The Production of Cercariae by *Schistosoma mansoni* and *S. haematobium* and Methods for Estimating the Numbers of Cercariae in Suspension

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The production of cercariae

Factors affecting the emergence of cercariae. There is general agreement that, at temperatures between 10°C and 30°C and possibly higher, light is the principal stimulus causing the release of cercariae of both *Schistosoma mansoni* and *S. haematobium.* However, cercariae are shed in smaller numbers in darkness, and periodic peaks of output occur even in the absence of light because of an innate rhythm. These characteristics were established by Schreiber & Schubert (1949a) and by Luttermoser (1955) in respect of *S. mansoni* cercariae, and by McClelland (unpublished data) for *S. haematobium*, and are shared by a variety of species of cercariae, including *S. bovis* (Lengy, 1962), *Cercaria elephantis* (Cort, 1922), *Cercaria limbifera* (Rees, 1931) and *Cercaria purpurae* (Rees, 1948). In all cases, elevated temperature has the supplementary effect of causing greater output than illumination alone but, within the range specified, temperature is of secondary importance, at least as far as *S. mansoni* and *S. haematobium* are concerned. Faust & Hoffman (1934) and Giovannola (1936) also considered light the more important stimulus causing the production of *S. mansoni* cercariae, and Bolwig (1955) agreed with regard to *S. haematobium* and noted the enhanced effect of elevated temperature and illumination simultaneously. While studying the development of *S. haematobium* in *Ferrissia tenuis*, Gadgil & Shah (1955), in India, concluded that light promoted the emergence of cercariae, but they also recorded that cercariae emerged at 27°C, but not if the temperature rose to 29°C, nor if it fell below 26°C. The cercariae were discharged intermittently, not on every day.

Barbosa, Coelho & Dobbin (1954) carried out a series of experiments in Brazil in which naturally and experimentally infected *Australorbis glabratus* were kept in aquaria in the open. The pattern of output of cercariae throughout the day was very clear-cut and corresponded to the curves for temperature and intensity of illumination. Large numbers of cercariae were produced from 9 a.m. onwards, and the output rose in each successive two-hour period to a peak at 3 p.m., then declined, and few cercariae emerged between 7 p.m. and 9 a.m. (The maximum temperature in this series was 37.5°C). These results are in close agreement with those of Faust & Hoffman (1934), Giovannola, (1936) Bolwig (1955) and McClelland (1961), and with the patterns of recovery of cercariae from natural waters in Puerto Rico recorded by Rowan (1958) and by Maldonado (1959), except that the peak of output recorded by Barbosa, Coelho & Dobbin (1954) was two to four hours later than other investigators have noted. However, during a hotter season (25°C-43°C), Barbosa and co-workers found that cercariae continued to emerge throughout the day and night although, if snails were moved inside the laboratory, the original pattern was resumed.

Gordon, Davey & Peaston (1934) and Kuntz (1947) were doubtful of the influence of light as a stimulus for emergence of cercariae, but their results are equivocal when compared with those obtained in more detailed later studies.

Under tropical conditions, daytime illumination is probably nearly always strong enough to stimulate emergence of cercariae, and when temperatures are very high, as in Brazil, this alone is sufficient stimulus for *S. mansoni.* Indeed, Maldonado (1959) noted that, under natural conditions in Puerto Rico, the recovery of cercariae from a pond was the same on dull days as on fine ones.

In the experience of almost all investigators, once a snail has started to produce cercariae, it does so whenever stimulated. The intermittent emergence of cercariae from *Ferrissia tenuis* and the extremely narrow temperature limits controlling their release have no parallel and other workers investigating *S. haematobium* (Gordon, Davey & Peaston, 1934; Bolwig, 1955; McClelland, unpublished data) did not observe such phenomena. Kuntz's (1947) results sug-

gest that if the stimulus is withdrawn after an hour the emergence of cercariae stops, since he was able to obtain cercariae by stimulating snails three times daily, but Duke (1952) recorded that once *Australorbis glabratus* had been stimulated to shed cercariae by a rise in temperature they continued to emerge after the temperature fell again to 18°C-20°C. (The duration of this observation was not recorded.)

