THE EFFECT OF TEMPERATURE ON THE RATE OF DEVELOPMENT AND EMERGENCE OF SCHISTOSOME CERCARIAE

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It has long been known (Porter 1938) that the distribution of the intermediate hosts of Bilharzia in South Africa is more extensive than is that of the disease, especially in the higher, colder parts of the country. It is thus strange that the conclusion reached by Stirewalt (1954) should have been allowed to lie dormant. She showed that after several sub-passages in *Australorbis* kept at low temperatures, sporocysts began to produce cercariae which had poor penetrative ability and often failed to mature. Since there are no data on cercarial emergence for South African strains similar to those of Kuntz (1947) in America, Standen (1952) in England and many others subsequently, it was thought advisable to become familiar with the factors influencing the "natural rhythm" of cercarial emergence before any study of long term temperature effects was undertaken. Such basic information should reveal the factors underlying the marked diurnal periodicity of cercariae reported by Rowan (1958) from Puerto Rico and noted locally in the field during rodent immersion experiments (Pitchford & Visser 1965). It is also essential for the regulation of cercarial emergence in laboratory investigations. The hope that emergence behaviour might prove to be sufficiently characteristic to form a basis for the identification of cercariae shed by wild snails was not realized. Rowan's buffer staining technique (1961) remains the only available method of differentiation.

MATERIAL AND METHOD

Local strains of *S. mansoni, haematobium* and *mattheei* were used to infect the local intermediate hosts, *Biomphalaria pfeifferi* and *Bulinus* (*Physopsis*) *globosus* with 5 miracidia per snail. The snails were cultured under conditions similar to those described in a previous paper (Frank 1963). During the experimental period groups of 5 snails were kept in 2 litre conical Fernbach flasks. They were fed all the dehydrated lettuce and lucerne they could consume. The medium used throughout was conditioned tap water, 500 ml per flask, changed at least once a week but more often in the period during which cercarial counts were being made. As Kuntz (1947) had reported that changes in the chemical nature of the water had no effect on the number of cercariae emerging from *Australorbis* no attempt was made to control the dissolved salt content of the medium; it was classified as a soft water (see Schutte and Frank 1964). The culture flasks were placed in constant temperature baths ($\pm$ 0·2C). Lighting consisted of daylight supplemented occasionally by fluorescent lighting; there was thus approximately 10 hours darkness in any 24 hour period.

To arrive at an accurate estimate of the number of cercariae produced by a group of snails in any particular time interval, the snails were rinsed and put in fresh medium (the flask and

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medium were always allowed to come to thermal equilibrium with that of the water-bath before the snails were transferred). No food was given during the test period if the time interval was short. At the conclusion of the test period the snails were removed from the flask, rinsed, the wash-water being added to that in the flask and the contents poured through coarse bolting silk to remove old food and faeces. At each transfer the snails, glassware and silk were thoroughly rinsed with a jet from a wash-bottle to ensure that as far as possible only those cercariae shed during the test period were collected and that none were left adhering to the snails or glassware. The entire contents of the flask plus wash-water was then passed, with the assistance of partial vacuum, through a No. 1 Whatman filter paper, or its equivalent, supported in a Buchner funnel. Thereafter, the damp filter paper was placed in a shallow dish with 1 ml 0·5% ninhydrin in aqueous solution and dried over a water-bath after the method of Rowan (1957). With this procedure the cercariae are sufficiently stained to be visible at low magnifications and the method has the advantage that the papers may be kept for future reference.

Experiment showed that when there were very large numbers of cercariae in the flask it was more convenient and accurate to count only a random sector of the filter paper rather than to filter an aliquot. A few tests on random sectors showed that variation was still well within experimental error. Further tests used to estimate the accuracy of the procedure are briefly commented on below.

(*a*) Six, 100 ml samples of a cercarial suspension were each filtered separately under exactly the same conditions and gave a mean count of 2,160 with an S.D. of 31. The filtrate was then heated to kill those cercariae which had penetrated the filter paper and filtered once more. It was found that 0·12% had succeeded in penetrating the paper. Several more tests of a similar nature were performed; these showed that, using a Buchner funnel with moderate suction to hasten filtration seldom more than 0·1% of the total number of cercariae escaped through the filter paper. However, when filtration was done in an ordinary conical funnel by gravity as much as 0·7% passed through the filter paper.

