PN-ABM-720 8.065
7-9271

FINAL REPORT

Covering Period: Oct.1989-Sept.1992

Submitted to the Office of the Science Advisor
U.S. Agency for International Development.

# A TRAP FOR THE DETECTION OF SCHISTOSOME CERCARIAE.

Principal Investigator: Clive Julian Shiff
Grantee Institution: Johns Hopkins University, Baltimore

Collaborator: Dr. Stephen Chandiwana
Institution: The Blair Research Institute, Harare.

Project Number: 8.065
Grant No. DPE-5542-G-SS-8058

A.I.D. Grant Project Officer: Dr. Bruce Harrison

Project Duration Sept 1989-1992.

REC'D IN R&D/R

NOV 4 1992 CC: CDME

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## EXECUTIVE SUMMARY

Schistosomiasis is contracted by people being attacked by free swimming parasitic larvae shed from infected snails in freshwater streams, ponds and canals. However, it is almost impossible to detect just where in the water these cercariae go, what attracts them, how they are affected by currents and diurnal changes in light. In the study of the transmission of this disease it is important to know this but it has been extremely difficult to detect the presence of schistosome cercariae in natural water. Filtration of water to detect these organisms is difficult because the apparatus becomes clogged with silt and cercariae cannot be seen easily. The only way to infer the presence of cercariae in the water is to look for snails and determine whether they are infected or to immerse mice or hamsters in the water, then to keep them alive for about six weeks and sacrifice them to see if they have become infected. This latter is difficult, expensive, inhumane and only provides information after the fact. What is needed is a simple quick method to locate cercariae in natural water. Then it will be possible to detect the presence of cercariae in the water and also it will help observers study diurnal periodicity and movements of cercariae and will also enable us to determine where in the habit these infective larvae collect to attack and infect the unwary human host.

A simple trap for schistosome cercariae has been developed using clear nail varnish as a matrix and linoleic acid as stimulant affixed to a 75 x 25 mm glass slide. Schistosoma mansoni cercariae will attach to the surface and can be easily visualised either stained or unstained. The trap works well under laboratory and field conditions where retrieval rates vary from 100% to 30%. Under laboratory conditions there appears to be some form of attraction of cercariae to the surface.

The trap has been developed at Johns Hopkins but has been tested in outdoor conditions in both Zimbabwe and Egypt. It involved visits to Baltimore by Dr. Chandiwana at the early stage of development and later by a junior member of his staff who spent three months at Johns Hopkins University perfecting the technique. Subsequently a series of field trials were carried out in Zimbabwe and with the collaboration of Dr. F. Yousif at the Theodor Bilharz Research Institute in Cairo. The method should now be tested as a research tool in the field in endemic areas. Well prepared individuals in Zimbabwe who have benefited from the project are now in country to carry out this work.

## RESEARCH OBJECTIVES

Schistosome cercariae are extremely difficult to detect in natural water bodies. Methods tried include filtration through a modified pressure cooker (Rowan, 1957), the overlay technique, (Sandt, 1972) and through a variety of sieves (Théron, 1974). These methods are effective in clear water, but are not efficient in water of moderate turbidity. Use of the continual action centrifuge (Barrett and Elison, 1965) improved recovery and has been used in Zimbabwe and Egypt. However it is time consuming to operate and requires a source of electricity and special apparatus. Detection of cercariae in transmission sites conducted by using sentinel rodent (mice or hamsters) immersion techniques requires large numbers of animals which need to be housed and maintained for long periods prior to sacrifice and detailed examination to detect small numbers of worms. Such methodologies have obliged workers to omit cercarial detection methods and look only for infected snails, (Ouma et al., 1989) the presence of which indicates to the observer that transmission is likely to occur.

This inability to study precisely where cercariae occur in the waterbody has precluded observation of actual transmission potential and the study of cercarial distribution in the habitat itself. In a recent review, Haas (1992) discussed response of different species of trematode cercariae to external stimuli which lead them into the appropriate regions of the habitat where they are likely to contact the appropriate host and which activate them or pre adapt them to active penetration. This applies as much to schistosome cercariae as to cercariae of other species. It is also relevant in defining areas and periods of high risk for infection when people are exposed to water.

