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The Ecology of Schistosome Transmission Foci

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The study of cercarial populations in the field can provide a useful means of locating schistosome transmission foci and of evaluating the success of bilharziasis control programmes. It also provides a means of learning about certain aspects of transmission that have hitherto been neglected.

In recent years six techniques have been successfully field-tested or employed in field research on the biology of the cercarial stage of schistosomes. They include several variations of paper filtration of the water, the use of phototaxy for separating cercariae from water, and bioassay by exposing mice to infective water. The various advantages and disadvantages of each method are discussed in this paper. The author suggests that an approach to the ideal cercariometric method may be achieved by combining a paper filtration technique with mouse exposure.

During the past 60 years of bilharziasis field research parasitologists the world over have been preoccupied with centrifuge tubes and egg counts. In Puerto Rico alone, between 1904 and 1955 64 faecal survey studies were done on groups ranging in size from 10 to 19 139 people, and one skin test survey of over 10 000 people was carried out during 1963. Yet the host survey, a tool in the analysis of trematode disease, reveals almost nothing about transmission. Unless such a survey covers an extremely long time period, it is impossible to know whether the disease caused by the trematode is spreading or contracting spatially; and, regardless of the length of time covered by the survey, it is impossible to determine whether the rate of transmission is higher or lower at one focus than another, whether transmission is continuous, intermittent, or spurious, or how extensive the transmission focus or foci may be within a local endemic area. Transmission can cease in a given area and a host survey may not reveal the change for from two to 10 years.

I had the opportunity in 1963 to visit about 25 areas in Puerto Rico where skin testing of schoolchildren had just been completed and had revealed the presence of *Schistosoma mansoni*. Twelve of the areas had a high endemicity (as indicated by the frequency of positive skin tests) and their discovery caused a good deal of excitement and comment among survey staff. Yet by cercarial survey (supported by a careful search for snails) I could show that only two of these 25 so-called endemic areas contained active transmission foci. Cercariae and infected snails were found in water for 800 m at one focus. The second focus was limited to less than 10 m of sluggish creek. I believe that most of the other 23 areas had been extinct as transmission foci for at least a year, and it is possible, since 10-12-year-old children constituted the survey group, that transmission had not occurred in some of these areas for several years. Snails were present in about one-third of these so-called endemic areas, but pollution patterns presumably had changed over the years so that they rarely, if ever, became infected. Yet, by typical "precontrol" survey standards, which at present make no use of the cercarial survey, these areas were judged to be dangerous and warranting immediate attention. In countries equipped to undertake control, the consequences of that judgement are familiar. Chemicals are purchased, control teams are organized, engineers calculate the velocity and volume of flow of rivers and streams, weirs are constructed, the waters run yellow for hours or days, fish culture is retarded for from two to five years and the snail population in that 10-metre section of sluggish creek, if it is accidentally included in the control zone, suffers a temporary population decline.

To control bilharziasis economically, it seems to me that we shall eventually have to become more selective in our efforts. We cannot continue to

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blanket whole watersheds with chemicals. The problem will inevitably become more acute as we attempt in future years to control other medically and economically important trematode diseases.

"Molluscicidologists" have argued that until we have some precise knowledge of the extent to which snails must be reduced to effect confinement of trematode-disease transmission, we have no choice but to eliminate the snails completely from entire watersheds. At the present time this may be partly true, but it is my belief that through extensive use of cercariometry in combination with other techniques the day will come when we shall have sufficient knowledge of thresholds in transmission to justify a more satisfactory phase of control. Meanwhile, cercariometry can serve as a rapid means of locating genuine transmission foci and of evaluating the success of control projects.

PRESENT STATUS OF CERCARIOMETRY

Six different techniques have been successfully field-tested or employed in field research on the biology of the cercarial stage of the schistosomes.

Millipore filtration (Pesigan et al., 1958)

This technique uses a millipore filter disc of type HA black grid without nutrient, 47 mm in diameter in conjunction with the commercially available millipore filter and a two-stage Welch Duo Seal vacuum air-pump with 1/3 hp motor. The cercariae are observed and counted on the disc by reflected light.