(*b*) Low pressures (approx. 24 in. Hg) were found to destroy the cercariae. In three test runs an average of 55 per cent of the cercariae disappeared when full procedure was followed with the filter paper omitted and the "filtrate" briefly exposed to the lowest pressure obtainable with an ordinary metal vacuum water pump. It is thus essential to employ uniform procedure if reproducible results are to be obtained.

TEMPORARY DEPRESSION OF THE TEMPERATURE

The intermediate hosts used in this experiment came from eggs laid by a single *Bulinus* (*Physopsis*) *globosus*. No special technique was used to raise the spat of which 60 per cent were still alive after 5 weeks when they were infected with *S. mattheei*. The exposed snails were kept in groups of 5 per flask with 4 flasks to an environmental condition. After 2½ weeks the snails were isolated twice a week in test tubes to determine the onset of cercarial emergence and infection rate. Results may thus have a 3 to 4 day error in timing. No cercarial counts were made in this preliminary test.

The first group of 4 flasks (20 snails) was maintained at 27C throughout and served as a control. These snails were found to be shedding cercariae 25 days after infection. Subsequentlv

they were examined twice a week on 10 separate occasions. Over this period 83 per cent of the snails shed cercariae. Egg counts showed that during the prepatent period the highest production was 496 eggs per 20 snails per week, but that during cercarial emission this dropped to 76 eggs, representing an 84 per cent drop in fecundity.

On the day after infection the other 3 groups (5 snails to a flask; 4 replicates to a condition) were gradually cooled down from 27C to 13C over a period of 4 days. These were kept at this temperature for 4, 8 and 12 days respectively and then returned to 27C for the rest of the

TABLE 1

THE EFFECT OF REDUCING THE TEMPERATURE OF CULTURES OF INFECTED *B.* (*Phys.*) *globosus* FROM 27C TO 13C FOR A LIMITED PERIOD ON THE DEVELOPMENT OF *S. mattheei* SPOROCYSTS AND INFECTION RATE IN THE SNAILS

Period for which temperature was reduced to 13C (in days)	0	4	8	21
Length of prepatent period (in days)	25	29	32	46
Number of prepatent days at 27C	25	25	24	25
Rate of infection (10 determinations)	83%	83%	86%	78%
Peak fecundity during prepatent period (eggs/20 snails/week)	496	141	51	352
Peak fecundity during cercarial emission (eggs/20 snails/week)	76	10	84	127
Percentage drop in fecundity	85%	93%	—	64%

experimental period. In all other respects their treatment was identical to that of the control group. The effect of this temperature reduction on the speed of development of the sporocysts and the fecundity of the snails is summarized in Table 1.

An experiment, similar to the one above, but using *Biomphalaria pfeifferi* infected with *S. mansoni* at six weeks was run concurrently. The minimum temperature in this case was, however, 15C and there was no control group. The results are shown in Table 2.

Conclusions

It would appear that in both cases sporocyst development ceased for the period that the snails were held at the lower temperature but neither their patency nor the speed of development of the sporocysts thereafter was affected. The duration of the exposure to low temperature seemed to have no noticeable effect on the infection rate of the snails which was uniformly high in the case of *B.* (*Phys.*) *globosus* infected with *S. mattheei* but considerably lower in *Biomphalaria pfeifferi* infected with *S. mansoni*. Except for the group of snails kept for 8 days at 13C which in any case laid very few eggs there was a very marked drop in the fecundity of *Physopsis* after the prepatent period. The fecundity of *Biomphalaria* was low throughout the experiment, actually increasing after the cercariae started to emerge. Unlike *Physopsis* probably only a

small percentage of these (despite being a week older) had reached full sexual maturity during the prepatent period since *Biomphalaria* does not respond as rapidly as *Physopsis* to higher temperatures (Shiff 1964; Shiff and Garnett 1965).

