

**SOME QUANTITATIVE DATA ON THE LABORATORY AND FIELD  
INFECTION OF *BIOMPHALARIA GLABRATA* FROM JABUTICATUBAS  
(STATE OF MINAS GERAIS, BRASIL) BY MIRACIDA OF  
*SCHISTOSOMA MANSONI***

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**S U M M A R Y**

On the strength of a series of laboratory and field data the Authors have attempted to outline some of the quantitative inter-relationships operating in the cycle of Schistosomiasis *mansoni*. The tentative conclusions supplied by the present paper are meant to apply strictly to one particular focus, namely an area in the town of Jabuticatubas (Minas Gerais, Brazil).

It was found that *B. glabrata* infected with *S. mansoni* will shed for an average of 7 days, and that total production of cercariae per snail is of the order of 16,000.

Miracidia exhibit a well-developed scanning power towards the intermediary host, even in large bodies of water. During one field experiment, undertaken in a drainage ditch 57 feet in length and with a current of 0.2 feet/sec, 19.9% of the miracidia succeeded in penetrating a snail. Laboratory experiments have shown that a rising miracidia: snail ratio will increase the infection rate, although a smaller proportion of the miracidia manage to penetrate a host.

On the other hand, no evidence of any taxis or tropism was seen in cercariae, especially in still water, where chance encounters seem to operate. In a body of water moving at a speed of 0.2 feet/sec, 5.2 per thousand cercariae approaching white mice exposed to the focus were recovered as adult flukes.

On the basis of the field experiments the Authors established a reproduction rate corresponding to 340 cercariae per egg of *S. mansoni* introduced into the focus. Notwithstanding the poor scanning power of the cercariae, a focus of Schistosomiasis can easily be self-perpetuating under these circumstances. No evidence of any self-regulating mechanism, leading to equilibrium in the epidemiological setting was ever found during these experiments.

**I N T R O D U C T I O N**

During the last few years efforts have been expended aiming at a mathematical epidemiology of Schistosomiasis, which would afford an insight into the quantitative relationships ruling the various phases of the cycle<sup>6, 7, 15</sup>. In addition to the unquestionable theoretical interest attached to such a venture, it is possible to predict its usefulness

in the appraisal of the priority to be accorded the different procedures advocated for prophylaxis or control.

The present Authors feel compelled to disagree with some of the conclusions drawn from these pioneer studies, while acknowledging their undisputable value as a doctrine. Owing to the vast differences presented by

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the ecology of the foci of Schistosomiasis, it would appear mandatory that any such analysis be carried out entirely on the basis of data collected within a single focus. What is lost in general applicability by such a procedure, will be amply repaid by its value in establishing a model for the transmission of this helminthiasis. It is likewise undesirable to mix field observations with data accruing from laboratory experiments, particularly with respect to the snail host, notoriously sensitive to changes in the environment.

While recognizing that the following are at best fragmentary contributions to epidemiology, and that they too have been unable to avoid recourse to laboratory experiments, the present Authors will report on some data bearing upon the transmission of *Schistosomiasis mansoni* in the town of Jabuticatubas during the period September-December, 1966. Transmission data for humans have been reported elsewhere<sup>11</sup>, other data are still in preparation. The present paper will merely emphasize that part of the cycle dealing with the "reproduction factor" in the snail host; in addition, a few observations on the exposure of white mice to a known number of cercariae will be presented. Owing to material difficulties these studies could not be pursued further.

The problem we sought to face can be summarized in the following question: how many cercariae are produced from one egg of *S. mansoni* entering the cycle?

Several are the variables involved:

- 1) Viability of the schistosome egg.
- 2) Probability of a susceptible snail being found by the miracidium.
- 3) Probability of infecting the snail.
- 4) Probability of survival of the infection.
- 5) Probability of survival of the snail during the prepatent stage of infection.
- 6) Duration of the infection in the snail (influenced by snail mortality, and by a possible extinction of the infection).
- 7) Number of cercariae shed each day.

The product of these various factors will yield the reproduction rate of the infection

in that part of the cycle extending from the schistosome egg polluting a focus to shedding of cercariae. Under field conditions, imposing not only practical difficulties for experiments, but handicapped by the requirement that interference with the focus be maintained at a minimum, not all of these variables are open to investigation. This, however, is not a mandatory prerequisite, since analysis of the initial and final stages of a chain of events will also supply the necessary information.