Numbers of cercariae produced. Numbers vary greatly, both from snail to snail and from day to day, differences of several hundred from one day to the next are common (Schreiber & Schubert, 1949b; McClelland, 1961). In general, few cercariae are produced daily when a snail first becomes infected, then the number increases over a period of days or weeks until it reaches a more or less constant level, which is maintained until a few days before the termination of shedding by death or cure.

The output from *A. glabratus* is usually very high, often in the range 1000-3000 cercariae per snail per day or more. Faust & Hoffman (1934) found that snails shed only small numbers in the first few days, but the counts soon reached 200-1500 daily in the first month, then rose to 1000-7500 per day for periods of up to nine weeks, but thereafter dropped again in some cases to around 500 per day. Two snails exposed to one miracidium each produced, respectively, 125 000 and 210 000 cercariae in three-and-one-half months and were still shedding when counts were discontinued. Two others shed 93 000 in just over two months and 75 000 in slightly less than two months, and both then died. Giovannola (1936) recorded that from 8 to 5721 cercariae were produced by single *A. glabratus* in a day. Schreiber & Schubert (1949a) counted between 14 and 4158 cercariae from single snails, and the average output per snail per day of snails examined in groups over a period of time varied from 209 to 997. Later figures (Schreiber & Schubert, 1949b) for the total observed output of single snails varied from 16 759 to 37 568 and the output per day from 729 to 1444. The numbers of cercariae produced by individual snails examined by Barbosa and his co-workers were very high; the greatest daily output of a single snail was 17 600, and the average for all 12 examined was 4598.

The average output from *Biomphalaria* spp. is usually about 500 per snail per day or less. Gordon, Davey & Peaston (1934) obtained 50-1000 cercariae daily from *B. pfeifferi*. The daily output from *B. sudanica* varied between 27 and 1361, with an over-all mean of 529; 58% of all daily counts were less than 500 and 80% less than 1000 (McClelland, unpublished data). The mean daily output per snail of *B. angulosa* was 280, but individual daily counts ranged from 2 to 4168 (Sturrick, 1964).

The difference between *A. glabratus* and *Biomphalaria* spp. is probably due to the different sizes of the snails. Barreto & Barbosa (1959) demonstrated that the mean daily output of *A. glabratus* 13-16 mm. in diameter was more than double the figure for snails 7-10 mm in diameter, and Standen (1949) published figures for output per snail per day of 290 for *B. boissyi* (=*B. alexandrina*) and 380 for *A. glabratus*. Both these figures are low, and this may be because of his use of alginate food. Erickson & Caldwell (1961) observed that only one-half as many cercariae were shed by *A. glabratus* on alginate food as by snails given food consisting of the same ingredients except alginate, and that the output rose when snails fed on alginate were given the other diet.

Snail species which are poor intermediate hosts produce only small numbers of cercariae and remain infected for a short period (Coelho & Barbosa, 1956; Richards, 1963).

Figures for the output of *S. haematobium* cercariae are limited: up to 1500 from a single *Bulinus (Ph.) africanus* (Porter, 1920), and 50-400 from *B. (Ph.) globosus* (Gordon, Davey & Peaston, 1934). McClelland (unpublished data) determined the mean daily output per snail per day from *B. (Ph.) nasutus* as 1068, with a few individual daily counts over 2000, the highest being 6245. Thirty-four per cent. of daily counts were less than 500, and 75% less than 1000 cercariae. Malek (1959) obtained large numbers of cercariae from *B. (Ph.) ugandae* and *B. (B.) truncatus*, but *B. (B.) forskalii*, which is smaller and could be infected only with difficulty, produced a small number of cercariae. Smithers (1956) also obtained a small number from *B. forskalii*, and Cowper (1953) reported that the output of *B. (B.) cernicus*, another small species, was low. Wright (1963) obtained 50 cercariae or less per day from *B. (B.) beccarii*, which is about the same size as *B. forskalii*, but *B. (B.) reticulatus*, which is even smaller, produced up to 200 cercariae per day from the first day cercariae were shed. *Ferrissia tenuis* was reported to shed only four or five cercariae at a time (Gadgil & Shah, 1956).