TABLE 2

THE EFFECT OF REDUCING THE TEMPERATURE OF CULTURES OF INFECTED *Biomphalaria pfeifferi* FROM 27C TO 15C FOR A LIMITED PERIOD ON THE DEVELOPMENT OF *S. mansoni* SPOROCYSTS AND INFECTION RATE IN THE SNAILS

Period for which temperature was reduced to 15C (in days)	3	7	14	21
Length of prepatent period (in days)	27	31	38	45
Number of prepatent days at 27C	24	24	24	24
Rate of infection (10 determinations)	19%	16%	42%	28%
Peak fecundity during prepatent period (eggs/20 snails/week)	14	3	78	16
Peak fecundity during cercarial emission (eggs/20 snails/week)	28	31	28	108
Percentage rise in fecundity	50%	90%	—	85%

CERCARIAL EMISSION AT CONSTANT TEMPERATURES

Eggs from a single *B.* (*Phys.*) *globosus* were used to provide young snails for infection. They were cultured at 27C until infected at approximately 7 weeks with *S. mattheei*. Temperature reduction was started on the day after infection and was spread over 2 days. In this experiment 4 separate groups with 8 flasks in each group were held at 27C, 23C, 19C and 15C respectively. There were, as usual, 5 snails to a flask and thus 40 snails exposed to each condition. The results of 270 cercarial counts are summarized in Table 3. As only weekly estimates were done there may be an error of up to 6 days in the timing of any result. These are based on a four-hour period from 8 a.m. to 12 noon. Snails were tested collectively in groups of 5 and the results expressed as the number of cercariae produced by such a group in 4 hours.

In view of the far reaching effects population pressure has on other aspects of snail biology (Chernin and Michelson 1957) it was thought essential during the course of the experiment to keep the number of snails in each flask constant. Thus, as the snails died off during the experiment(see Table 3), the survivors had to be moved together and, especially towards the end of the experiment were perforce not in the same group as they were in the beginning. Weekly cercarial counts were also only done on flasks which had contained 5 snails during that week. The results are consequently not equally accurate and the effects of mortality on total emission are entirely obscured in Fig. 1.

Conclusions

The results of this experiment confirm Stirewalt's conclusion (1954) that during the first parasite passage at reduced temperature the absolute number of *S. mansoni* cercariae produced by a snail is almost constant and, within limits, independent of the temperature. Thus, the

TABLE 3

THE EFFECT OF CONSTANT MAINTENANCE TEMPERATURES ON THE FECUNDITY AND MORTALITY OF *B.* (*Phys.*) *globosus* INFECTED WITH *S. mattheei* AND ON THE RATE OF DEVELOPMENT OF THE LATTER

Maintenance temperature	27C	23C	19C	15C
Length of prepatent period (in days)	24	25	42	—
Period from infection to peak cercarial emission (in days)	28	49	84	—
Total output of 5 surviving snails/4 hrs/day over 50 days max. output	93,450	105,800	115,800	56
Maximum cercarial emission on one day by 5 snails in 4 hrs.	2,649	4,003	3,500	7
Peak fecundity during prepatent period (eggs/20 snails/week)	146	167	98	22
Peak fecundity during cercarial emission (eggs/20 snails/week)	15	36	19	7
Percentage drop in fecundity	90%	78%	79%	68%
Percentage original snails left after 50 days	15%	20%	25%	85%

snails cultured at 27C produced only 20 percent fewer cercariae than did those cultured at 19C, but produced them in far less time. However, below 19C the production rate was severely curtailed, at 15C it was only 0·05 percent of that of snails at 19C. When represented graphically (Fig. 1.) the onset of shedding is marked by a peak, whose height seems to depend on the temperature. The greatest number of cercariae produced by any flask of 5 snails in one day was shed by snails maintained at 23C, but over a long term (50 days) those flasks maintained at 19C produced more cercariae than any other group. The optimum temperature for the development of *S. mattheei* sporocysts in *B.* (*Phys.*) *globosus* under the conditions described above would thus appear to lie somewhere between 19C and 23C. The number of cercariae produced daily by these snails under optimum conditions and peak emission is very similar to the average production of *S. mansoni* cercariae by *A. glabratus* reported by Schreiber and Schubert (1949). Under their conditions (unfortunately they do not state their maintenance temperature) the number of infected snails (23 percent) surviving for more than 56 days is also of the same order.