Advantages. The pore size of the disc probably allows 100% recovery. The sample is dipped from any point in the stream or skimmed from the surface and is then poured through the millipore filter.

Disadvantages. The practical size of the sample is only one litre. Filtration using the HA disc is slow. Turbidity interferes with an accurate count and reduces the sample size. Many cercariae lose their tails in the process of filtration, thereby becoming difficult to recognize. If samples are run in the field, a 110-volt generator is required, and it is impracticable to move many large samples to the laboratory for processing.

Pressure paper filtration (Rowan, 1957, 1958)

Cercariae are trapped on Schleicher & Schuell 404 (18.5 cm diameter) filter-paper in a pressure filtration chamber activated by a double-action bilge pump (45 litres per minute capacity). The sample (20-100 litres) is drawn directly from the pond or stream through a hose into the pump. Cercariae trapped on the paper are heat-stained in the field with ninhydrin reagent. The dry paper is brought to the laboratory for a detailed cercarial count, but a preliminary estimate can be made in the field using a hand lens.

Advantages. An average of 64%±14% of the cercariae in the sample are recovered, and 45%±8% are sufficiently well oriented on the paper to be recognized as brevifurcate cercariae. The technique employs a large sample size (20-100 litres). The sample is collected by means of a hose to any point in the pond or stream. Turbid water can be flocculated before filtration without important reduction of the cercarial recovery rate. The blue-stained cercariae are readily visible against a white background. The dry filter-paper disc forms a permanent record which can be filed for future use. No electrical power source is required. All parts are commercially available and easily assembled and maintained. The sampling time is 10-15 minutes. A trained technician can read an 18.5-cm paper in 10-15 minutes.

Disadvantages. In direct sampling, turbidity is a severe problem. The equipment is bulky and weighs about 16 kg complete. This is considered by many to be too heavy for extensive field use in remote areas.

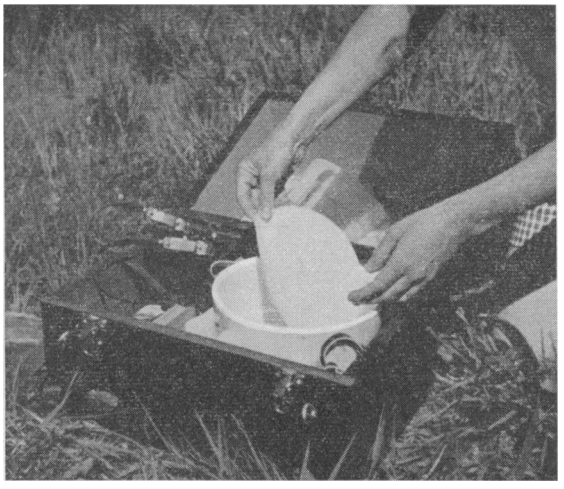
Remarks. I have replaced this equipment in my own work with the two vacuum filtration methods next described; these give a higher cercarial recovery and the equipment is easier to construct and to use.

Aluminium sulfate ($Al_2(SO_4)_3.18H_2O$; commercial filter alum) as a flocculant was suggested in the original description of the equipment. This addition to the basic technique under review is not inconvenient and it has the advantage that the water sample can be collected in batches over several hours at a given focus in order to level out the effect of hourly fluctuations in cercarial density.

Electrically operated vacuum paper filtration (Rowan, unpublished)

The device, as indicated in Fig. 1, consists of a wooden box containing a table-type Büchner funnel (18.5-cm diameter paper size) connected to a Jabsco Co. self-priming, 6-volt, "Mini-Puppy" water-pump. The pump is activated by a small motor-cycle battery. The cercariae are trapped on Schleicher & Schuell 404 or similar filter-paper and handled as in the pressure paper filtration method described

FIG. 1
ELECTRICALLY OPERATED VACUUM PAPER FILTRATION APPARATUS



above. The equipment was used successfully in the field in Puerto Rico during 1963.

Advantages. Water is dipped from any point in the stream or skimmed from the surface and is poured into the Büchner funnel. The motorization of the unit is very helpful. The small batteries, fully charged, are useful for approximately half a day of sampling.