#### MATERIALS AND METHODS

The miracidia used in these experiments were obtained from fresh feces of the individual L. F., who exhibited one of the highest egg counts found in the population of Jabuticatubas (range 950-1240 eggs/g). After an egg count made by a modified Stoll-Hausheer method, the chosen weight of feces was stirred into a large volume of pond water, then exposed to sunlight from 10-12 A. M. During the laboratory experiments (Tables III and IV) an average temperature of 28.0°C prevailed in the glass cylinders used for egg hatching, the field experiments being carried out at a water temperature of 22.5°C. Viability of the schistosome eggs was determined by exposing an aliquot of the egg suspension for a period of 5 hours to the same conditions as above, then centrifuging the sample and under the dissecting microscope counting the proportion between empty egg shells and unhatched eggs.

Laboratory infection of *B. glabrata* was carried out in cylindrical vessels 10 inches in diameter, and containing 3.7 liters of pond water. At 12 A.M. the snails were added to the chosen number of miracidia, and exposure to sunlight resumed till 3 P.M.. During these last 3 hours the average water temperature reached 30.5°C.

In one set of experiments (Table III) *B. glabrata* from focus A4 of Jabuticatubas was used, another series dealing with laboratory-reared snails of a line originally collected in Sabará, Minas Gerais (Table IV). After a 3-hour contact between miracidia and snails the latter were transferred to aquaria put at our disposal by Dr. J. Pellegrino of the Instituto de Biologia, University of Minas

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Gerais. These tanks are provided with temperature control, and maintain constant recirculation of filtered and oxygenated water. Mortality both of the infected and the control *B. glabrata* from Jabuticatubas was very high under these conditions, demanding that 14 days after infection 15 snails of each group be fixed in preservative and dissected for determination of the infection rate. Only a small number of animals survived to 45 days after infection, and on that day were examined for shedding of cercariae. *B. glabrata* reared in the laboratory survived in larger numbers through the prepatent period, and were periodically examined by exposure to artificial light.

Cercariae shed from at least 10 *B. glabrata* were used for the mouse infections in the laboratory (Table V). Animals six weeks in age were exposed inside a wooden cage 11 by 6.1/2 inches in cross-section. This box was provided at the bottom with a wide-mesh wire screen, with edges turned up so as to form a pan 1/2 inches deep.

Only one experiment was undertaken in a 11 by 14 feet cement tank, containing 6,700 liters of pond water. The mice were exposed at a point halfway along the longer side of the tank, and 4,700 cercariae released (without stirring of the water) at a point directly opposite, on the other side. Another set of experiments was carried out in a 27.1/2 by 41 inches canvas tank, 2.1/2 inches deep and stretched between a wooden frame. After distributing the cercariae evenly over the surface of the tank, the mouse cage was introduced into the center, water reaching to the top of the wire screen. Freshly-shed cercariae were used throughout the series. Exposure lasted from 12 A.M. to 1 P.M., and was carried out under direct sunlight, the water temperature reaching an average of 22.5°C.

Forty-five days after exposure the mice were killed by ether, the portal vein cut between two ligatures, and liver and intestines removed. The liver was crushed between glass plates, the flukes then teased out with needles and collected in saline. The portal vein and its tributaries were slit open with fine scissors and the parasites likewise removed and counted, all operations being done under the dissecting microscope.

When cercarial output was to be determined, the snails were exposed individually to artificial light from 10 A.M. to 2 P.M., at water temperatures never in excess of 30.0°C. The suspension of cercariae was then transferred to Petri dishes, stirred, and aliquots removed. This sample was distributed in discrete drops upon a glass plate, and counts performed under the microscope. Determination of the cercaria output by the snails collected on 10.15 in focus A7 (see field experiments) was carried out by exposing the individuals previously identified as infected in colourless glass vials, introduced into the focus and left during all the daylight hours.

Sex of the infection in the snail was established by infecting white mice individually with the cercariae shed by a single snail.

Field experiments were undertaken in an abandoned vegetable garden entirely surrounded by a barbed-wire fence. The inhabitants of the neighboring houses, nearly all of which had stools positive for *S. mansoni*, were warned of the hazards of exposing themselves to infection in the drainage or irrigation ditches of this piece of ground.

Focus A4 consists of a drainage ditch confined within the garden, and entirely isolated from other bodies of water. It served for the purpose of supplying the *B. glabrata* used for laboratory experiments; in addition it spontaneously became infected at one time, periodical snail captures yielding the data used in Table VIII.

Focus A7 is an irrigation ditch 57 feet in length, 9 inches deep on average, and presenting a cross-section of 220 square inches. The average water velocity during the period of the experiments was 11.2 feet/min, as determined by the float method. Large amounts of water weed grow in this canal. It is fed by a natural spring through another ditch, entirely devoid of vegetation or snails. Drainage is effected into another stretch of canal offering little opportunity for snail populations, and then on to a cement culvert.