The length of the prepatent period in snails living outside at the same mean but ambient temperature (unpublished data) was similar especially in the higher temperature range. The discrepancies between results for low constant temperatures and similar low ambient temperatures is probably due to the fact that low mean temperatures in the Eastern Transvaal Lowveld are invariably the result of a combination of cold nights, far below 15C (when sporocyst development ceases), coupled with high day temperatures (when development must be rapid). This results in sporocyst development in the colder months which is far more rapid than superficial examination of mean ambient temperatures, calculated from simple maximum-minimum readings would lead one to expect from results in the laboratory.

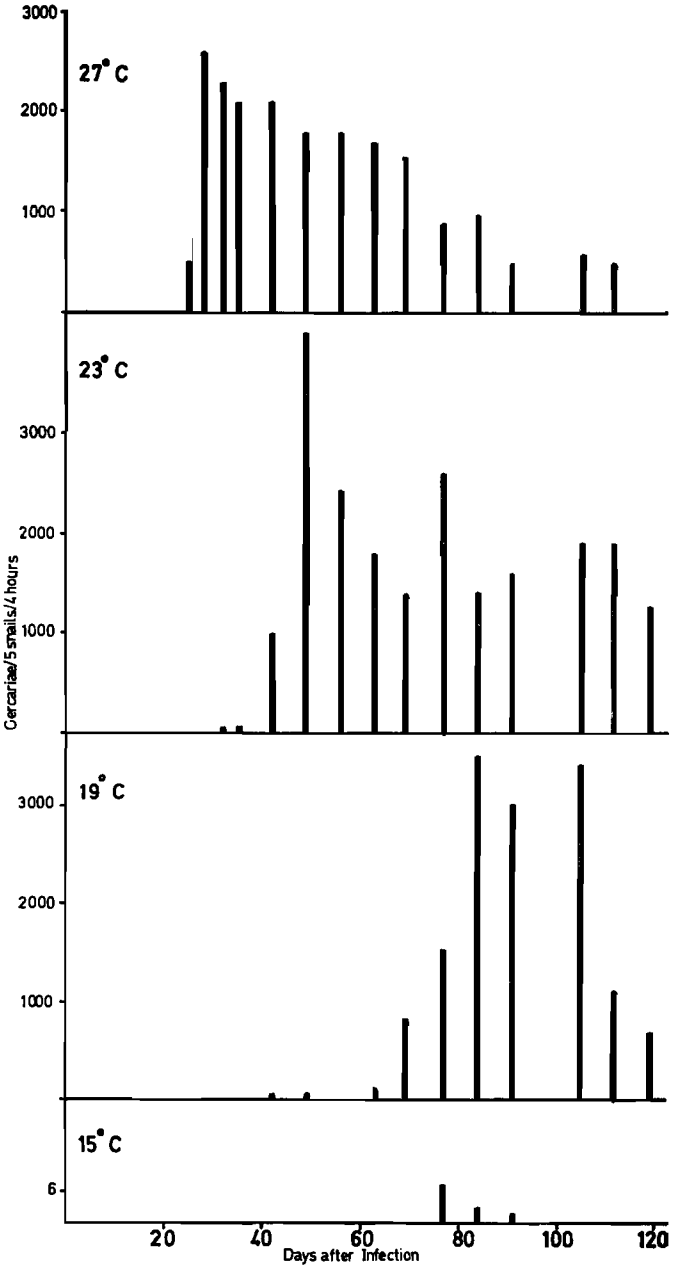

Figure 1. Comparative histograms depicting the effect of different constant temperatures on the length of the prepatent period and the number of *S. mattheei* cercariae produced by *B. (Phys.) globosus.*

Again in this experiment the end of the prepatent period was marked by a sharp drop in fecundity, most marked at the higher temperatures. After 50 days the fecundity of the few survivors gradually improved but did not seem to be related to cercarial emission in them.