Disadvantages. Although the instrument is easily packed and unpacked for use, it weighs about 16 kg. Turbidity interferes with the sample size as usual, and it is necessary to resort to preflocculation when sampling turbid waters. This adds to the weight and bulk of the equipment. The small batteries are not very satisfactory. They seem to be difficult to recharge and they do not withstand misuse.

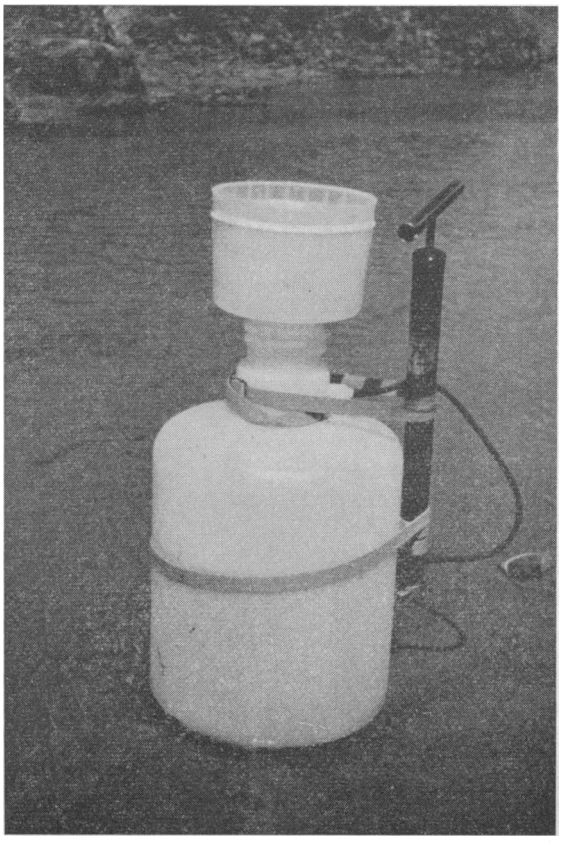
Remarks. Motorization of the unit is so helpful that we have begun a study of other possible means. The use of a small (2.4 kg) gasoline-driven pump is being explored.[1]

Vacuum paper filtration (Rowan, unpublished)

As indicated in Fig. 2, this device consists of a 20-litre, polyethylene bottle with an improvised plastic Büchner-type funnel sealed to the opening, and with a drilled side vent provided with a plastic one-way valve (Cole-Palmer Co., item 6308) for attachment of a modified bicycle air-pump. The valves of the air-pump (Wizard, A-6405, Western Auto Supply) are reversed so that it serves as a simple vacuum pump. A hole drilled in the bottom of the bottle and closed with a rubber stopper provides a drain. All parts are glued together using any of the paste-form elastic rubber compounds. Water is poured into the funnel. The pump provides a gentle vacuum. The filtered water collects in the bottle and the cercariae remain on the filter-paper (S & S 404) and are stained with ninhydrin as indicated above. This equipment was developed and used extensively in Puerto Rico during 1963. With correct use, essentially 100% of the cercariae are trapped on the paper.

Advantages. The equipment is light in weight (2-4 kg) and unbreakable. It can be used in the most

FIG. 2
VACUUM PAPER FILTRATION APPARATUS



[1] This equipment is now available and will be reported on in a subsequent paper. — W.B.R., 30 July 1965.

difficult terrain by one man working alone if necessary. It is less expensive than any of the equipment thus far described.

Disadvantages. The equipment is subject to the same turbidity restrictions as described above. One hundred litres would seem to be a reasonable sample volume to aim at and this would require five papers per station with this equipment.

Remarks. A metal container substituted for the plastic bottle would give more rigidity to the vacuum chamber and speed the filtering process. Some data comparing preflocculation with direct sampling when using this equipment are available from parallel 20-litre field tests done at the Rio Barranquitas, Puerto Rico:

	Cercariae per litre	
Trial No.	*Direct sampling*	*Preflocculation*
1	2.9	2.5
2	2.4	2.7
3	2.9	2.7
4	2.8	2.2

Apparently, when silt is flocculated with commercial filter alum in a second 20-litre plastic water-bottle and the supernatant is filtered, no significant loss of cercariae from the sample occurs.

