0/16
	% Infection rate	100	0.0	0.0	0.0	0.0
B.(P.) globosus/ S. haematobium						
	Mean Rate of movement ± S.D. (no. of trials)	1.66±0.20 (21)	0.67±0.20 (15)	0.43±0.13 (12)	0.26±0.05 (5)	
	1	8/8	0/9	0/4	0/6	
	2	8/9	0/6	0/4	0/4	
	Totals	16/17	0/15	0/8	0/10	
	% Infection rate	94.1	0.0	0.0	0.0	

was therefore not surprising that no infections occurred among snails exposed to miracidia irradiated for more than 60 seconds, some of which had become 'lethargic' and some even motionless. It was found that infection was interrupted at the penetration phase as many lethargic and dead miracidia were found in petri dishes together with their susceptible snails after the exposure period.

d. Penetration of ultra-violet radiation into clean clear still water

The depth to which ultra-violet radiation penetrates in water depends upon the degree of impurity or the amount of suspended matter in the water. This study was made in order to determine the depth to which ultra-violet radiation will penetrate in clean clear still water.

Advantage was taken of the positive geotaxis of S. haematobium miracidia which in a column of water tend to concentrate at the bottom, and the characteristic effect of irradiation on miracidia was used as an indicator of the depth to which the radiation penetrated. The columns were irradiated from the top and the degree of activity of the miracidia at the bottom of the columns was checked; if miracidia were still active after the irradiation it was assumed that the irradiation did not reach that depth.

Large specimen tubes of diameter 3.5 cm and varying heights (5.0 cm, 7.7 cm and 15.5 cm) and two graduated cylinders (100 ml and 250 ml) provided water columns of 2, 4, 7, 10, 15, 20 and 30 cm. By means of slabs of wood the distance between the ultra-violet lamp bulb (source) and the surface of the water column was kept at a constant distance of 25 cm. Active S. haematobium miracidia (60-80) were pipetted in minimum fluid into these columns. An interval of about 20 minutes was allowed for the miracidia to concentrate at the bottom. Each column was irradiated for 3-5 minutes, or more in some cases. The bottom of the column was especially searched for moving miracidia and the degree of activity in each case was carefully noted. The columns containing 2 cm and 4 cm of water were examined further under

TABLE 41

Depth in clean clear still water to which ultra-violet radiation penetrated using the effect of irradiation on S. haematobium miracidia concentrated at various depths as an indicator

Depth in cm	Period of Irradiation	Effect of irradiation on miracidia	Penetration +ve, -ve
2	3 mins.	All killed. Tadpole shape assumed.	+ve
4	3 "	" " " " "	+ve
7	3 "	Very lethargic.	
	plus 2 "	All killed. Tadpole shape assumed.	+ve
10	5 "	Some killed, others very lethargic.	+ve
15	5 "	Few active miracidia moving at bottom. Bottom sample had both slow and lethargic individuals.	+ve/-ve
20	5 "	Miracidia seen moving in bottom 2 cm.	-ve
	plus 3 "	Miracidia unaffected, situation unchanged, no slow or lethargic miracidia in bottom sample.	
30	5 "	No observable effect, no change in degree of activity.	-ve
	plus 5 "	Still no change.	

the dissecting microscope and a 1ø ml sample of water was taken from the bottom 1 cm of each of the other columns and examined in 5 cm petri dishes under the dissecting microscope for the degree of activity of the miracidia. The results of two sets of experiments have been combined to form Table 41.

The experiments indicate that in clean clear still water ultra-violet radiation penetrates as far as 15 cm. Under natural circumstances, however, suspended organic and inorganic matter will further reduce the depth to which the radiation penetrates.

c. <u>The absorption of ultra-violet radiation by turbid water using the effect of irradiation on the rate of movement of miracidia as indicator</u>

Preparation of water samples of different turbidities: A few pieces of ordinary garden soil (previously washed, sieved and evaporated to dryness) were ground up into a fine powder. A 1% soil suspension was prepared by putting 5 gms weight of the soil powder into 500 ml of distilled water and shaking the mixture thoroughly. It was allowed to stand in a 700 ml urine glass for 1 hour for all the heavy particles to settle leaving a "permanently" turbid supernatant fluid. From this (1%) stock "solution" a series of dilutions were prepared (0.5%, 0.1% and 0.05%). The amount of light transmitted by the water samples of different turbidities and distilled water was determined by means of a "Turbidity meter" (Fig. 20). This consists essentially of a rectangular glass box, C, into which the turbid water was poured and a light meter, L, placed just behind C to measure the amount of light transmitted through the turbid water. Both C and L are fitted into a black box B with a round opening A in front of it. In use, a 60 watt lamp was placed about 20 cm (8 inches) in front of the box and the amount of transmitted light was read from the light meter. The amount of light transmitted through distilled water and that for each sample of turbid water were recorded for comparison (Table 42).