TEMPERATURE AND DIURNAL PERIODICITY

Two groups of *B.* (*Phys.*) *globosus* and one of *B. pfeifferi* infected at approximately 6 weeks with *S. haematobium, mattheei* and *mansoni* respectively were subsequently exposed, in open aquaria outside, to the normal diurnal temperature fluctuations occurring in the spring in the Eastern Transvaal Lowveld. Three well-developed adult snails actively shedding cercariae, were selected from each of the *S. haematobium* and *S. mattheei* groups and 2 from the *S. mansoni* group. The 8 snails were placed individually in Fernbach culture flasks and treated in much the same way as the snails in the previous experiments. As these had shown that, for *S. mattheei*, the optimum probably lay between 19C and 23C and that sporocyst development was sharply curtailed below the former temperature, experimental fluctuation was limited to temperatures from 12C to 23C.

All cercariae shed by the snails were collected and counted at regular 4 hourly intervals except in the middle of the experiment when only a single count was made after 48 hours. With the one exception mentioned, the medium was thus changed at relatively short intervals, and it is possible that the generally disturbed culture conditions could have affected the maturation of the sporocysts. Schreiber and Schubert (1949) found that the liberation of *S. mansoni* cercariae by *A. glabratus* was generally higher after a rest period during which the snails were not stimulated to liberate cercariae. The snails were therefore exposed to experimental conditions for only 12 days.

The eight snails, though in separate culture flasks, were exposed as far as possible to identical conditions throughout the experiment. Initially they were held for 72 hours at 23C. This was followed by a diurnal temperature rhythm with "cold nights" and "hot days" for another 72 hours. During daylight they were maintained at 23C but on the first night they were cooled to 18C, the second to 20C and on the third night to 22C to see if reduction in temperature fluctuation would disorganize the rhythm of cercarial emission. The snails were then held at 18C for 48 hours and immediately thereafter brought to 23C during the hours of darkness, thus inverting their usual "hot day–cold night" rhythm. Finally, they were exposed to a rhythmic 5 degree diurnal temperature fluctuation but with both the maximum (day) and minimum (night) temperatures reduced by 2 degrees in each successive 24 hour period.

Results

The data are presented as comparative histograms in Fig. 2.

(*a*) *Normal "hot day–cold night" diurnal rhythm* (*22nd to 24th and 27th to 31st Oct.*)