All the filtration devices have the disadvantage that related cercariae are difficult or impossible to distinguish on the paper. In some locations non-pathogenic species could be confused with pathogens and *vice versa.* A method has been described for differential staining of cercariae of *S. mansoni* and *S. mattheei* on filter-paper (Rowan, 1961). It is possible that an extension of this line of research will make determination of several different cercarial species possible.

Phototaxic response equipment (Klock, 1961)

An apparatus that relies on the phototaxic response of *Schistosoma mansoni* cercariae to separate the cercariae from river water has been developed. It includes a series of flood lamps that cause the cercariae passing through the apparatus to be attracted up into a side arm, where they can be counted. The designer claims a very high efficiency in sorting cercariae from the water.

Advantages. Turbidity is probably not an important hindrance to use of the equipment, but no data are available on the effect of various turbidities on the efficiency of recovery.

Disadvantages. The equipment is heavy and breakable. A 110-volt power source is required. The equipment has not been used in extensive field studies, although it has been field-tested.

Remarks. It is possible that the principle of phototaxis can be applied more efficiently and made to work even with those species such as *S. haematobium* which are said not to respond to bright light (Raybould, 1963). Bourns (1962) notes that some species of cercariae "which have been thought not to exhibit phototaxis will respond positively to certain light intensities. Cercariae of *Schistosomatium douthitti*, for example, do not respond to dark or to bright light, but will swim quickly from an area of darkness to a spot lighted by approximately one candle-power of incandescent light."

Mouse exposure tests

Laboratory mice, partially immersed in standing or running water, are subject to infection. This method has been used extensively by Pesigan (1958) and by Pitchford & Visser (1962a, 1962b).

Advantages. Turbidity does not interfere. The method imitates natural conditions of exposure. Differentiation of species of *Schistosoma* is usually possible.

Disadvantages. The mice must be maintained for six weeks after exposure before the results can be determined. This usually does not interfere with research studies but may be a factor limiting use in control projects.

Remarks. The recovery varies with the age of the mice, the density of the cercariae, the velocity of the water, and probably many other factors. At cercarial densities ranging from 69 to 95.4 per litre and flow velocities ranging from 2.7 to 50.0 cm per second, groups of mice exposed for 30 minutes acquired an average of from 1.0 to 70 worms, a percentage recovery (of cercariae approaching the skin of the mouse) of from 0.17 to 1.79 (Rowan & Gram, 1959). In later studies, at cercarial densities of 0.75-1.05 per litre and stream velocities of 30 cm per second, groups of mice exposed for 30 minutes acquired an average of from 1.0 to 2.5 worms, a percentage recovery of from 0.3 to 1.0 (Rowan & Rodriguez, Rio Barranquitas, Puerto Rico—unpublished). The mice failed to become infected under these conditions at cercarial densities of 0.1, 0.2, 0.8, 0.45, 0.15 and 0.60 per litre, measured and tested at different times during the same day. Yet these latter levels of density almost certainly have epidemiological significance. Radke et al.

(1961), using a similar method of calculation, noted percentage recoveries of from 0.021 to 0.24 at a velocity of nearly 150 cm per second. Pesigan et al. (1958) found an average of 0.2, 3.5 and 2.25 worms per mouse in mice exposed for 1½ hours at three different times of the day to water in "a small stream" with a cercarial density of 0.33, 1.0 and 3.0 per litre, respectively. Data from these several sources agree remarkably well, but indicate that low percentage recoveries can be expected using mice and that mouse exposure techniques may fail to give the necessary sensitivity when cercarial densities are low.

ECOLOGY OF SCHISTOSOME CERCARIAE

Periodicity

Until a few years ago practically nothing was known of the biology of schistosome cercariae under natural conditions. Laboratory observations had demonstrated a marked daily periodicity in the escape of cercariae of *S. mansoni*, *S. haematobium* and *S. japonicum* from snails, but these data had not been confirmed by field observations.