On 10.14, start of the field experiment, infectivity of focus A7 was investigated by exposing lots of 10 mice each, at various hours of the day. The animals were exposed for one hour in individual wire-mesh cylinders so close-fitting that the mice had to remain in the erect position. The set of cages

was exposed to the lower end of the irrigation canal, and in such a way as to sample the infectivity across its entire width.

On 10.15 three one-meter length of ditch were marked off with wooden slats, all vegetation as well as surface soil within these areas being then removed. Careful count of *B. glabrata* recovered by this sampling permitted an estimate of the snail density along focus A7. The snails were exposed to artificial light on the same day, the individuals identified as harbouring schistosome infection being separated for further experiments.

On 10.16 positive snails were exposed to the focus, as indicated above, in order to determine their cercaria production. On this day, also, experimental infection of focus A7 was undertaken. At 10 A.M. a glass vessel containing a feces suspension with 7,000 fresh *S. mansoni* eggs was immersed into the focus; at 12 A.M. the contents of the cylinder were slowly emptied at the upper end of the irrigation ditch A7.

At irregular intervals this focus was revisited, and a random sample of *B. glabrata* captured for examination in the laboratory. Size distribution of the snails in these samples did not change significantly during this period (average diameter of 15 millimeters, with 37% of the individuals 14-16 millimeters in diameter). Some of the snails were dissected after fixing, others exposed to artificial illumination, those found not to eliminate cercariae then being reexamined by crushing of the shell.

No heavy rainfall occurred in Jabuticatubas during the entire course of the field experiments, and the volume of water in focus A7 remained essentially unchanged.

## RESULTS

Our past experience with *B. glabrata* from Jabuticatubas shipped to a laboratory in São Paulo already had shown how sensitive this snail is, even when not more than 2 days had elapsed between removal from the natural habitat and transfer to the aquaria. Average survival of the infected individuals was 6.8 days, with a range of 2-14 days; the non-infected survived an average of 14 days. At that time this was attributed to an unsuitable environment in the aquaria.

Notwithstanding the ideal conditions of the aquaria used in the present experiments, mortality of *B. glabrata* from Jabuticatubas remained high, even though the snails were never kept out of the water longer than 5 hours. Table I reproduces the mortality rate of the snails used for the infection experiments:

No correlation was found between mortality in the various groups and the ratio between number of miracidia and number of snails, mortality of the controls likewise being very high. Forty-five days after infection no more than 19 snails remained alive, 17 of which shed cercariae of *S. mansoni* when exposed. Two weeks later only 2 individuals remained, both negative.

This high mortality rate for snails captured in the field was attributed at first to infection by leeches, which multiplied in the aquaria and were probably brought in from the focus. It became likewise apparent that 20% of the snails harboured infection by a trematode of the genus *Halipegus*, though it is still uncertain whether this can materially affect snail survival. This trematode infection is

TABLE I

Survival in the laboratory of *B. glabrata* from Jabuticatubas (Minas Gerais), used for the experiments in Table III

| Groups of snails | Initial number | Number alive           |                         |
|------------------|----------------|------------------------|-------------------------|
|                  |                | 5 days after infection | 13 days after infection |
| Infected         | 400            | 220(55.0%)             | 79(19.7%)               |
| Controls         | 50             | 50(100.0%)             | 14(28.0%)               |

exceedingly common in *B. glabrata* from Belo Horizonte (Minas Gerais) and surrounding areas, subsequent captures in Jabuticatubas disclosing *Halipegus* infection in an average of 47% of the snails.

Laboratory-reared *B. glabrata* exhibited better resistance towards infection by *S. mansoni* (Table II).

The difference in mortality between the infected group and the controls is not statistically significant.

In view of the short survival of the snails from Jabuticatubas, infection rate was determined by dissection at an early date. The results are given in Table III.

The results of the infection experiments with laboratory-reared *B. glabrata* are given in Table IV.

Seventy days after infection the negatives of each group were examined by crushing between two glass plates, and the absence of infection confirmed.

The schistosome eggs used for the two sets of experiments were found to be viable in the proportion of 51%.

With respect to the production of cercariae by the field snails, the individuals remaining at 45 days after infection eliminated an average of 57 cercariae per day. In the experiments of Table IV cercaria production at 49 days after infection was 162 per day for Lot 1 (2 snails positive), 440 for Lot 2 (12 snails), and 1395 for Lot 3 (16 snails). This was not a consistent relationship, however. At 42 days Lot 2 shed an average of 673 cercariae, Lot 3 an average of 280 (10 positive snails in each lot). And on day 80 the average daily output for the 3 groups was respectively 90 (2 snails), 2396 (3 snails), and 171 (3 snails).