S. haematobium seldom liberated cercariae during the 6 p.m. to 6 a.m. period.

S. mattheei sporocysts released cercariae almost continuously but by far the majority from 6 a.m. to 6 p.m.

S. mansoni, like *S. haematobium* produced very few cercariae from 6 p.m. to 6 a.m.

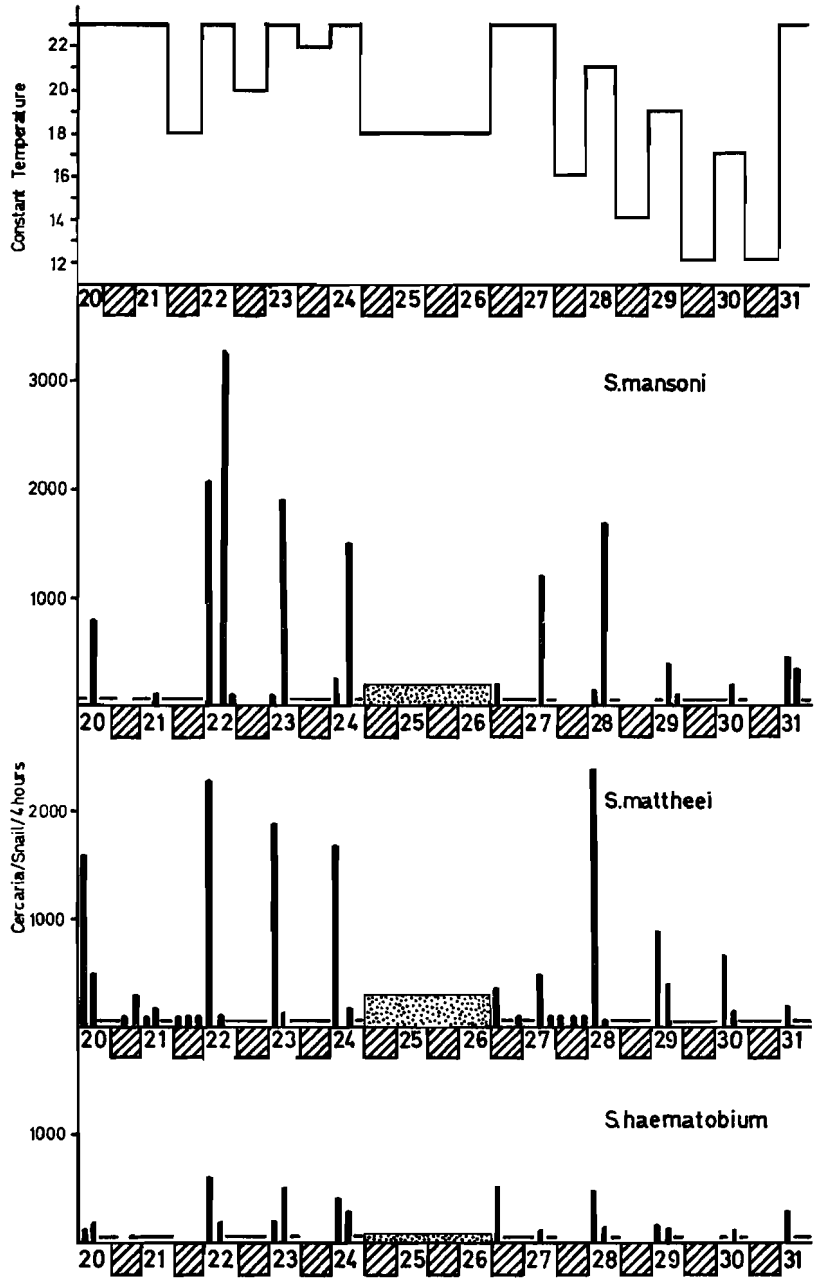

Figure 2. The short term effect of diurnal temperature fluctuation on the time of emergence and rate of production of *S. mansoni, mattheei* and *haematobium* cercariae from *B. pfeifferi* and *B.* (*Phys.*) *globosus*. The thin horizontal line represents less than 100 cercariae per snail per four hours, the shaded blocks 12 hours darkness and the number, the date in October.

(*b*) *Constant high temperature* (*e.g.* 20*th to* 21*st Oct.*)

S. haematobium sporocysts cercaria production (when the diurnal rhythm of hot and cold was replaced by a constant temperature of 23C) dropped from 200 cercariae per snail per 4 hours to 7 and the difference between day and night emission was less marked.

S. mattheei while still showing some diurnal rhythm, began to liberate cercariae in a more uniform stream which fell off slowly (1,600 cercariae/snail/4 hours to 320 cercariae/snail/4 hours).

S. mansoni, under the same conditions liberated fewer cercariae than usual but the diurnal rhythm was maintained.

(*c*) *Inversion of diurnal rhythm; "cold day–hot night"* (25*th*, 26*th and* 27*th October.*)

When the temperature cycle was upset by subjecting the snails continuously to low temperature (18C) for 48 hours followed immediately by a "hot night and a hot day" at 23C, all sporocysts produced more cercariae during the hours of darkness (26th to 27th Oct.) than would have been the case under the more usual regimen.

S. haematobium sporocysts reversed their cycle almost completely under these conditions producing approximately as many cercariae during the 6 p.m. to 6 a.m. period as at any other time during the experiment. However, during the succeeding daylight hours which, in this case, were also "hot" they continued to produce fair numbers of cercariae. The usual cycle was rapidly re-established when they were returned to a "hot day—cold night" diurnal rhythm.

S. mattheei sporocysts under the same conditions again showed that when the "normal" diurnal rhythm is upset they tend to produce less cercariae but more evenly there being less difference between the numbers shed at night and those shed during the day than was the case with the other two schistosomes.

S. mansoni was the least affected by this reversal of the temperature cycle, showing low cercarial emission on the first "hot night" followed by a relatively high rate during the daylight hours which followed.