During 1955 and 1956 the research group led by Pesigan in the Philippines began a study of the daily periodicity of *S. japonicum* cercariae. Using the millipore filter technique at several research sites and sampling at four-hour intervals, these authors noted an evening peak in cercarial density (7-11 p.m.). Mice exposed in the stream from 7 to 8.30 p.m. developed more worms than mice exposed from either 11 a.m. to 12.30 p.m. or 3 to 4.30 a.m. The cercarial density was found to vary throughout the day by tenfold, being lowest at 3. p.m. (Pesigan et al., 1958).

During 1957 Mr Carrion and I studied daily fluctuations of *S. mansoni* cercariae in a variety of field situations in Puerto Rico using the pressure paper filtration method described above (Rowan, 1958). Sample volumes varying from 30 litres to 115 litres were taken at one-and-a-half hour intervals from small, medium and large streams and from a pond. In these tests some of the sample papers were found to have trapped over 500 cercariae so that reliable trends could be noted from one time to another. Cercarial densities were found to vary during the day from 0 to 35 per litre at the test sites. In flowing water, a consistent and sharp peak was noted in each case between 11 a.m. and 12 noon. No cercariae were recovered from flowing water before 9 a.m. and cercarial densities diminished and remained at zero after 5.p.m. Observations at the pond were somewhat different. The cercariae began to appear in significant numbers in the water at 10 a.m. and increased in numbers to 4 p.m. Subsequently, the numbers of cercariae declined sharply from 4 p.m. to midnight and gradually to zero between midnight and 10 a.m. Mice exposed in the pond during the low density period at night failed to develop infections.

I believe that *S. mansoni* cercariae in both streams and ponds are shed daily in increasing numbers from 9 a.m. to noon and then in decreasing numbers from noon to 4 p.m. (this is the usual fluctuation reported in the laboratory). In streams the cercariae are probably quickly dispersed by the current from the point of their release so that samples taken in a transmission focus reveal clearly the daily cycle in shedding. In both streams and ponds the cercariae possibly are eaten in large numbers by *Lebistes* sp. and other fish, just as they are in the laboratory (Rowan, 1958). However, in ponds the cercariae probably continue to accumulate until the rate of consumption by predators exceeds the rate of release from the snails. Apparently, *S. mansoni* cercariae are present in significant numbers in Puerto Rican waters for a limited portion only of the 24-hour day. This conclusion should be useful to health educators.

From 1959 to 1961 Pitchford & Visser (1962a, 1962b) carried out an excellent, detailed study of daily and seasonal periodicity of *S. mansoni* and *S. mattheei* cercariae in standing and flowing water in South Africa using mouse exposure as their recovery technique. The daily periodicity studies indicated that *S. mansoni* is present in both waters from 10 a.m. to 4 p.m. This confirms our findings in Puerto Rico. *S. mattheei* was found to be present in both standing and flowing water in South Africa from 7 a.m. to 7 p.m.

The seasonal studies of Pitchford & Visser indicated that *S. mattheei* is also the less periodic of the two. Adult *S. mattheei* were recovered from mice exposed during every month of the year, with June exposures providing low worm yields during the period of the study. *S. mansoni*, on the other hand, was found to have a definite peak of transmission from November to January and a low point in transmission from May to July. In these studies, water temperature seemed to have little influence on the infectivity of mice. The peak of transmission coincided partly with the rainy season.

Location of cercariae in the water

Laboratory tests by Pesigan et al. (1958) indicate that cercariae of *S. japonicum* usually locate on the surface film of the water. On the other hand, Rowan (unpublished) noted that cercariae of *S. mansoni* in standing water under laboratory conditions scattered rather evenly throughout a 1000-ml graduated cylinder during the day if undisturbed but seemed to collect towards the lower half of the cylinder at night. They shifted slightly upwards again as a group with the morning light. When disturbed by agitation of the water at any time of the day, the cercariae congregated at the surface. In gently flowing water (0.5 cm per second) through large glass laboratory tubing (3 cm external diameter) cercariae of *S. mansoni* moved with the current, showing no obvious effort to oppose the flow or to swim to the surface film at a rate faster than in standing water. When the tubing was arranged vertically, and the flow was from top to bottom, the cercariae (at flow rates of 0.5 cm per second and faster) were invariably carried out of the bottom of the tube. When the flow was reversed they were carried out of the top of the tube. They seemed to be unable to maintain their position against minimal currents (Rowan, unpublished).