There are conflicting views on whether the number of parasites harboured by a snail affects the number of cercariae produced. Cort (1922) dissected *Planorbis trivolvis*, producing markedly differing

numbers of *Cercaria elephantis*, and established a relationship between the numbers of cercariae discharged and the number of sporocysts in the snails. On the other hand, Byrd & Scofield (1954), who carried out a detailed investigation of four species of ochetosomatid in *Physa gyrina*, failed to establish any correlation between the number of cercariae released by snails and either the number of eggs to which the snails had been exposed or the number of daughter sporocysts which developed. Pesigan et al. (1958) recorded that the mean daily output of cercariae from *Oncomelania quadrasi* into which one *S. japonicum* miracidium had penetrated was nearly twice as high as the output from snails into which 2-5 miracidia had penetrated, and suggested that this was due to sporocyst-crowding, leading to slower development of cercariae, a view also favoured by Kendall (1949) in connexion with *Fasciola hepatica* and *Lymnaea truncatula*. McClelland (1965) noted that the mean output of *S. haematobium* cercariae per day from snails with infections that gave rise to bisexual infections in hamsters was higher than the figure for snails with unisexual infections, and that the difference was statistically highly significant. However, this does not necessarily support the view that multiple infections result in increased production of cercariae, because the incidence of bisexual infections (approximately 50%) was so high that up to 40% of the unisexual infections must also have been due to the development of two or more miracidia of the same sex.

As far as *S. mansoni* and *S. haematobium* are concerned, the number of cercariae produced by single-miracidium infections is so great that any increase due to multiple infections is probably unimportant and, in view of the equivocal results obtained to date, it is doubtful if the matter deserves special investigation, since this would inevitably be lengthy and detailed but might not be conclusive.

Duration of infection. Numerous authors have mentioned figures for duration of infection, but generalizations are extremely difficult because of variations in methods of maintaining snails and differences in frequency of handling, as well as because it is impossible to know when naturally infected snails became infected. Some of the available figures are presented in the accompanying table.

Infected *A. glabratus* often live for two months or more, but the normal duration of infections in other species is one month or less.

Usually infection is terminated by death, but a few *A. glabratus* and *Biomphalaria* become cured spontaneously, and this effect is more pronounced at low temperature, which also causes shorter duration of infection and a reduction in the number of cercariae produced soon after the peak of output has been attained (Stirewalt, 1954). There is no record of a cure of *Bulinus* infected with *S. haematobium*.

Fate of cercariae after emergence. One question that has been neglected concerns the fate of cercariae in natural waters. Although output figures appear to be spectacular, they must be considered in relation to the volumes of water involved in natural sites and injury due to the effects of physical and chemical agents.

Krakower (1940) investigated the effect of a number of these on *S. mansoni* cercariae in the laboratory by visually judging their behaviour and the number found swimming. Exposure to temperatures of 32°C and 34°C for up to eight hours did not affect activity or life-span, but heating to 36°C for one hour or more caused greater mortality at the end of 24 hours than among controls. Exposure to ultraviolet light for 20 minutes impaired activity to the same extent as exposure to strong sunlight at 34°C for half an hour, while exposure to ultraviolet light for 45 minutes killed all cercariae outright, and exposure to strong sunlight for one hour killed nearly all. Even on a relatively cloudy afternoon, the cercariae were severely injured and many were dead one hour after exposure to sunlight.