In the single attempt at infecting mice in the cement tank, none of the 4,700 cercariae was recovered at autopsy of the 10 animals. As for the experiments undertaken in the

TABLE II

Survival in the laboratory of laboratory-reared *B. glabrata*, used for the experiments in Table IV

| Groups of snails | Initial number | Numbers alive, days after infection |           |           |           |
|------------------|----------------|-------------------------------------|-----------|-----------|-----------|
|                  |                | 29                                  | 39        | 49        | 70        |
| Infected         | 120            | 76(63.3%)                           | 72(60.0%) | 71(59.2%) | 54(45.0%) |
| Controls         | 30             | 21(70.0%)                           | 21(70.0%) | 19(63.3%) | 17(56.8%) |

TABLE III

Number of mother sporocysts of *S. mansoni* on dissection of *B. glabrata* from Jabuticatubas, 14 days after experimental infection

| Lot no. | Number of snails | Number of miracidia | Miracidia per snail | Number snails dissected | Number positive | Total number mother sporocysts in lot | Per cent of miracidia developing in snails |
|---------|------------------|---------------------|---------------------|-------------------------|-----------------|---------------------------------------|--|
| 1       | 100              | 50                  | 1:2                 | 15                      | 5               | 6                                     | 80.0                                       |
| 2       | 100              | 250                 | 5:2                 | 15                      | 11              | 20                                    | 53.3                                       |
| 3       | 100              | 1250                | 25:2                | 15                      | 15              | 37                                    | 19.7                                       |
| 4       | 100              | 2500                | 25:1                | 15                      | 15              | 51                                    | 13.6                                       |
| 5       | 50               | Controls            | —                   | 15                      | 2               | 2                                     | —  |

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TABLE IV

Infection rate by *S. mansoni* of laboratory-reared *B. glabrata*, at various intervals after experimental infection

| Lot no. | no. of snails | no. of miracidia | Miracidia per snail | Per cent of surviving snails eliminating cercariae, days after infection |      |      |      |
|---------|---------------|------------------|---------------------|--|------|------|------|
|         |               |                  |                     | 29   | 39   | 49   | 70   |
| 1       | 30            | 60               | 2:1                 | 0.0  | 0.0  | 8.3  | 8.7  |
| 2       | 30            | 300              | 10:1                | 3.8  | 52.2 | 61.0 | 61.5 |
| 3       | 30            | 1500             | 50:1                | 3.7  | 76.0 | 66.8 | 75.0 |
| 4       | 30            | Controls         | —                   | 0.0  | 0.0  | 0.0  | 0.0  |

TABLE V

Number of *S. mansoni* adults recovered at autopsy in white mice exposed in an artificial focus to different numbers of cercariae

| Lot no. | no. of mice exposed | no. of cercariae in tank | no. of mice surviving | no. of <i>S. mansoni</i> recovered in lot | Flukes per mouse | Per cent of cercariae recovered * |
|---------|---------------------|--------------------------|-----------------------|---|------------------|-----------------------------------|
| 1       | 10                  | 400                      | 8                     | 9   | 1.1              | 2.7                               |
| 2       | 10                  | 800                      | 9                     | 32  | 3.6              | 4.5                               |
| 3       | 10                  | 1500                     | 10                    | 52  | 5.2              | 3.5                               |
| 4       | 10                  | 4900                     | 9                     | 150                                       | 16.7             | 3.4                               |

\* Admitting that average worm load applies to 10 mice

canvas tank, the results are as follows (Table V).

A total of 187 *B. glabrata* was recovered on 10.15 while sampling focus A7,22 of which were positive for cercariae of *S. mansoni* (11.8%). Eighteen were exposed to artificial illumination, revealing an average daily output of 2347 cercariae.

By extrapolating these findings, we will assume that the number of snails present in this focus on 10.14 (before removal of the sample, therefore) was 1,077, and that a total of 234,700 cercariae were shed during that day. Table VI gives the autopsy results on the various lots of mice exposed at the lower end of focus A7.

The feces of the patient L.F. on 10.16 con-

tained 52% viable eggs, thus 3,640 miracidia were released into focus A7.

Twenty-two per cent of the *B. glabrata* sampled 14 days after this experimental infection presented mother sporocysts on dissection, with an average of 3.7 per infected snail. Therefore 724 miracidia succeeded in developing in the 890 *B. glabrata* still remaining in focus A7 after removal of the initial sample, giving a success rate of 19.9%.

The data yielded by the periodical sampling of the snails in focus A7 is reproduced in Table VII.