Cercariae have been shown to be stimulated by skin surface lipids (Stirewalt, 1971; Austin et al., 1972; Shiff et al., 1972). The latter showed that unsaturated fatty acids such as oleic and linoleic acid would also stimulate this response. More recent studies have confirmed the long chain unsaturated fatty acids to be the active constituents (Granzer and Haas, 1986, Salafsky et al 1984, Salafsky 1988). When cercariae are thus stimulated they exhibit a number of precise behavior changes culminating in a penetration response during which the acetabular and post acetabular glands discharge, and penetration is initiated. We have used this response to develop a simple trap which causes schistosome cercariae to adhere to a prepared surface in a way that they can be trapped in the aquatic environment and subsequently counted after simple staining procedures. We have used the trap to detect schistosome cercariae preferenda for certain environmental modulators.

## METHODS AND RESULTS

1. Trap Surface:

The trap consists of a glass slide 25 mm x 75 mm, with the

stimulant matrix applied close to one end. The stimulant matrix comprised clear nail varnish base (Clear Nail Protector, Pavlon Ltd, Nyack, NY) and linoleic acid (99% pure, Aldrich Chemical Co. NY). The actual preparation process was as follows. One drop of the nail varnish was placed on the slide and 5 $\mu$l linoleic acid (= $1.78 \times 10^{-2}$ mM) added. The compounds were mixed on the slide and spread over about 4 $cm^2$ of the surface. The slides were dried under vacuum for 1 hr to remove organic solvents and were ready for use. Storage in the dark for 24 hrs did not appear to alter the efficacy.

For immersion in water, the slides were placed individually on the bottom of the container or in a triangular plexiglass stand 25 cm high with slots cut in the hypotenuse at 1.0 cm centers (see Fig. 1). The frame with slides was then immersed in the water and left in situ for 2 to 3 hrs depending on the experiment. After the exposure period, the frame was removed gently from the water with minimum washing effect on the surface of the slides, the slides were allowed to dry for 30 min, then removed from the frame, stained in haematoxylin for 60 min. and examined under low power microscopy using glycerine as mounting medium. Cercaria bodies, tails or both were easily detected and counted. Following exposure in field conditions with much detritus, cercariae were best observed dry without any staining al all. The stain appeared to obscure the cercariae by highlighting plankton and other detritus.

2. Laboratory Exposure Conditions.

For all experiments freshly emerged Schistosoma mansoni cercariae were used. Experiments under laboratory conditions were carried out in a variety of containers (Table I.). Dechlorinated tap water was used and the filled receptacles were allowed to stand for several hours to permit dispersal of air bubbles which seemed to interfere with cercarial swimming activity. The cercariae were then added, slides introduced and left for the designated period of time.

3. Outdoor Conditions:

Two concrete ponds situated outside the Blair Research Laboratory in Harare were used for these experiments. The ponds are approx. 2m diam. and 0.75m deep. Surface area of Pond A was 71 634 $cm^2$ and pond B was 52 685 $cm^2$. When filled to 50cm depth Pond A had a volume of 358.2 l and Pond B a volume of 263.4 l.

An experiment was conducted to determine the number of traps needed to sample the ponds effectively. Pond A was divided using string into 94 partitions, 70 of which were squares 30cm x 30cm. A trap was suspended in each of the 74 full squares after the pond had been filled to a depth of 16cm. The water was left to settle for 60 min. when 1080 cercariae (90/litre) were added. The traps were exposed for 60 min. and then removed, stained with haematoxylin and examined.

The resulting data were processed as described by Southwood (1979):

$$\text{Number of full quadrats with traps } n = \frac{SD * t}{Ex}$$

Where n = number of traps
SD = standard deviation
t = t-value from the t tables at E
E = 0.05
x = mean.

$$\text{substituting } n = \frac{1.634 * 2.02}{0.05 * 2} = 33$$

Therefore, for the trials, traps were suspended in 33 randomly chosen full quadrats. Trap holders 50 cm long were used in the experiments, three traps placed in each at a depth of 5, 25 and 45 from the surface. After the ponds were filled with dechlorinated tap water they were allowed to settle for 60 min after which the cercariae (19 cerc./l) were added. The traps were left in situ for 60 min, removed, stained and examined. Three replicate trials were carried out in each pond.