The effect of ultraviolet light on the infective capacity of cercariae is probably more rapid, since cercariae can survive 24 hours in water in the laboratory, but only a small proportion continue to be infective. In this connexion, the figures for output of cercariae from *A. glabratus* calculated by filtering pond water (Maldonado, 1959) are low, compared with figures obtained in the laboratory, and the life-span of the cercariae was calculated at only about 10 hours.

There appears to be scope for more work on the effects of ultraviolet light and high temperatures on the infectivity of cercariae, particularly of *S. mansoni*, which are concentrated near the water surface. Cercariae of the strain of *S. haematobium* around Mwanza, Tanzania, are distributed fairly evenly in the water, with some tendency to concentrate at the bottom, which may protect them from irradiation in the turbid waters of the area. Not all strains exhibit this characteristic; Bolwig (1955) referred to upward-swimming movements of a South African strain, with resultant concentration at the surface.

SUMMARY OF DATA ON DURATION OF *SCHISTOSOMA MANSONI* AND *S. HAEMATOBIUM* INFECTIONS IN SNAILS

Host	Usual duration of infection[a]	Longest duration of infection	Authors
Australorbis glabratus *Australorbis olivaceus*	—	78 days	Lampe (1927)
Biomphalaria alexandrina	—	58 days	Archibald & Marshall (1932b)
Australorbis glabratus	50-98 days	98 days	Faust & Hoffman (1934)
Australorbis glabratus	2 months	—	Schreiber & Schubert (1949b)
Biomphalaria pfeifferi	28-42 days	90 days	Gordon, Davey & Peaston (1934)
Australorbis glabratus	—	7 months	Newton (1953)
Australorbis glabratus	3 months	over 1 year	Stirewalt (1954)
Australorbis glabratus	40 days	135 days	Barbosa, Coelho & Dobbin (1954)
Biomphalaria pfeifferi	up to 30 days	103 days	Teesdale (1962)
Biomphalaria sudanica	10-20 days	29 days	McClelland (Unpublished data)
Biomphalaria angulosa	1-73 days	156 days	Sturrock (1965)
Bulinus truncatus	10-14 days	4 ½ months	Archibald & Marshall (1932a); Archibald (1933)
Bulinus cernicus	8-9 days	53 days	Cowper (1953)
Bulinus africanus	14-21 days	—	Edwards & McCullough (1954)
Ferrissia tenuis	—	4 weeks	Gadgil & Shah (1955)
Bulinus ugandae	—	over 2 months	Malek (1959)
Bulinus truncatus	—	over 2 months	
Bulinus forskalii	3-12 days	12 days	
Bulinus africanus	less than	49 days	Teesdale (1962)
Bulinus globosus	30 days	45 days	
Bulinus forskalii	less than 9 days	9 days	
Bulinus beccari	—	21 days	Wright (1963)
Bulinus reticulatus	—	21 days	
Bulinus nasutus	1-26 days	63 days	McClelland (Unpublished data)

[a] — = no observation.

Estimation of numbers of cercariae

Methods of counting cercariae fall into three main groups, corresponding to the main purposes for which the information is required:

(*a*) assessment of the number of cercariae in natural waters under field conditions;

(*b*) determining the total number in a suspension of small volume in the laboratory;

(*c*) delivery of a known number from a suspension for infecting animals.

All the methods applicable to (*b*) and (*c*) have involved counting all the cercariae present or, more usually, the number in aliquots taken from the suspension. Different methods of taking aliquots and of counting the cercariae in them have been used, often with no indication of the accuracy of the prediction.