The transience exhibited by the infection in the *B. glabrata* experimentally infected within focus A7 is further supported by data obtained from focus A4, at various intervals

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TABLE VI

Number of *S. mansoni* recovered at autopsy in white mice exposed at different hours of the day to a natural focus in Jabuticatubas

| Lot no. | Exposure at hours | no. of mice exposed | no. of mice surviving | Total number of <i>S. mansoni</i> recovered | Flukes per mouse | Type of infection |        |          |
|---------|-------------------|---------------------|-----------------------|---|------------------|-------------------|--------|----------|
|         |                   |                     |                       |   |                  | Male              | Female | Bisexual |
| 1       | 10-11 A.M.        | 10                  | 9                     | 0   | 0.0              | 0                 | 0      | 0        |
| 2       | 12 A.M.<br>1 P.M. | 10                  | 10                    | 3   | 0.3              | 3                 | 0      | 0        |
| 3       | 2-3 P.M.          | 10                  | 9                     | 47 *  | 5.2              | 4                 | 0      | 5        |
| 4       | 4-5 P.M.          | 10                  | 10                    | 2   | 0.2              | 2                 | 0      | 0        |

\* Total recovery 30 male and 17 female *S. mansoni*

TABLE VII

Infection rate by *S. mansoni* of *B. glabrata* from Jabuticatubas, at various intervals after an experimental field infection

| Days after infection | no. of snails exposed to light or crushed | Positive for cercariae of <i>S. mansoni</i> |      | no. of snails dissected | no. with mother sporocysts | no. with daughter sporocysts |
|----------------------|---|---|------|-------------------------|----------------------------|------------------------------|
|                      |   | no.   | %    |                         |                            |                              |
| 0                    | 187                                       | 22  | 11.8 | —                       | —                          | —                            |
| 7                    | 69  | 1   | 1.4  | —                       | —                          | —                            |
| 14                   | —   | —   | —    | 50                      | 11(22.0%) *                | 1(2.0%)                      |
| 42                   | 30  | 5   | 16.6 | 22                      | 0                          | 2(9.1%)                      |
| 54                   | 57  | 2   | 3.5  | —                       | —                          | —                            |
| 85                   | 53  | 0   | 0.0  | 32                      | 0                          | 0                            |
| 139                  | 48  | 0   | 0.0  | 24                      | 0                          | 0                            |

\* Average of 3.7 mother sporocysts per positive snail

after a natural infection by *S. mansoni*, first surprised on 11.18 (Table VIII).

The five positive snails in the sample collected in focus A7 on day 42 after infection, shed an average of 1,249 cercariae per day, with a range of 37-5,100.

Sixteen of the snails from focus A4 were exposed to light, and an average daily production of 1,632 found, with a range of 34-5,520. By exposing mice to the individuals *B. glabrata*, it was found that 9 snails harboured male infection, in 4 only female cercariae were eliminated, and infection was bisexual in 3.

TABLE VIII

Infection rate by *S. mansoni* of *B. glabrata* from Jabuticatubas at various intervals after a natural infection was first surprised

| Days | no. of snails exposed to light or crushed | Positive for cercariae of <i>S. mansoni</i> |      |
|------|---|---|------|
|      |   | no.   | %    |
| 0    | 129                                       | 39  | 30.3 |
| 8    | 55  | 3   | 5.5  |
| 42   | 90  | 2   | 2.2  |
| 78   | 54  | 1   | 1.9  |
| 115  | 79  | 0   | 0.0  |

## DISCUSSION

While we will endeavor to give some coherence to these fragments of experimental data, no claim is made that a thorough mathematical analysis of the schistosome cycle can be attempted on their basis. Nor do we intend that general validity should be attached to our rough calculations; these results apply only to miracidia hatching from the feces of one given individual, to one particular focus within the town of Jabuticatubas, and possibly would have to be modified somewhat were we to investigate a period other than that extending from September to December, 1966.

Furthermore, when undertaking field studies it will often be necessary to waive the rigour of the methodology used in the laboratory. When aiming at the determination of the cercaria output by naturally infected snails, for example, the investigator will have to satisfy himself with studying but a few individuals, since under normal circumstances the number of infected snails within a focus of limited dimensions will be small. He will likewise find himself handicapped in his efforts to study this production of cercariae at various intervals after infection, since the behaviour of *B. glabrata* exhibits marked changes when the snail is transferred to the laboratory, frequently also presenting a substantial reduction in survival rate.

The first fact to call attention in our data is the surprisingly short life-span disclosed by *B. glabrata* from Jabuticatubas under laboratory conditions, notwithstanding the elaborate aquaria available. No relationship to "miracidial pressure" was apparent, and we still ignore whether predation by leeches or infection by *Halipegus* was instrumental in this.