Fifty active <u>S. mansoni</u> miracidia were pipetted into 10 ml of each sample

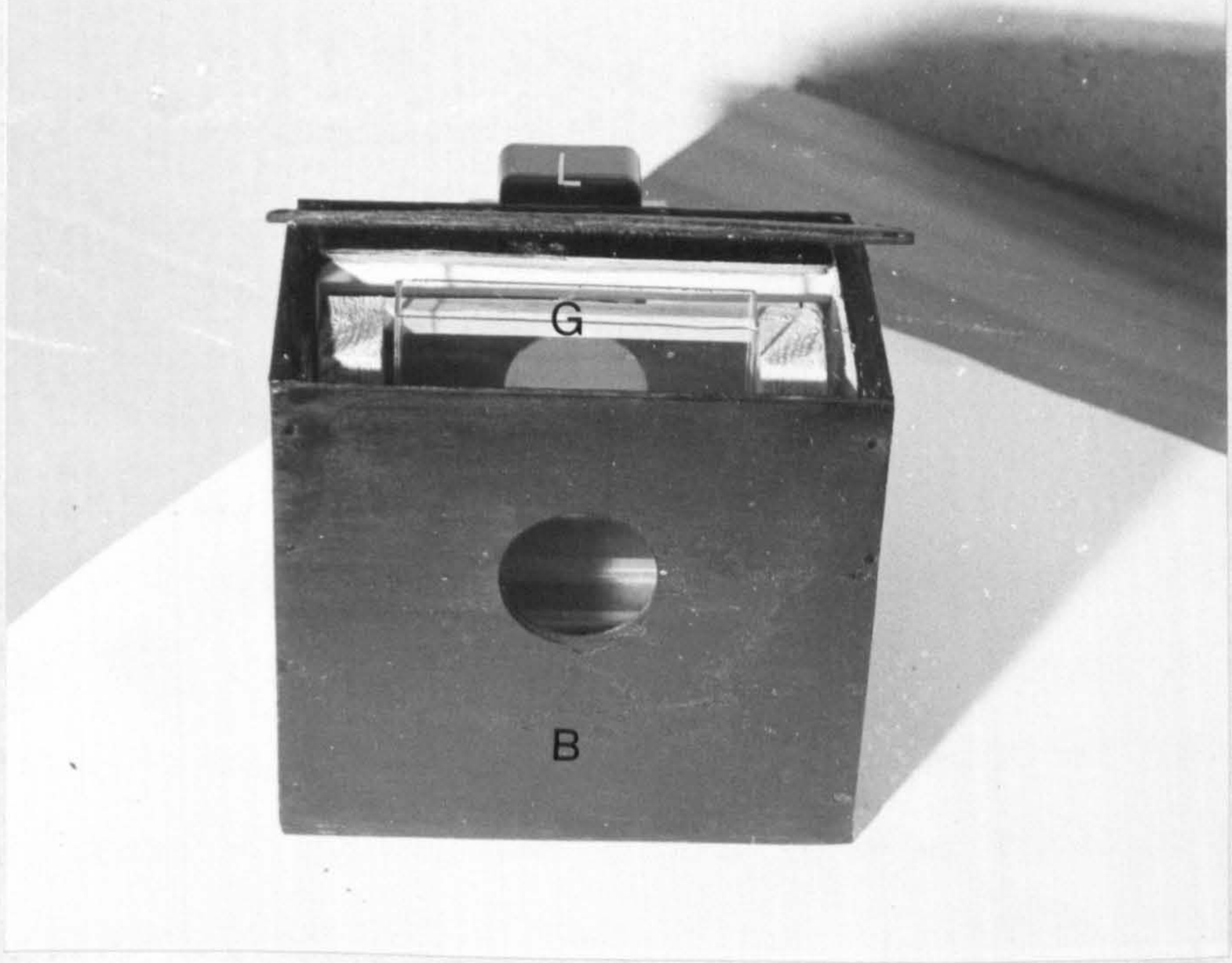


Fig. 20. "Turbidity meter".

B. - Black box.

G. - rectangular glass box containing sample of turbid water.

L. - light meter.

of turbid water in a 5 cm petri dish. The rate of movement of 5-10 miracidia was timed for each dish. All dishes were irradiated for 50 seconds and the rate of movement of the miracidia was timed again. The degree of activity of the miracidia was also inspected.

Table 42 shows the mean rate of movement of S. mansoni miracidia before and after irradiation and the corresponding turbidity of the water sample.

Before irradiation all the miracidia were moving at an average rate of 2.06 mm/sec but after irradiation considerable changes in the rate of movement became apparent. In distilled water and water sample of turbidity 0.05% the rate of movement fell from 2.06 mm/sec to 0.83 mm/sec and from 2.09 mm/sec to 0.86 mm/sec respectively, i.e. less than half the rate of movement before irradiation. There was no significant difference between the reduction in the rate of movement of miracidia in the distilled water and that in turbidity 0.05% ($t_{12} = 0.1169$, $P > 0.90$). But there was a highly significant difference in the reduction in the rate of movement of miracidia in distilled water and those in water samples of turbidity 0.1% ($t_{11} = 3.672$, $P < 0.005$) and 0.5% ($t_{15} = 4.140$, $P < 0.001$). At turbidity 1.0% there was only a slight reduction in the rate of movement which did not appreciably affect the degree of activity; the miracidia were as active as they were before irradiation ($t_{14} = 0.2848$, $P > 0.70$).

Irradiation for 50 seconds rendered miracidia in distilled water 'slow' and 'lethargic', while those in the most turbid water sample (1.0%) remained 'active' and moved at a rate twice as fast as those in the distilled water (1.77 mm/sec and 0.83 mm/sec respectively).

Fig. 21 shows the % reduction of mean rate of movement in the different samples of turbid water after the irradiation. It is clear that greater turbidity afforded more protection to the miracidia and consequently less reduction in the rate of movement.