Under field conditions, *S. mansoni* cercariae can be found in nearly equal numbers in streams at any depth from zero to 50 cm (lowest depth tested) when the velocity of flow is between 10 and 50 cm per second (Rowan, unpublished). One study was made to compare cercarial density in samples dipped from positions along the edge of a stream with samples taken from the main flow of the stream (Rowan & Rodriguez, Rio Barranquitas, Puerto Rico—unpublished). The stream under study varied in width from 2 m to 5 m and in depth from a few centimetres to 0.8 m. The velocity of flow at the sampling points varied from zero to 40 cm per second. Vacuum paper filtration as described above was used in these studies and water was dipped from depths of up to 10 cm. The length of the section studied was 250 m. Infected snails were present along this entire distance and for 100 m upstream. Seven midstream samples of 10 litres each provided an average of 11 ± 7 cercariae (range 5-25) as compared with 13 edge samples with an average of 29 ± 24 cercariae (range 5-70). Although cercariae, and snails as well, were more abundant near the edge of the stream, the results from the edge were more erratic. From the practical standpoint in most survey work, it would seem that samples dipped from the centre of a stream probably give more consistent and usable estimates of cercarial density.

Distribution of cercariae downstream

Few conclusive studies have been done concerning the distribution of cercariae downstream from the point of release.

Early work in the Philippines by Sullivan & Ferguson (1946) indicated that many American soldiers became infected during bridge-building operations at some distance downstream from snail-breeding foci. The possibility that in this instance a few infected snails might have been washed downstream from a colony to serve as "substations" for release of cercariae should be considered, since it is practically impossible to verify the total absence of snails along a kilometre or more of river bank.

A study was done by Pesigan et al. (1958) in the Malirong river and its tributaries on Leyte. On one occasion, between 7 p.m. and 9 p.m., five mice were exposed to the water at a focus of transmission, five were exposed in midstream in the Malirong river 150 m downstream from the focus, and five were exposed near the bank of that river 150 m downstream from the focus. All three groups of mice received heavy and nearly equal infections. The experiment was repeated in turbid water following heavy rains with the result that higher rates of infection were noted at the downstream sites. A similar experiment was done exposing the mice between 9 a.m. and 11 a.m. at the focus and in midstream at the Malirong site, as above, and about one kilometre downstream from the Malirong site in the Palo river. In this instance the infection rate and worm recovery were high at the focus and nearly zero (average of 0.2 and 0.4 worm per mouse, respectively) at the other two locations. Again, one cannot state that infected snails were absent from the downstream areas.

During 1958 Radke et al. (1961) released cercariae into the flow of a small concreted channel at Fort Buchanan in Puerto Rico. Mice were exposed downstream and water samples were tested downstream by the pressure paper filtration method described above. Worm recovery in mice at 330 m was only one-fifth the recovery at 33 m from the cercarial release point. By 660 m the worm recovery had halved again. A few worms were recovered from mice exposed 1700 m below the release point. In contrast, the cercarial density of the water, as determined by the same method, remained at the

initial level for the first 660 m of the channel. No cercarial counts were made beyond that point. It should be pointed out that, owing to frequent use of molluscicide, the channel was essentially devoid of *Lebistes* during these studies. Furthermore, on occasion, the channel was observed to receive army-kitchen scrub-water with detergent. Cercarial testing was delayed once on this account. In spite of these artificial conditions, the study provides some useful information. It would appear that in the absence of a normal population of predaceous fish cercariae can persist in the water at least 1700 m downstream. In these experiments the marked decline in the infectability of cercariae downstream may have been due to fatigue or to chemical poisoning. No chemical tests of the water were done.