As far as the transience of field infection is concerned, this might either be due to death of the infected snails, or to the phenomenon of "self-cure". (Unpublished personal observations with these snails, however, indicate that infection nearly always ends with death of the intermediary host). Regardless of the mechanism responsible, the effect upon transmission will be the same. Data in Table VII justifies the statement that *B. glabrata*

in Jabuticatubas will shed for 7 days only, on average. The decline in shedding was faster in focus A4 (see Table VIII), but chances are remote that this natural infection was detected on the day shedding first started.

If we adopt as average daily output the data supplied by the snails captured in focus A7 on 10.15, that is, 2,347 cercariae, we can calculate that each infected snail that survives to the end of the prepatent stage will on average produce some 16,000 cercariae. A few snails, however, die before shedding starts: from Table VII this proportion is 24.5%, between days 14 and 42 after infection.

Only some of the more pertinent references will be given. BARRETTO<sup>2</sup>, for instance, showed that the average duration of infection in artificially infected *B. glabrata* from Salvador was 9 days, an average of 367 cercariae being produced daily. Working with the snail from Paulista, Pernambuco, BARRETTO & BARBOSA<sup>3</sup> reported a survival of 9 weeks after infection in the larger-sized individuals, 382 cercariae being produced each day. In a latter paper, however, BARBOSA<sup>4</sup> cites a shedding period of 39 days, and a daily output of 4,598 cercariae.

A second point to be noted is the exceedingly efficient scanning capacity of the miracidium when in presence of the intermediary host, close confinement apparently not being mandatory<sup>4</sup>. Once again we find support for those laboratory experiments suggesting a chemotaxis between snail and miracidium<sup>5,8,9</sup>. It strikes us as indeed remarkable that 19.9% of the miracidia introduced into a ditch overgrown with weeds succeeded in finding and penetrating their prey along the 57 feet of its course. WEBBE<sup>21</sup> reports similar results. The fact that 78% of the *B. glabrata* were overlooked by miracidia, while the balance presented evidence of penetration by an average of 3.7, should not be construed as indicating that some individuals are more "attractive" than others, since the laboratory experiments did not disclose any such preference. Probably the snails were simply more exposed to attack.

Table III indicates that, as miracidial pressure is increased, the failure rate likewise mounts. An attractive explanation would



be retraction of the snail within its shell after penetration, thus exposing less surface for further attack.

Scanning capacity of the cercariae, on the other hand, seems to be poor, a fact already well-illustrated by literature<sup>17, 18, 19, 20</sup>. This seems particularly true for stagnant bodies of water. As reported above, no evidence of infection could be detected on autopsy of 10 mice kept during one hour at a distance of 11 feet from a point at which 4,700 cercariae had been introduced. In keeping with the postulate that infection by cercariae is a random phenomenon, the data in Table V likewise fail to demonstrate any kind of taxis. During these experiments, carried out in a 44-liter tank with cercaria densities ranging from 9 to 112 per liter, the recovery in per cent of introduced cercariae did not vary significantly. The area of the cage being 1/16 of the whole surface of the canvas tank, we can calculate that about 57% of the cercariae assumed to be distributed beneath the wire-screen succeeded in penetrating.

As concerns the field experiment, by extrapolating from Table VI we find that a total of 113 flukes would be recovered were mouse exposure to be continuous from 10 A.M. to 5 P.M. We are in agreement with RADKE et al.<sup>17</sup> that efficiency of infection in moving water should be determined on the basis of the number of cercariae approaching the area under consideration, and that for mice a reasonable estimate for this area would be 2 square inches. Since 10 mice will occupy 1/11 of the cross-section of focus A7, 21,300 cercariae will be carried through that area during the day, and recovery in our experiment is seen to be 5.3 per thousand cercariae, at the water velocity of 0.2 feet/sec. This is somewhat higher than the data published elsewhere.

While aware that these are just preliminary data, still it behoves us to make as good use of them as possible, even though only tentative conclusions can be drawn.

During the field experiment it was demonstrated that 7,000 eggs of *S. mansoni* yielded 3,640 miracidia, that these succeeded in initiating infection in 16.6% of a group of 890 *B. glabrata*, that 16,000 would be a reasonable estimate for total cercaria pro-

duction per snail. Thus the final figure of 2,370,000 cercariae and a multiplication factor of 340 cercariae per schistosome egg is reached.