TABLE 42

The effect of ultra-violet radiation on the rate of movement of S. mansoni miracidia in water samples of different turbidities

Light intensity using "Turbidity meter"	% Turbidity of water samples	Mean rate of movement of miracidia ± S.D. (no. of readings)		% Reduction of rate of movement
		Before irradiation	After irradiation	
60 lumens/sq.ft.	0.00 (Distilled water)	2.06 ± 0.34 (8)	0.83 ± 0.16 (8)	60.3
58 "	0.05	2.09 ± 0.33 (6)	0.86 ± 0.13 (6)	58.7
52 "	0.1	2.02 ± 0.17 (5)	1.01 ± 0.15 (5)	50.0
32 "	0.5	2.06 ± 0.25 (9)	1.19 ± 0.23 (9)	40.8
20 "	1.0	2.09 ± 0.15 (6)	1.77 ± 0.25 (10)	15.3

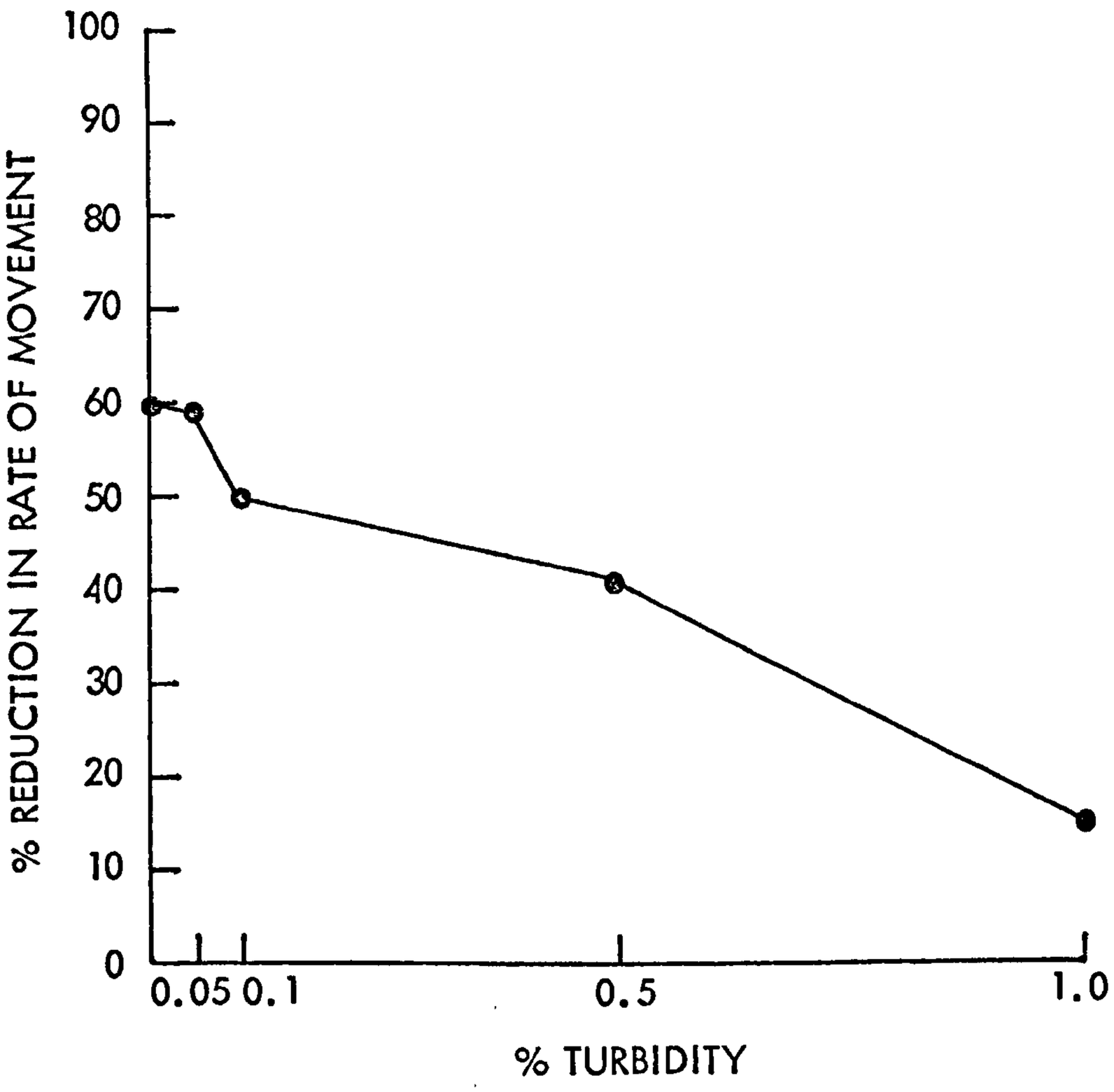


Fig. 21. Percentage reduction of rate of movement of *S. mansoni* miracidia in water samples of increasing turbidity exposed to 50 seconds of ultra-violet radiation.

Conclusions

While there was no significant difference between survival of miracidia in the covered and exposed plates during the first hour in the sun ($P > 0.32$), there was a highly significant difference between them in the second ($P < 0.0001$) and the third ($P < 0.001$) hours. Solar ultra-violet radiation affected the degree of activity, rendering some of the miracidia in the exposed plate "lethargic" even in the first hour, and by the second hour there were no "active" miracidia in the exposed plate while almost all the miracidia in the covered plate remained active.