Mr Rodriguez and I studied cercarial distribution in two transmission foci in Puerto Rico during 1963 (unpublished). At one focus (Malpica, upper section) cercariae were present at a concentration of approximately 27 per litre in a pool of a sluggish creek. The exit from the pool had a flow rate of less than one litre per second. Ten metres downstream from the exit no cercariae could be found in the water. *Lebistes* were abundant in the pool and downstream. At the other focus (Rio Barranquitas) infected snails were more or less evenly distributed along an 800 m length of the stream. In the heavily polluted upstream section of this study area, near the fouled city septic tank, the cercarial density was a rather constant 1.0 per litre. But the cercarial density dropped sharply from 1 to 0.25 per litre about midpoint in the 800-m study area. Above the midpoint *Lebistes* sp. were not abundant and those present were confined to grassy pools at the edges of the stream. At the 600 m point and beyond the *Lebistes* sp. were present in large numbers and were actively feeding in midstream. The accompanying table compares cercarial density, oxygen tension, and snail infectivity rates at eight points along the length of this study area. The density of cercariae continued to decline even though the snail infectivity rate remained high throughout the length of the study area. I believe that the predaceous *Lebistes* were largely responsible for the cercarial density decline.

The possibility that fish effectively limit cercarial density in nature should not be treated lightly. The data collected thus far tend to support this possibility. In the laboratory it has been shown that four *Lebistes* in one litre of water are capable of consuming 7000 *S. mansoni* cercariae in less than three hours (Rowan, 1958). Cover in the form of vegetation and suspended matter was not provided for the larvae in this experiment. Such cover probably plays an important role in cercarial population dynamics in nature. The role of turbidity as cover for cercariae against predation by fish should receive more study. Several scattered reports indicate unusually high cercarial counts during turbid water conditions (e.g., Pesigan et al., 1958, second Malirong river test; Rowan, 1958, during heavy rains at the Mayaguez pond when a record density of 64 cercariae per litre was recorded). It is interesting to speculate on the over-all impact of periodic, total-watershed molluscicide application if *Lebistes* and other species of fish do have an important role in limiting cercarial density and in eradicating each day's crop of cercariae. Perhaps the chemicals which destroy the minnows are the ally of bilharziasis. This possibility must be explored by further studies of cercarial bionomics at transmission foci.

RELATIONSHIP BETWEEN CERCARIAL DENSITY, SNAIL INFECTIVITY, AND OXYGEN TENSION IN AN 800-M SECTION OF THE RIO BARRANQUITAS, PUERTO RICO, 1963

Distance from septic tank (m)	Cercariae per litre	Oxygen tension (ml/l)	Snails examined	Snails infected
100	1.0	2.6	80	36
200	1.4	2.8	58	18
300	1.1	3.2	64	27
400	0.8	3.4	184	34
500	0.8	3.6	123	46
600 [a]	0.25	4.0	80	20
700 [a]	0.3	4.8	12	2
800 [a]	0.08	4.8	70	12

[a] *Lebistes* were actively feeding in midstream at the 600 m point and beyond.

Water velocity and infection

The velocity of flow of a stream is an important factor to consider in relation to infectivity. It is a crucial factor when using the mouse exposure cercariometric method described above. Obviously, faster flowing water will carry more cercariae past a given point per unit of time. Assuming that the cercariae are not inconvenienced by the velocity of flow of the water, higher infections should occur at higher velocities. Dr Gram and I found this to

be the case both in the field and in the laboratory with velocities varying from 2.7 to 50 cm per second (Rowan & Gram, 1959). Curiously, the percentage recovery (of the theoretical number of cercariae approaching the skin of the mice) also increased with increasing velocity, a finding that we are unable to explain adequately. Radke et al. (1961) continued these studies at higher velocities and found a drop-off in the recovery when the velocity of flow reached about 130 cm per second.

CONCLUSION

The usefulness of cercariometry is obvious, but the methods must be further improved. The ideal cercariometer might be a mechanized unit weighing less than 6 kg that would sample 100 litres of water regardless of turbidity and produce a record that would permit differentiation between closely related and morphologically identical species of cercariae. Preferably, the water sampled should be collected automatically over a 6-8-hour period so as to erase hourly population fluctuations.

Actually, these requirements are not far from being met if two of the methods described above are combined—namely, electrically operated vacuum paper filtration (with preflocculation) and mouse exposure tests (with differentiation of species at autopsy).

For purposes of control work and routine survey, these methods might be combined in a four-step operation:

1. A survey unit truck would deliver a worker, several 100-litre cans, flocculation solutions and a number of white mice to the study area in the morning.