Estimation of cercariae in natural waters. Rowan (1957, 1958) devised apparatus consisting of a pressure filtration chamber made by drilling holes in the bottom of a pressure cooker. Water was drawn in by a hand-operated pump and forced

through a filter-paper (grade S & S 404) placed over the holes and into a large container below. After the required volume had been passed, the filter paper, which trapped cercariae, silt, zooplankton and other suspended matter, was transferred to a shallow pan containing 5 ml of 0.5% ninhydrin solution and heated until dry. In the laboratory, the papers were saturated with xylene containing excess sodium bicarbonate and examined with a dissecting microscope, and *S. mansoni* cercariae, which were stained blue and red, were counted. The degree of efficiency and consistency with which the apparatus trapped cercariae was established experimentally; 64%±14% of cercariae in the water were recovered, but only 45%±8% were oriented well enough to be clearly recognizable. When water contained less than two ppm of suspended silt, 20-30 US gallons (77-116 litres) could be pumped through one paper but, when there was 15 ppm suspended matter, only five US gallons (20 litres) could be pumped, and more turbid waters (25-250 ppm) had first to be treated with alum before filtration, which reduced the recovery of cercariae to 37%±7% (24%±8% well oriented). Maldonado (1959) recovered 76.5%± 21.3 % of cercariae with the same type of equipment.

The apparatus has been tested in two places in Tanzania (Mwanza and Amani) without success. In the Mwanza area, many natural waters are very turbid, and this caused almost immediate blockage and disintegration of the filter paper. Alum flocculation was not satisfactory, apparently because all of the cercariae were trapped in the floc.

At Amani, two other methods have been investigated. One involved preliminary filtration through a stainless steel mesh with effective pore size of 23 μ, which removed much suspended matter but allowed *S. haematobium* cercariae to pass through to be filtered on nylon fabric on a ceramic support (Raybould, 1962). Details of the other method are not available. Trials have been discontinued.

Pesigan et al. (1958) skimmed the surface of the water in a stream and in still water and obtained 1-litre samples that were passed through a millepore filter that detected a density of one cercaria per litre. The method would appear unsuitable for use with turbid water or when cercariae are evenly distributed through a water-body.

Klock (1961) devised a method that involved concentrating cercariae by passing water through a chamber with illuminated glass cones in the top into which the cercariae rose. The apparatus requires an electrical power supply, is too complicated for wide field use, and is evidently only suitable for use in flowing water. Since it depends on phototropism in cercariae, it would not be suitable for use in turbid water or in areas where cercariae do not exhibit this characteristic.

One is obliged to conclude that no filtration method yet devised is applicable to conditions in many parts of Africa.

Estimation of total numbers in a suspension of cercariae from one or more snails. Faust & Hoffman (1934) isolated individual snails in an unspecified volume of water and then added acetic or hydrochloric acid to the suspension to kill the cercariae. The container was shaken well, and an unspecified number of samples of unspecified volume taken and the cercariae in them counted. An accuracy of 10% was claimed. Giovannola (1936) placed snails in 98 ml of water and, after removing them, added 2 ml of 5% iodine in 70% alcohol to stain the cercariae, then took two or three l-ml samples after shaking the suspension. Krakower, Hoffman & Axtmayer (1940) used suspensions of about 2-litres' capacity containing cercariae from many snails. After stirring the suspension, they took two or three 1.5-ml samples, killed the cercariae with hydrochloric acid, and counted them in a watch-glass marked off in squares. The mean number of cercariae per millilitre was calculated, and so the volume containing the number of cercariae required to infect animals was determined. As a check, the cercariae in an extra sample taken in the same way as those used for infection were counted. The counting error was ± 10%, but was later reduced to less than 5%. The principal source of error was thought to be adherence of cercariae to the apparatus. Barbosa, Coelho & Dobbin (1954) took samples of 10% of the total volume of five litres. Abdel Azim & Cowper took a 5-ml sample from a suspension of unspecified volume, placed it in a circular glass dish with the bottom marked off in quadrants to facilitate counting, and added a few drops of Bouin's fluid to kill and stain the cercariae. Luttermoser (1955) used a similar method and counted the cercariae in three 5-ml aliquots drawn from a suspension of unspecified total volume.

McClelland (1961) modified Rowan's technique by filtering the suspension of cercariae produced by a single snail through a Whatman No. 1 filter-paper 4.25 cm in diameter, supported on a sintered glass plate on a filter funnel connected to a water-pump. Hot water was used for rinsing the glassware, as both

formol and acetic acid interfere with the ninhydrin staining reaction. A sampling method was devised to take a 2.2-ml sample from 10 ml of suspension while it was being agitated.