The effects of artificial ultra-violet radiation on the miracidia were determined by the reduction of the mean rate of movement and changes in size (length) and shape. Sublethal dosages of irradiation caused a clear reduction of the mean rate of movement which followed a linear trend. Increased time of exposure resulted in increased reduction of the rate of movement. A significant elongation of the body accompanied irradiation and a characteristic tadpole shape was assumed on prolonged irradiation.

The infectivity of irradiated miracidia was quite unlike that of irradiated cercariae, where penetration of the vertebrate host occurred but development of cercariae into adult worms was inhibited (Standen and Fuller, 1959). In the case of miracidia the effect was at the penetration phase but once the snail was penetrated development proceeded unimpeded.

Ultra-violet radiation penetrated to a maximum depth of 15 cm in clean, clear water. Miracidia were, however, protected to different extents by water samples of different turbidity; the more turbid the water, the less the effect of ultra-violet radiation. In nature, therefore, the effect of solar ultra-violet radiation upon miracidia in turbid waters is likely to be negligible.

X. LENGTH OF CONTACT TIME

Introduction

The length of time miracidia spend in the immediate vicinity of susceptible snails depends upon their degree of activity and selective capacity. Schistosome miracidia have been observed to complete the process of penetration in periods varying from 10-45 minutes (Gordon et al, 1934; Maldonado, 1957; Lengy, 1962; Smyth, 1966), the most favoured sites of penetration being the head, foot and base of the tentacles. However, other soft parts of the body are penetrated (Adams, 1934; Gordon et al, 1934; Maldonado, 1957; Lengy, 1962). Penetration by miracidia is not limited to susceptible snail hosts alone; S. mansoni miracidia have been shown to penetrate a wide variety of insusceptible snails (Barbosa, 1960; Sudds, 1960; Richards, 1963) and even non-molluscan organisms such as tadpoles (Barbosa and Carneiro, 1965). However, miracidia have also been observed to remain in close proximity to susceptible snails for a considerable time without making any attempt to penetrate them.

In a natural environment miracidia have to select their susceptible snail hosts from among a large number of other organisms and penetrate them in order to establish infections and maintain transmission. Penetration of insusceptible snails is a waste of miracidia since further development in such snails does not occur and the miracidia are destroyed by phagocytic cells. Chernin (1968) studied the scanning capacity of S. mansoni miracidia by interfering with their host finding process by interposing insusceptible snails as decoys between the point of miracidial release and susceptible target snails. Recently Upatham (1972) studied the interference with infectivity of S. mansoni miracidia under field-simulated conditions in St. Lucia, West Indies.

The present study was designed to compare the penetration efficiency and the selective capacity of S. mansoni and S. haematobium miracidia based

on the infection rates produced in B. pfeifferi and B. (P.) globosus respectively.

Experiments and Results

a. Penetration of B. pfeifferi and B. (P.) globosus by S. mansoni and S. haematobium miracidia respectively during exposure periods of 15, 30 and 60 minutes

Young miracidia were pipetted in the ratio of 10/snail into 9 cm petri dishes containing 40 ml of dechlorinated water at 25-28°C. The appropriate number of host snails were placed into the dishes and were exposed for 15, 30 or 60 minutes. After the exposure period the snails were removed, carefully blotted on tissue paper to remove excess water and free miracidia, and then maintained in aquarium tanks as described under "Materials and Methods". A fresh batch of snails was dropped into the petri dishes and allowed to remain there for about 2 hours, after which they were also removed and maintained.

The results of infections produced are shown in Table 43.

B. pfeifferi/S. mansoni: Almost all surviving snails were infected; high infection rates of 91.2-100% occurred at the three exposure periods of 15, 30 and 60 minutes. This indicates that penetration successfully took place even at the shortest exposure period of 15 minutes. The infections which occurred among snails placed in the petri dishes after the exposure periods of 15 and 30 minutes showed the presence of miracidia which had retained their infectivity beyond the test period. After 60 minutes contact with susceptible snails, however, no S. mansoni miracidia remained that were capable of successfully penetrating snails, and consequently no infections occurred. There was no significant difference between infection rates at 15 and 30 minute exposure periods ($P > 0.40$), neither was there any significant difference between the infection rates at 15 and 60 minute exposure periods ($P > 0.50$).

B. (P.) globosus/S. haematobium: High infection rates ranging from

TABLE 43

Infections produced in B. pfeifferi and B. (P.) globosus by S. mansoni and S. haematobium miracidia respectively at different exposure periods

	Exposure periods in Minutes						Temperature
	15	Post 15	30	Post 30	60	Post 60	
B. pfeifferi/ S. mansoni	17/17	2/5	12/12	2/9	16/17	0/6	25.5
	12/12	1/8	-	-	18/18	0/6	27.0
	16/16	2/12	13/13	1/9	12/12	0/4	27.0
	7/12	0/6	10/10	0/6	-	-	26.5
Total infections	52/57	5/31	35/35	3/24	46/47	0/16	
% total infection rate	91.2	16.1	100.0	12.5	97.9	0.0	
B.(P.) globosus/ S. haematobium	4/7	0/3	8/8	1/4	3/3	0/3	25.5
	3/3	0/4	5/6	1/1	7/9	0/5	26.5
	3/3	1/3	9/11	0/2	5/5	0/2	27.0
	0/9	0/2	-	-	7/7	0/3	27.0
	10/11	1/4	12/13	0/3	10/10	0/4	27.0
Total infections	28/33	2/16	34/33	2/10	32/34	0/17	
% total infection rate	84.8	12.5	89.4	20.0	94.1	0.0	