4. Natural Irrigation Canals

The cercaria traps were tested by immersion in an experimental canal at the Theodor Bilharz Research Institute Field Research Station at Kanater, Egypt. (Yousif et al. 1992). The experimental canals are 30 m long 1.5 m wide and approx 70 cm deep. A portion of one canal was isolated by metal partitions 1 m apart and exposure took place in that sector. There was no water flow during the experiment. Following closure of the canal with the partitions, 60 000 freshly shed S. mansoni cercariae were introduced and after 30 min dispersal time, the triangular frame loaded with 50 slides was immersed in the canal so that the apex was about 5 cm below the surface. Slides were left in situ for 2 hrs. The water contained large numbers of diatoms and seeds with a turbidity of 30 Nephelometric Turbidity Units (NTU).

Slides were visualised either stained with haematoxylin or dry. Cercariae were seen best in the dry, unstained preparations.

## RESULTS

The results of many series of laboratory and field experiments are given in Table II. It can be seen clearly that cercariae are effectively trapped and clearly observed and enumerated in the experiments both indoors and outdoors. Whether the test vessel was a petri dish (Dish 1) or large aquarium (Tank 1) cercariae were retrieved with remarkable efficiency. In fact under several conditions, particularly when densities were high, the trap surface appears to collect more cercariae than expected from a randomly distributed population (see column 6, Table II). Here the ratio between the theoretical number of cercariae per 4.84 $cm^2$ and the actual trap recovery is given.

Under field conditions in the irrigation canal in Egypt, recovery per trap approximates 30 percent of the population of cercariae distributed randomly per surface area. This recovery rate is reasonably consistent and occurs not only in the outdoor experiments, but also in the higher dilutions of cercariae used in the large laboratory container, Tank 1. Recovery rates in the Zimbabwe trials (Pond A and B, Table II) were close to the expected value, assuming random distribution of cercariae in water.

## DISCUSSION

Our work has clearly indicated that an artificial matrix containing chemical stimulants can stimulate and entrap normal swimming schistosome cercariae and that irrespective of the volume of container and density of the organisms, the recovery rate is good and reasonably consistent. It can provide a mechanism by which one could investigate ecological factors which may influence the distribution of schistosome cercariae within water bodies. With such apparatus both laboratory based or field studies can be carried out.

It does also pose certain questions about the mechanisms of entrapment and stimulation. Attachment of S. mansoni cercariae to the host can be clearly distinguished from other behavioral patterns classified as swimming movements and host finding activities. In his recent review, Haas, (1992) mentioned that patterns of swimming movements of S. mansoni cercariae were influenced by several physical factors such as light stimuli and temperature, but not by chemical gradients of stimulant. In his opinion, cercarial swimming movements are determined by chemical gradients only in species represented by relatively large, slowly swimming cercariae. This is because water turbulence created by the cercarial tail, prevents detection of any chemical gradient of the stimulant.

Haas (1992) has suggested that host finding reactions of S. mansoni cercariae involve: 1. Detection of the host surface stimulated by temperature gradient, water turbulence and 0.05 L-arginine in the substrate. 2. Remaining on the host and creeping on the surface (exploration) for entry sites is stimulated by heat, with a temperature gradient as little as 0.07 C (Haas, 1990; 1992) and several neutral and polar fractions of human skin surface lipids (Haas et al., 1987). 3. Penetration of the host is stimulated by free unsaturated fatty acids as shown by numerous authors e.g. Stirewalt, 1971; Shiff et al., 1972; Austin et al., 1972,; Salafsky, 1984a, 1984b).