Most of these cercariae will be swept away and wasted. Assuming not 5 per thousand but 5 per million to ever reach their definitive host (and that this host behaves like our white mice), we find that 12 *S. mansoni* will develop in the animal. If the proportion between male and female flukes is similar to that found in our field data, this will correspond to 4 pairs of parasites. Previous experiments by the senior Author<sup>12</sup> demonstrated that in the feces of white mice a total output of 3,000 eggs is achieved per pair of *S. mansoni*. Some 50% of these eggs will be viable<sup>14</sup>, thus we attain the final result of 6,000 miracidia in our hypothetical infection which assumed that only one in each 200,000 cercariae ever developed into an adult fluke! Therefore the cycle of transmission in this instance not only will be self-perpetuating, but will reach ever increasing levels of endemicity, providing that the white mouse (or other susceptible rodent in the role of wild reservoir) deposits all its feces into or close to a focus.

It is likewise noteworthy that in order to start the experimental infection in focus A7 not more than 1/30 of the daily egg output of the individual L.F. was used.

As usual, nature operates with largesse. And the mind is drawn to invoke a limiting factor involved in the cycle of transmission of Schistosomiasis, otherwise fluke infection would obviously take on epidemic proportions. A feed-back mechanism has been postulated, which keeps the level of infection in a habitat within narrow boundaries. Is there any evidence for such a phenomenon?

MACDONALD (personal communication) believes that this mechanism lies within the intermediary host, whose mortality would increase as miracidial pressure in a focus mounts. Nevertheless, in none of the Brazilian foci studied by the senior Author is any such evidence to be found: the infection rate in the intermediary host is never even close to 100%, indicating that the capacity of this reservoir for absorbing further miracidia is far from exhausted. And in certain areas,

in the presence of an intermediary host relatively unsusceptible to a given strain of *S. mansoni*, a sufficiently large miracidia: snail ratio can make all the difference whether a focus is to be established or not. Experiments by PARAENSE & CORRÊA<sup>16</sup>, for example, who investigated *A. tenagophilus* of a certain area of Brasil, have demonstrated the need for a ratio of 1,000:1 before the resistance of this snail towards a certain strain of *S. mansoni* could be overcome.

It is not our intention to minimize the need for thorough investigations on these matters; the relationship operating at various levels of miracidial pressure between input into the cycle and total output of cercariae is a subject of great interest. Such a study would attempt to deal not only with the effect upon death rate of the snail, but with length of the period of shedding, and daily output of cercariae as well.

If the epidemiological model is drawn up for the white mouse alone, a limiting factor to be contended with is an increasing mortality rate in the definitive host as cercarial load increases. But this point is largely of theoretical interest, since infections by less than 50 cercariae are perfectly well tolerated<sup>12</sup>.

Infection of man by a number of cercariae large enough to cause death must be highly exceptional. When groups of population are surveyed, a steady rise in fecal egg counts will be demonstrated during the first 15 years or so after the initial infection, a sharp drop in counts then occurring<sup>10, 11, 13</sup>. This turning point, however, appears after a fixed period, and seems to be independent of the intensity of infection by *S. mansoni*, therefore lacks the characteristics of a feed-back mechanism.

Those investigators admitting that self-regulating mechanisms are involved in establishing the level of endemicity of Schistosomiasis see it as an oscillating phenomenon, presenting plus and minus swings around a base-line. In none of the foci of Jabuticatubas did we ever find any evidence that such a concept ought to be taken seriously. Schistosomiasis in that area appears as an all-or-none phenomenon, one focus dying out after a few days, another cropping out elsewhere. We believe that the only obstacle preventing

expansion of Schistosomiasis in Jabuticatubas and vicinity is the improving socio-economical level of the community, thus limiting pollution, and restricting exposure to the foci to a minority of the population.

#### RESUMO

*Alguns dados quantitativos referentes à infecção de campo e no laboratório, de Biomphalaria glabrata, de Jabuticatubas (Minas Gerais, Brasil), com miracídios de Schistosoma mansoni*

Baseando-se numa série de experiências de campo e de laboratório, efetuamos uma primeira tentativa para a conceituação quantitativa de algumas das interações do ciclo da esquistossomose mansônica. Desejamos ressaltar que as conclusões deste trabalho referem-se somente a um determinado foco da helmintose, no caso uma área da cidade de Jabuticatubas, Minas Gerais.

Foi verificado que a eliminação de cercárias pela *B. glabrata* daquela região efetua-se durante um período médio de 7 dias, podendo avaliar-se em 16.000 o número total de cercárias produzidas pelo molusco infetado.

O miracídio de *S. mansoni* exibe boa capacidade discriminatória, mesmo em coleções d'água mais extensas encontrando o hospedeiro intermediário. No decurso de uma experiência realizada numa vala de 17,3 metros de comprimento, e com correnteza de 3,6 metros/minuto, 19,9% dos miracídios introduzidos conseguiram penetrar o molusco.