84.8-94.1% occurred when S. haematobium miracidia were in contact with B. (P.) globosus for 15, 30 and 60 minutes. There was no significant difference between infection rates at the different exposure periods; ($P > 0.80$) and ($P > 0.30$) for 15 and 30 minutes and 15 and 60 minutes respectively. However, not all the miracidia had penetrated in the exposure periods of 15 and 30 minutes: those that remained produced low infection rates in the second batch of snails provided. There were no infections among snails placed in petri dishes after the 60 minute exposure period.

b. The selective capacity of S. mansoni and S. haematobium miracidia in a test system

In this test system B. (P.) globosus was used as the insusceptible snail for S. mansoni, and B. pfeifferi was used as the insusceptible snail for S. haematobium. The experiment, divided into three groups, was carried out in petri dishes as described in a. above.

In group 1 the miracidia of each species spent the first hour with its own snails and after this period the susceptible snails of the other species of miracidia were supplied. In group 2 the miracidia of each species spent the first hour with the insusceptible snail and after this period their susceptible snails were provided. In effect group 1 of S. mansoni is also group 2 of S. haematobium and vice versa. In group 3 miracidia of both species were together with both B. pfeifferi and B. (P.) globosus. All exposures were allowed to continue for a further period of 2-3 hours, when snails were removed and maintained as already described.

Results of the infections produced in B. pfeifferi and B. (P.) globosus in the three test groups are shown in Table 44.

B. pfeifferi/S. mansoni: Infection rates in the three test groups were high and ranged between 88 and 91%. There was no significant difference between infection rates in groups 1 and 2 ($t_6 = 0.5046$, $P > 0.60$) and groups 1 and 3 ($t_5 = 0.1410$, $P > 0.00$). This result shows that S. mansoni miracidia

TABLE 44

Infections produced in B. pfeifferi and B. (P.) globosus by S. mansoni and S. haematobium miracidia respectively in a system to test their selective capacity

B. pfeifferi/ S. mansoni	Group 1 1st hour B.pfeifferi with S. mansoni and S.haematobium	Group 2 1st hour B.(P.)globosus with S. mansoni and S.haematobium	Group 3 Mixed group B.pfeifferi & S.mansoni B.(P.) globosus and S. haematobium
	0/9 (10)	7/9 (10)	-
	-	13/15 (16)	12/16 (16)
		13/13 (14)	15/16 (16)
	11/11 (12)	9/11 (12)	9/10 (12)
	10/12 (12)	11/12 (12)	12/12 (12)
Total infections	29/32	53/60	43/54
% total infection rate	90.6	88.3	80.9

B.(P.)globosus/ S. haematobium	Group 1 1st hour B.(P.)globosus with S.haematobium and S.mansoni	Group 2 1st hour B.pfeifferi with S.haematobium and S.mansoni	Group 3 Mixed group B.(P.)globosus and S.haematobium B.pfeifferi & S.mansoni
	3/3 (0)	0/6 (0)	-
	-	4/7 (12)	10/11 (12)
	-	5/10 (16)	8/13 (16)
	4/5 (0)	6/10 (12)	8/11 (12)
	7/8 (12)	1/5 (0)	8/10 (12)
Total infections	14/16	15/30	34/45
% total infection rate	85.7	39.5	75.6

were unaffected by the presence of B. (P.) globosus for 1 hour in their second group and retained their infectivity until the susceptible snail hosts, B. pfeifferi, were supplied.

B. (P.) globosus/S. haematobium: The overall infection rates of 85.7% and 75.6% produced in groups 1 and 3 respectively were high and there was no significant difference between them (t_5 = 1.465, $P > 0.20$), but a low infection rate of 39.5% produced in group 2 where S. haematobium miracidia spent one hour with B. pfeifferi, an insusceptible snail host, was significantly different from group 1 (t_6 = 3.412, $P < 0.02$). S. haematobium miracidia were able to select their appropriate snail host from a mixture of susceptible and insusceptible snails producing high infection rates but were incapable of retaining their infectivity in the presence of insusceptible snails only.

Conclusions

There was no significant difference between the penetration efficiency of S. mansoni and S. haematobium miracidia even at the shortest exposure period of 15 minutes studied ($P > 0.80$). High infection rates of 85% and above occurred at all the three exposure periods, although some miracidia in infective condition remained after the 15 and 30 minute periods. After 60 minutes exposure any miracidia that remained did not produce infections in host snails and therefore one hour may be considered as a minimum adequate period for exposing snails to miracidia at such moderately high temperatures. S. mansoni miracidia have been known to complete the process of penetration in 10-15 minutes (Gordon et al, 1934) and in 2-10 minutes (Maldonado, 1957) but S. bovis miracidia did not penetrate in less than 30-45 minutes (Lonsy, 1962). The results obtained in the present studies agree with these casual observations.

In the selective capacity experiments both S. mansoni and S. haematobium miracidia produced high infection rates when they were either alone with their susceptible snails or together with the other insusceptible snail in a mixture,

indicating their ability to select their own snail host. But while S. mansoni miracidia were able to retain their infectivity in the presence of B. (P.) globosus, an insusceptible snail host, S. haematobium miracidia wasted their energy in the presence of B. pfeifferi. The mechanism controlling loss of infectivity is not properly understood. It has been suggested that this may be due to the miracidia exhausting themselves by repeated attempts to perforate the skin of the snail (Chernin, 1963; Kinoti, 1970; Wajdi, 1972) while those that actually penetrate are destroyed by phagocytic cells in the snail. Chernin (1968) and Upatham (1972) found that in a mixture of susceptible and insusceptible snails the scanning capacity of S. mansoni miracidia was interfered with, resulting in reduced infection rates in B. glabrata. In the present studies, however, high infection rates produced in the mixed group (group 3) were not significantly different between the two species ($t_6 = 1.578$, $P > 0.20$).