All these activities stimulated by the presence of a host surface have been described as occurring after contact with various natural or experimental substrates containing unsaturated fatty acids. In a typical attachment response, after contact with the substrate containing fatty acid, cercariae alter their swimming pattern from a tail first activity to forward swimming.

Ascending cercariae approach the substrate tail first, attach to the substrate using the penetration organ (Haas, 1986) then commence the complex behavior of penetration. What then is the mechanism of our trap? Purely mechanical followed by chemical stimulation, or is there an alternative selective process operating.

In our designs the trap has been represented by a single slide in small volume or multiple slides (up to 50 slides) per exposure in larger volumes. This is designed to improve the probability of an initial contact by cercariae with unsaturated fatty acid in the substrate to stimulate them and consequently initiate penetration reactions. However, it may be that individual cercariae detected on a particular slide have been stimulated by fatty acid signals in the water and not necessarily from the slide on which they were found. They may be activated by the presence of large areas of stimulant or micelles of fatty acids in the water. Certainly the high level of recovery seen in the smaller vessels and tank experiments suggest a selective attraction of cercariae to the surface.

It is difficult to conceive that fatty acids restricted to a surface will influence swimming movements of S. mansoni cercariae because of the instability of a chemical gradient of fatty acids in water. Even under laboratory conditions the effective range of chemical gradients are small (LaRue, 1951). However there is ample evidence (Haas and Schmitt, 1982a, 1982b; Haas, 1984, 1986) that host finding activities can be initiated by passive permeation of the fatty acid molecules into the cercarial body. The presence of free unsaturated fatty acid molecules in the water may act as a trigger to initiate host finding reaction of cercariae and should be considered in explanation of the high number of cercariae detected in the trap surface which operates only under fatty acid signal. This would mean that free molecules of stimulant derived from substrate or surface can be present and act on the cercariae even if there is no real chemical gradient present.

Figure 1. Triangular stand used for immersion of slide traps in large laboratory aquaria and in field experiments.

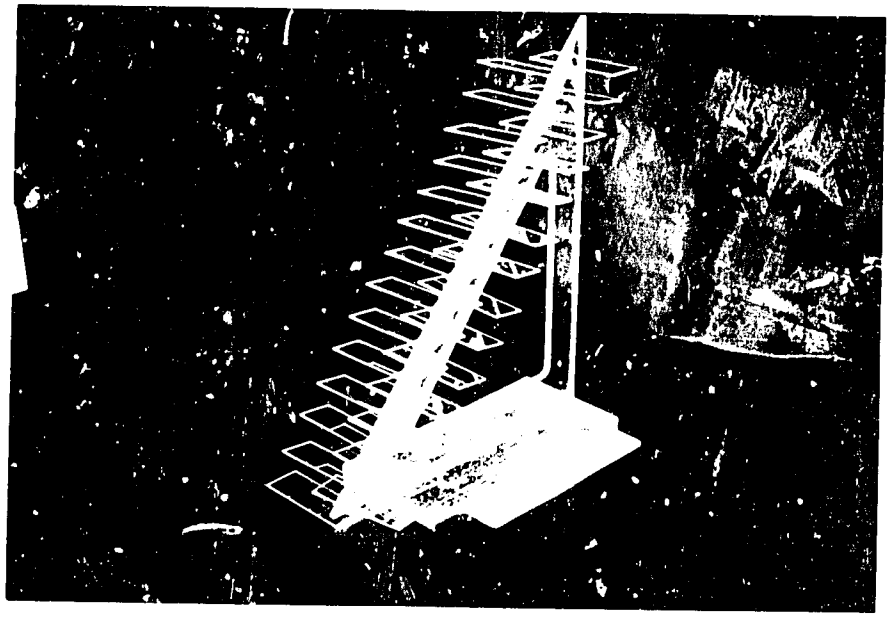

Table I. Volume of water containers used for recovering *Schistosoma mansoni* cercariae in the laboratory.

| | Area of bottom | Size | Capacity* of container |
|---|---|---|---|
| | $cm^2$ | cm | litres |
| Dish 1 | 23.7 | 5.5 | 0.05 |
| Dish 2 | 308.2 | 22x14x4 | 1.23 |
| Dish 3 | 900.0 | 30x30x10 | 9.00 |
| Tank 1 | 2100.0 | 30x70x29 | 60.90 |