2. The worker would distribute the cans to a number of suitable sampling stations along the study area. He would expose mice to the water for 15 minutes out of each hour at each sampling station and collect 20 litres of water into the 100-litre can each hour at each sampling station.

3. At the time of addition of the 100th litre of water to a can he would add flocculant and stir the contents to produce a suitable floc.

4. The truck would return at this time with the cercariometer and all samples would be filtered through paper and the papers stained.

The operation would require a three-man team. Presumably, control teams could be trained to conduct these tests before and after the application of molluscicide and at various intervals throughout the year. Undoubtedly, work now in progress and future studies will greatly improve the equipment for performing these tests. However, I believe that the questions that can be answered by cercariometry are sufficiently important to recommend its more extensive field use.

This brief review of cercarial biology not only illustrates the use that has been made of cercariometry up to the present time but also points out how little we really know about transmission of this disease and about the ecology of the transmission focus. We have not taken time to learn anything about schistosome egg-load thresholds at different snail densities in natural waters and are unable, therefore, to evaluate pollution objectively. We cannot speak quantitatively about transmission in terms of cercarial density, or even descriptively of the dozens of factors that directly or indirectly influence cercarial density. Most important of all, we have not gained an adequate understanding of the size and extent of the transmission focus in relation to the total watershed. Many workers hold the probably unfounded belief that all water downstream from a focus is hazardous. Scraps of information discovered in the field tend to refute this view, but the matter needs determined study. On the basis of my observations thus far, it seems to me probable that the usual transmission focus of *S. mansoni* is rather limited in size, an average of 100 m or less of stream. Nothing is known of foci in lakes.

In 1954 Meleney compared our present stage in the conquest of bilharziasis to the Paris green stage of malaria control. Each year that has elapsed since then has strengthened my belief that certain of the methods of the malariologist cannot be applied to the control of this metazoan disease. It seems to me that massive chemical application is one of these. With further study of the intricacies of the transmission focus by means of cercariometry in combination with other techniques, we may find it profitable eventually to abandon the utopian concept of snail eradication in favour of more conservative and more subtle means of control.

RÉSUMÉ

L'étude de la biologie des cercaires, sur le terrain, peut permettre de localiser les foyers de transmission active de la bilharziose et faciliter l'évaluation des résultats de la lutte par les molluscicides.

La recherche en ce domaine utilise différentes techniques que l'auteur décrit et dont il énumère les avantages et les inconvénients. Certains appareils sont basés sur la filtration d'échantillons d'eau, d'autres tirent parti du phototactisme des cercaires (notamment des cercaires de *Schistosoma mansoni*) et comportent des sources lumineuses qui permettent la récolte et la numération du matériel d'étude. Le principal inconvénient de ces techniques est le poids et l'encombrement du matériel. Un autre procédé consiste à immerger partiellement des souris de laboratoires dans l'eau contenant les cercaires et à procéder ultérieurement, par autopsie des animaux, à la numération des schistosomes. Par ce moyen, il est ordinairement possible de préciser l'espèce parasitaire. Ce procédé allonge cependant la durée de l'expérimentation; il est en outre peu sensible et échoue lorsque la densité cercarienne est trop faible. Le choix de l'auteur se porte sur une méthode mixte, combinant les techniques de filtration de l'eau et d'exposition d'animaux à l'infection.

Des observations effectuées dans différentes régions montrent que le nombre des cercaires dans l'eau des sites naturels varie fortement au cours d'une période de 24 heures. Dans le cas de *S. mansoni*, les chiffres de la densité cercarienne peuvent osciller entre 0 et 35 en l'espace d'une journée, avec une valeur maximale entre 10 et 16 heures. De même, les cercaires de *S. japonicum* sont plus abondantes pendant la nuit.

En épreuves de laboratoire, les cercaires de *S. mansoni* n'opposent qu'une faible résistance aux déplacements de l'eau et sont facilement entraînées par un léger courant. En milieu naturel, on observe la présence des cercaires dans l'eau de sites situés à 1700 m au moins de l'endroit d'émission. Certains prédateurs, comme *Lebistes*, interviennent activement pour réduire la densité des cercaires et contribuent à réduire l'intensité de la transmission.

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