None of these methods is very accurate, particularly when the volume of suspension is large, but this is rarely of importance because the wide natural variations in cercarial output and the time-consuming nature of total count techniques do not normally justify their use. Accuracy is usually required only when animals are being exposed to cercariae under controlled conditions. The method outlined in the next section affords a reasonable standard of accuracy in such circumstances.

Delivery of a known number of cercariae from a suspension. Schubert (1948) devised a method by which he agitated a heavy suspension of 60-ml volume and then drew off 4 ml into a 10-ml syringe. The contents of the syringe were frequently agitated while drops were expelled, and the cercariae in each drop counted. The total number in 0.5 ml was counted in this way. The standard deviation was 15% of the mean number per ml.

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Seasonal Variations of Cercarial Output from *Biomphalaria pfeifferi* and *Bulinus (Physopsis) globosus* in a Natural Habitat in Southern Rhodesia

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Pesigan et al. [a] in the Philippines and Pitchford & Visser [b, c] in South Africa have described the exposure of mice to bilharziasis by immersion in natural waters. From the lack of further information on this subject in the literature, it would appear that little further investigation has been carried out to determine any seasonal variations of cercarial output from infected snails. It was therefore decided that an attempt be made to discover what seasonal variations, if any, exist in the cercarial density of natural water-bodies.

A suitable site, 4 miles (7 km) east of Salisbury, Southern Rhodesia, on a small stream flowing into the Makabusi River, was chosen from which to collect a total of 100 snails on the same day of every alternate week, at approximately the same time of day and from the same place, for an entire year (April 1963 to March 1964). This particular site was selected because of its large snail population and the considerable human contact with the water.

The stream is fed from a fairly large catchment of a spongy nature and from a water reservoir, seepage from which keeps the stream barely flowing throughout the long, dry winter season.

The site of the sampling was a number of pools through which the water moves slowly, being held up by a series of rocky shelves, the stream trickling over bare granite surfaces into sedge-lined pools about 4-6 feet (1-2 m) wide and of the same length. It was estimated that the dry-weather water-flow was approximately 0.25 ft³/sec (0.0762 m³/sec).

Within about 150 m of the site there is a large housing area used as transit accommodation for African labourers and their dependants going to and from Malawi, Zambia, and the Johannesburg gold mines. At this point, the stream is much used by the women for washing clothes and for personal ablution. It is obvious, from the state of the ground and of the vegetation on either bank of the stream, that the whole site is used as an outdoor latrine, particularly by children. The stream is perennial, with the vegetation on the banks and in the river-bed being fairly profuse and dense but not more than three feet (one metre) in height.

Materials and methods

A total of 100 snails, or as near to that number as could be found, was collected of *Biomphalaria pfeifferi* and *Bulinus (Physopsis) globosus*, in as near to equal numbers as possible, and placed in a polyethylene bag containing a little water. At the laboratory, they were transferred to a glass aquarium 12 inches (20.5 cm) in diameter and 6 inches (10.25 cm) high, containing matured water.[d] A few pieces of dried lettuce, prepared according to the methods of Claugher,[d] were offered as food.

On the following day, at about 10 a.m., the snails were removed from the tank and placed individually into 3-inch × 1-inch (75-mm × 25-mm) glass specimen tubes, each tube containing 10 ml of matured water. These tubes were then exposed to intense illumination for two hours, after which time the

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[b] Pitchford, R. J. & Visser, P. S. (1962) *Trans. roy. Soc. trop. Med. Hyg.*, **56**, 126.
[c] Pitchford, R. J. & Visser, P. S. (1962) *Trans. roy. Soc. trop. Med. Hyg.*, **56**, 294.
[d] Claugher, D. (1960) *Ann. trop. Med. Parasit.*, **54**, 333.