Note*: filled with water

Table II. Recovery of Schistosoma mansoni cercariae by linoleic acid/matrix substrate in various containers of water.

| Exp. | Density of cerc. (c/l) | No of cerc. in trial | Random. disper. per 4.84 $cm^{2*}$ | Mean No of cerc. recovered per slide ± SD | Ratio |
|---|---|---|---|---|---|
| Dish 1 | 1000 | 50 | 10.22 | 29.9±4.70 | 2.93 |
| Dish 2 | 1000 | 1000 | 15.70 | 109.0±8.27 | 6.94 |
| | 1000 | 9000 | 48.41 | 119.7±17.11 | 2.47 |
| Dish 3 | 500 | 4500 | 24.22 | 65.3±7.21 | 2.70 |
| | 125 | 1125 | 6.05 | 31.1±3.92 | 5.14 |
| | 1000 | 61000 | 140.59 | 121.7±15.72 | 0.87 |
| | 500 | 30500 | 70.30 | 78.7±17.15 | 1.12 |
| Tank 2 | 125 | 7600 | 17.51 | 29.0±10.21 | 1.66 |
| | 60 | 3600 | 8.30 | 2.8±0.34 | 0.34 |
| | 30 | 1800 | 4.15 | 1.5±0.18 | 0.36 |
| Pond A | 19 | 7000 | 0.47 | 0.51±0.39 | 1.09 |
| Pond B | 19 | 5000 | 0.46 | 0.48±0.38 | 1.04 |
| Kanater Irrig. Canal | 120 | 60000 | 20.74 | 7.1±1.45 | 0.34 |

Note: * area of treated surface examined under 22x22 mm coverslip

Ratio = Number expected (per 4.84 $cm^2$): Number seen on trap surface.

## IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

These finding will be useful in developing countries where schistosomiasis is endemic because they provide a tool to study the epidemiology of this disease. When we know where in the habitat cercariae move, it may be feasible to discourage contact with the water in specific areas and at specific times.

The project enabled close liaison between the PI and the Director of the Blair Research Institute, Zimbabwe to develop. Both visited each other and will be planning further research work in the epidemiology of schistosomiasis with particular respect to modelling transmission patterns using this trap. It also enabled Mr Peter Chibatamoto, Research Officer from the Blair Research laboratory to work for three months at Johns Hopkins University and to help significantly in the development of this trap.
It provided Mr Mark Bradley with resources to employ a student to carry out the experiments in the outdoor ponds at the Blair Research Institute as part of her Honours degree in Zoology.

## PROJECT ACTIVITIES AND OUTPUTS

| | | |
|---|---|---|
| Meetings attended | | NIL |
| Publications: | | Manuscript submitted to Journal of Parasitology; July 1992 |
| Training: | a | Mr. P. Chibatamoto trained in scientific investigation at Johns Hopkins University. (Three months). |
| | b | Dr. S. Chandiwana spent one month in the US visiting Johns Hopkins and the laboratory of Dr. Salafsky in Cincinnati |
| | c | Miss F. Mutapi spent six months at the Blair Research Institute completing her research project for Honours degree in Zoology. |

## PROJECT PRODUCTIVITY

The project achieved its proposed goals

## FUTURE WORK

Now that we have a tool to detect schistosome cercariae in water and estimate numbers in particular parts of the waterbody, larger scale field experiments should be undertaken to study the ecology of schistosomes in natural conditions. There should be three major objectives in this work.

- This will help validate mathematical models currently being proposed for the transmission of the parasite. Until now, there has been no method for including the exposure patterns, seasonality and diurnal rhythms of these infectious organisms.

- Studies can be carried out to identify attractants and repellents in both semi field and field situations.

- In controlled field situations studies can be carried out to observe the distribution of cercariae with reapect to the position of snails which emit them within the natural habitat. This should enable us to identify risk factors assoicated with infection.

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