XI. GENERAL DISCUSSION

In the present study physical factors in the environment which are likely to influence the behaviour, survival and infectivity of the miracidia of S. mansoni and S. haematobium were examined. These have been divided into two main groups: those that directly affect the metabolism of the miracidia (temperature and ultra-violet radiation), and those that affect a "system" in the miracidia which then react in a particular manner (light, gravity, pressure, rate of flow of water, turbulence and dispersion). The influence of contact time and the physical presence of other organisms on the behaviour of miracidia have also been examined.

The behaviour of miracidia is mainly orientated towards the location of snail hosts in order to establish infections in them. It is therefore important for the survival of the parasite that this orientation should include reactions which guide them to areas where most of their snail hosts are found. Etges and Decker (1963) expressed the opinion that both light and gravity are far more powerful stimuli in determining the orientation of S. mansoni miracidia than any chemical factors produced by their molluscan hosts. The chemical aspects of the environment were not included in the present study. Although the ultimate behaviour of the miracidia may be the result of interaction of several physical, chemical and biological factors, in the present observations the influence of each physical factor has been studied in order to estimate its value in the dynamics of transmission.

The initial orientation of miracidia in an environment is most likely to be reaction to light and gravity - they either move towards the surface (in positive phototaxis and negative geotaxis) or towards the bottom (in positive geotaxis and negative phototaxis), or they may even distribute themselves randomly in the medium showing indifference to gravity and light.

A striking difference was found in the responses of S. mansoni and

S. haematobium miracidia to the intensity and direction of light and to gravity. S. mansoni miracidia were positively phototactic and were more sensitive to light, while S. haematobium miracidia were negatively phototactic and showed less sensitivity to low light intensities. In spite of their positive phototaxis S. mansoni miracidia effectively scanned both the illuminated and darkened halves of a vessel and infected B. pfeifferi located there. S. haematobium miracidia on the other hand preferentially scanned the shaded end of the vessel. When light and gravity acted together S. mansoni miracidia showed positive phototaxis but indifference to gravity, while S. haematobium miracidia showed negative phototaxis and a strong positive geotaxis.

The usual method of evaluating the efficacy of chemotherapy by hatching schistosome eggs from excreta and concentrating the miracidia in the illuminated side-arm of a darkened flask will therefore give quite misleading results in the case of a S. haematobium infection.

It is evident that the responses of miracidia to light and gravity guide them into the niches where most of their snail hosts are located. There is evidence to indicate that the behaviour of the miracidia is correlated with the ecology of their snail host (Porter, 1938; Wright, 1956; Chernin et al, 1962; Wajdi, 1972). Biomphalaria spp are commonly found on the surface of the water, on leaves, branches, etc., but some are found at the bottom and some species, such as B. choanomphala in Lake Victoria, are found at considerable depths. B. (P.) globosus have been observed to prefer sluggish streams with muddy bottom and shaded banks (Blacklock and Thompson, 1924; Gerber, 1952). Schistosome miracidia have no eyespots nor any morphologically recognizable photoreceptors that are known to respond to light so the whole of the body surface may be involved in this diffuse photosensitivity, generally known as dermal light sense (Steven , 1963). Wright, Lavigne and Ronald (1971) showed that Schistosomatium douthitti miracidia respond to monochromatic light in a manner similar to other invertebrates with

dermal light sense.

In the water columns both S. mansoni and S. haematobium miracidia dived to the bottom to infect target snails. A depth of two metres - the maximum tested - offered no barrier to host location.

Hydrostatic pressure at the depth of 2 m in the column did not affect miracidia and infections produced were comparable to those obtained when exposures were carried out under an equivalent pressure in the pressure apparatus. Hydrostatic pressure in water is directly proportional to depth. Pressures up to 5.0 m depth had no adverse effect upon the miracidia, which were observed to behave in the same manner in the apparatus either with applied pressure or without. Infection experiments using miracidia upon which pressure had been applied for 3 and 5 hours did not show any alteration in their infective capacity. Snail hosts, however, appeared to have been affected by pressures equivalent to depths of 3.6 m and 5.0 m, since the observed withdrawal of their soft bodies into the shells resulted in reduced infection rates. The evidence of infected snails living at considerable depths may suggest an adaptation to bottom living conditions. Examples are B. choanomphala in Lake Victoria (Webbe, 1962; Coles, 1970; Hagendorn, personal communication 1972) and B. truncatus rohlfsi, the principal intermediate host of S. haematobium in the Volta Lake, which has successfully adapted to deep static water in association with the submerged aquatic weed Ceratophyllum. As a result, human prevalence rates in some areas around the lake have risen to epidemic proportions in only about two years (Webbe, personal communication, 1972).