(*d*) *Diurnal temperature fluctuation of less than* 5 *degrees* (22*nd*, 23*rd and* 24*th Oct.*)

Under these conditions all schistosome sporocysts tended to release fewer cercariae than usual during daylight but again only *S. mattheei* sporocysts released appreciable numbers during the relatively "hot" night of the 22nd to 23rd of Oct., (*S. mattheei:* 54 cercariae, *S. haematobium:* 3 cercariae, *S. mansoni:* 0 cercariae).

(*e*) *Overall depression of temperature* (27*th to* 30*th Oct.*)

With a diurnal temperature fluctuation of 5C but with a 2 degree drop in the maximum and minimum temperature on each successive day all three schistosomes produced fewer cercariae at the lower temperatures. *S. mattheei* sporocysts, because they tended to lose their diurnal cycle easily, showed the poorest recovery when high temperature (23C) was resumed during daylight.

Conclusions

Bearing in mind that these results are based on observations over a comparatively short period it would seem that all three schistosomes depend to a greater or lesser extent on a diurnal temperature fluctuation in order to synchronise the release of their cercariae. However,

distinction must be kept between the short term and long term effects of light and heat. When a steady rhythm of light and heat is maintained, cercarial emergence may be induced by a sudden change in heating or lighting (Kuntz 1947), but when subjected to uniform conditions the exact rhythm of heat and light to which the infected snails had previously been exposed probably determines when exactly emission will take place.

Thus *S. haematobium* appeared to be the schistosome most heavily dependent on this temperature cycle, while *S. mansoni* appeared the least dependent. When diurnal temperature rhythm was normal i.e. when a "cold night" was succeeded by a "hot day", *S. haematobium* released most of its cercariae in the period 6 a.m. to 10 a.m. (6 out of 8 tests), *S. mattheei* also had a maximum at this time (8 out of 8 tests) but the peak for *S. mansoni* was later, occurring in the period 10 a.m. to 2 p.m. (6 out of 8 tests). Unfortunately Schreiber and Schubert's (1949) results for *S. mansoni* are based on different time intervals and are thus not easily comparable with these. They report a greater average number of cercariae per snail per hour for the period 9.30 a.m. to 11 a.m. than for the period 11 a.m. to 3 p.m. This discrepancy can only be resolved if we assume that peak cercarial emission actually occurs only between 10 and 11 a.m. which seems improbable.

On the other hand when the temperature cycle was inverted, of the three schistosomes, *S. mansoni* was the least affected. Despite high temperatures during the hours of darkness cercarial emission was delayed until the illumination had also increased. The converse was reported by Faust and Hoffman (1934) viz., that on dark days little emergence of *S. mansoni* cercariae occurred, but Kuntz (1947) found that with the combined effect of increased light and elevated temperature the snails yielded only a few more cercariae than when exposed to increased temperature alone. It would be interesting to see whether *S. mansoni*, like *S. douthitti* (Olivier 1951) can be made to invert its cycle by an alteration in the succession of light and dark.

SUMMARY

The optimum for *Schistosoma mattheei* sporocysts in *Bulinus* (*Physopsis*) *globosus* at constant temperature lies between 19C and 23C. Development virtually ceases when the sporocysts of *S. mattheei* in *B.* (*Phys.*) *globosus* and *S. mansoni* in *Biomphalaria pfeifferi* are maintained for short periods at 13C and 15C respectively. However, the lowered temperature seems to have no effect on subsequent development or rate of infection. The sporocysts of *S. mansoni*, *haematobium* and *mattheei* all show a reduced cercarial output when maintained at a constant temperature. A regular diurnal fluctuation seems to be necessary for optimum cercarial output. Maximum cercarial emergence under normal conditions occurs between 6 and 10 a.m. in *S. haematobium* and *mattheei* and 10 a.m. and 2 p.m. in *S. mansoni*. The emergence of the latter seems to be influenced by light.

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. R. Elsdon-Dew for his helpful criticism in the preparation of this paper.

This paper is published with the permission of the South African Council for Scientific and Industrial Research.

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