Schistosome miracidia have a remarkable scanning capacity. In large volumes of water and in vessels where miracidia and snails were separated by considerable horizontal and vertical distances miracidia were capable of locating and infecting their snail hosts. Although there was a gradual decline in infection rates with a decrease in miracidial density, fairly high infection rates occurred even at densities as low as 0.42-0.04/litre

both in B. pfeifferi and B. (P.) globosus. Small numbers of miracidia may still therefore be capable of producing infections in a large body of water where snails may be sparsely distributed in particular niches. By extrapolating the results it may be possible to reach a point where no infection takes place but in view of the efficient seeming capacity of miracidia this must be very near to a situation where no miracidia reach the environment. In endemic areas transmission may be stopped if no contamination occurs. But is this possible? Chernin et al (1962) and Shiff (1968) have shown in the laboratory that single miracidia are capable of infecting single snails and that an increase in the number of miracidia significantly increases the chances of many snails becoming infected. The present experiments also showed that a decline in infection rate occurred as the distance traversed by miracidia to reach snails was increased. While miracidia may traverse considerable distances in small circumscribed habitats, in flowing waters miracidia are conveyed over considerable distances downstream.

Flowing water is therefore of significant importance in the epidemiology of schistosomiasis, since miracidia in an infective condition may be distributed over long distances thus giving them greater opportunity of coming into contact with host snails. Snails are unable to maintain themselves in fast flowing waters such as occur in the flooding conditions of the rainy season in endemic areas, and may be flushed downstream, leading to marked seasonal fluctuations in snail numbers (McCullough and Duke, 1954). Flow rates exceeding 33 cm/sec dislodge B. glabrata at the solid boundary of a canal (Jobin and Ipen, 1964) but where stable conditions exist in fast flowing water, such as still backwaters, coves and crevices along the margins, snail-miracidia contact is enhanced. Infected Biomphalaria spp have been collected from streams in some parts of which flow rates as high as 122-176 cm/sec have been recorded in the rainy season (Webbe, 1965). The results of the present study and those of other workers agree in that infections

occur at flow rates of 10 cm/sec and below, while infections are rare above this rate (Shiff, 1968; Webbe and James, personal communication; Upatham, personal communication 1972).

A degree of turbulence may aid distribution of miracidia in the environment and enhance snail-miracidia interaction (Shiff, 1969) but above a certain limit turbulence may sweep miracidia away from susceptible snails and thus physically prevent contact. In the present study the turbulence produced was inimical to snail-miracidia contact and prevented the infection of snails located in the turbulent zone. In the natural environment the presence of stones, tree trunks, branches of plants, etc. in a stream may produce turbulence which may aid host-seaming by miracidia. The high infection rates produced in both B. pfeifferi and B. (P.) globosus in the flowing water system studied and the recorded occurrence of infected snails in flowing water habitats clearly indicate the dangers which may result from the development of irrigation systems for agricultural purposes in endemic areas. If flow rates in irrigation channels provide a suitable habitat for the snails, then their infection certainly cannot be prevented unless contamination is eliminated.

The influence of temperature and ultra-violet radiation on the survival and infectivity of schistosome miracidia is profound. These factors affect metabolic processes in the miracidia and therefore directly affect their degree of activity, and consequently their survival and infectivity. Miracidia of S. mansoni and S. haematobium were affected equally.

Miracidia survived longer at moderate temperatures than at high or low temperatures. Within the biokinetic zone of 10-45°C (Gleso, 1960) a rise in temperature resulted in an increased degree of activity. As food stores are utilised to provide the energy for the increased activity the life-span of the miracidia is proportionately shortened. Decline in the degree of activity was therefore sharper at high temperatures and more gradual at moderate and low temperatures. Very high temperatures made miracidia very

active but a possible irreversible inactivation of enzymes in the cells leads to rapid exhaustion and early death. At very low temperatures (5-10°C) miracidia were "lethargic", the result of suppressed metabolism which, when prolonged, led to the suspension of essential life processes and a considerably shortened life-span.

The penetration of succeptible snails depends on the activity of the miracidia, only the "active" and "slow" miracidia being capable of penetration. Miracidia that were "lethargic" as a result of low temperature or old age were incapable of penetrating, and consequently no infections occurred at temperatures below 10°C. Both S. mansoni and S. haematobium miracidia retained their capacity to infect for about 17 hours at moderately low temperatures. This is of significant importance and indicates how long miracidia may remain infective in natural waters.

Although the survival and infectivity of miracidia is greatly affected by temperature, the temperatures encountered in the field fall well within the limits tolerable to them. The temperature, however, limits the distribution of the snails and thereby the distribution of the schistosomes. In some areas high temperatures in the dry season result in the drying up of snail habitats, leaving small pools in which susceptible snails abound. Increased contact with such waters leads to increased human infection and further contamination of the habitat, which in turn leads to further infection of snails. In habitats that dry up completely snails aestivate and transmission is interrupted. Thus anywhere that snail hosts manage to survive can become a focus of transmission when contaminated with miracidia. It has been suggested that the absence of S. mansoni on the coastal plains of East and West Africa is due, at least in part, to the high temperature, intolerable to Biomphalaria species.

Sunlight contains ultra-violet radiation which significantly shortens the life-span of miracidia. Artificially produced ultra-violet radiation had a quick damaging effect on miracidia exposed to it.

Although ultra-violet light can penetrate to a depth of about 15 cm in still clear water, penetration is considerably reduced in turbid waters. Therefore when waters in nature are turbid very few miracidia of S. mansoni, which congregate near the surface, will be affected and none of the positively geotactic S. haematobium.

In the environment of the snail miracidia "determine" the susceptibility of different snails and, depending on their activity, proceed to penetrate them. Experiments in the present study show that S. mansoni and S. haematobium miracidia were equally efficient in penetration.

The presence of other molluscan species in the environment together with susceptible snail hosts has been known to reduce infections among the latter. It has been suggested that this is a result of some miracidia exhausting themselves with repeated attempts to penetrate the insusceptible snails, or of the destruction of those that successfully penetrate unsuitable snail hosts. It seems reasonable to infer that in the natural environment miracidia are wasted in this manner.

Thus none of the physical factors studied could be applied to interfere effectively with the infective capacity of schistosome miracidia. However, the apparent reduction in infection rates obtained with decreasing miracidial densities suggests that there is a threshold below which no infection will occur. Any measures taken to reduce the number of miracidia entering the snail habitat would therefore be of significant value in the control of the disease.

The importance of the eradication of the snail intermediate host in the control of schistosomiasis has been emphasized but this should be carried out in combination with other methods such as: mass and selective chemotherapy to suppress egg output in man, reduction of contact with water by providing safe water supplies for domestic and recreational purposes, and provision of sanitation. Education in the value of these practices would reduce transmission and also yield long-term benefits since the schistosome life

cycle would thus be interrupted at several points and the benefits of combined control measures would be achieved.

The present study has shown that S. mansoni and S. haematobium miracidia are affected in a like manner by the various physical factors, their behaviour, survival and infectivity being very similar, and that any differences are adaptations which help the miracidia to find the snail host. The opinion that S. haematobium miracidia are less capable than those of S. mansoni of locating and infecting their snail hosts is a gross underestimation of their scanning capacity.

Miracidia of the two species of schistosomes studied showed remarkable versatility. Their ability to dive to the bottom of tall columns of water and to withstand pressure at considerable depths is unlimited. They are capable of traversing long distances and scanning large volumes of water in order to locate and infect their snail hosts. In flowing water miracidia are able to locate and infect their snail hosts, and in the presence of other snails and organisms they are capable of selecting their own snail hosts. It is evident that the response to some of the physical factors encountered actually assists the miracidia to reach their snail hosts. Miracidia are therefore efficient parasites which have evolved a behaviour pattern that enables them to successfully complete their role in the life cycle of the schistosome.

SUMMARY

1. Temperature has a profound effect on the activity of schistosome miracidia. Within the range of 10-43°C a rise in temperature resulted in increased activity and shortened life span. Their infectivity was directly influenced by their degree of activity; "active" and "slow" miracidia were capable of infecting snail hosts, while "lethargic" ones were not. No infections occurred at temperatures below 10°C, but above it a rise in temperature resulted in higher infection rates in snail hosts. At the normal aquarium temperature of 23-29°C both S. mansoni and S. haematobium miracidia retained their infectivity for about 15 hours, but at the moderately low temperature of 19°C infectivity was retained for 17 hours.

2. S. mansoni miracidia were positively phototactic and indifferent to gravity, while S. haematobium miracidia were negatively phototactic and strongly positively geotactic. S. mansoni miracidia, however, scanned both the illuminated and darkened ends of a vessel, producing high infections, while S. haematobium miracidia preferentially scanned the darkened end of the vessel.

3. A depth of 2 metres, the maximum studied, was no barrier to host location and infection by S. mansoni and S. haematobium miracidia.

4. Hydrostatic pressure equivalent to a depth of 5 metres had no effect on the infective capacity of S. mansoni and S. haematobium miracidia. High infection rates occurred among snails exposed to miracidia at depths of 3.6 and 5.0 m for 3 and 5 hours. But high pressures did affect snail hosts, which were observed to retract most of their soft bodies into their shells.

5. Snails became infected at miracidial densities as low as 0.42-0.84/litre but an increase in miracidial density resulted in increased infection rates. Miracidia located and infected snails placed at a horizontal

distance of 5.1 m away from the point of introduction of miracidia.

6. In flowing water both S. mansoni and S. haematobium miracidia located and infected snails when water was flowing at 10 cm/sec and below, but infections were rare at higher flow rates. Although some degree of turbulence may enhance host seeming capacity of miracidia, the turbulence produced in this study prevented snail-miracidial contact.

7. Solar ultra-violet radiation significantly affected the survival of miracidia. Artificially produced ultra-violet radiation killed miracidia in about two minutes. Irradiation for one minute made miracidia "lethargic" and consequently incapable of penetrating susceptible snails. Sublethal dosages resulted in significant elongation of the body with the final assumption of a characteristic tadpole shape.

8. S. mansoni and S. haematobium miracidia penetrated their susceptible snail hosts B. pfeifferi and B. (P.) globosus respectively with equal efficiency, high infection rates occurring at the shortest exposure period of 15 minutes. Although S. mansoni and S. haematobium miracidia were equally capable of selecting their snail hosts, S. haematobium miracidia were less capable of retaining their infective capacity in the presence of unsusceptible snails only.

9. These studies have shown that the survival and infectivity of S. mansoni and S. haematobium miracidia were influenced in a very similar manner by the various physical factors and that both parasites have a remarkable capacity to locate, select and infect their snail hosts. Miracidia effectively scan large volumes of water and even flowing water, and they are highly selective in the choice of snail hosts. Their overall activity, however, is greatly influenced by the temperature of the surrounding water. The different responses to light and gravity of S. mansoni and S. haematobium miracidia appear to increase the opportunities of contact with their particular snail hosts.

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