Por outro lado, não foi possível evidenciar qualquer tropismo ou cinesia por parte da cercária, especialmente em águas paradas, nas quais a infecção do hospedeiro definitivo parece depender de seu encontro fortuito com a cercária. Numa coleção d'água movendo-se com a velocidade de 3,6 metros/minuto, de cada mil cercárias levadas de encontro a camundongos-sentinela apenas 5,2 foram recuperadas como vermes adultos por ocasião da autópsia.

Os resultados das experiências de campo nos levaram a admitir um "fator de reprodução" correspondendo a 340 cercárias produzidas por ovo de *S. mansoni* introduzido no ciclo, o que facilmente permite a perpe-

tuação de um foco, não obstante a ausência de capacidade discriminatória da cercária.

Êstes estudos não sugerem a existência de qualquer mecanismo auto-regulador, capaz de manter em equilíbrio estável uma situação epidemiológica.

#### REFERENCES

1. BARBOSA, F. S. — Os transmissores da esquistossomose mansônica no nordeste do Brasil. *J. Brasil. Med.* 8:263-268, 1964.
2. BARRETTO, A. C. — *Esquistossomose mansônica na cidade do Salvador*. Tese. Salvador, 1960.
3. BARRETTO, A. C. & BARBOSA, F. S. — Qualidade de vetor dos hospedeiros de *S. mansoni* no nordeste do Brasil. IV — Eliminação de cercárias de *Schistosoma mansoni* por *Australorbis glabratus* de diâmetros diversos. *An. Soc. Biol. (Pernambuco)* 16: 13-18, 1959.
4. CHERNIN, E. & DUNAVAN, C. A. — The influence of host-parasite dispersion upon the capacity of *Schistosoma mansoni* to infect *Australorbis glabratus*. *Amer. J. Trop. Med. Hyg.* 11:455-471, 1962.
5. ETGES, F. J. & DECKER, C. L. — Chemosensitivity of the miracidium of *Schistosoma mansoni* to *Australorbis glabratus* and other snails. *J. Parasit.* 49:114-116, 1963.
6. HAIRSTON, N. G. — *Population Ecology and Epidemiological Problems*. New York, Ciba Foundation Symposium on Bilharziasis, 1962.
7. HAIRSTON, N. G. — On the mathematical analysis of schistosome populations. *Bull. W. H. O.* 33:45-62, 1965.
8. KLOETZEL, K. — Observações sobre o tropismo do miracidio de *Schistosoma mansoni* pelo molusco *Australorbis glabratus*. *Rev. Brasil. Biol.* 18:223-232, 1958.
9. KLOETZEL, K. — Novas observações sobre o tropismo do miracidio de *Schistosoma mansoni* pelo molusco *Australorbis glabratus*. *Rev. Inst. Med. trop. São Paulo* 2:341-346, 1960.
10. KLOETZEL, K. — Sobre a conveniência da quimioterapia da esquistossomose em populações em contínuo contato com os focos. *Rev. Inst. Med. trop. São Paulo* 5:106-110, 1963.
11. KLOETZEL, K. & SILVA, J. RODRIGUES — Alguns dados clínicos e epidemiológicos da esquistossomose em Jaboticatubas, Minas Gerais. Presented at the *Third Congress of the Brazilian Society of Tropical Medicine*. Salvador, Bahia, 1967.
12. KLOETZEL, K. — Egg and pigment production in *Schistosoma mansoni* infections of the white mouse. *Amer. J. Trop. Med. Hyg.* 16:293-299, 1967.
13. KLOETZEL, K. — A rationale for the treatment of schistosomiasis mansoni, even when reinfection is expected. *Trans. Roy. Soc. Trop. Med. Hyg.* 61:609-610, 1967.
14. KUNTZ, R. E. — Passage of eggs by host infected with *Schistosoma mansoni*, with emphasis on rodents. *J. Parasit.* 47:905-909, 1961.
15. MACDONALD, G. — The dynamics of helminth infections, with special reference to schistosomes. *Trans. Roy. Soc. Trop. Med. Hyg.* 59:489-506, 1965.
16. PARAENSE, W. L. & CORRÊA, L. R. — Susceptibility of *Australorbis tenagophilus* to infection with *Schistosoma mansoni*. *Rev. Inst. Med. trop. São Paulo* 5:23-29, 1963.
17. RADKE, M. G.; RITCHIE, L. S. & ROWAN, W. B. — Effects of water velocity on worm burdens of animals exposed to *Schistosoma mansoni* cercariae released under laboratory and field conditions. *Exp. Parasit.* 11:323-331, 1961.
18. ROWAN, W. B. — The ecology of schistosome transmission foci. *Bull. W. H. O.* 33: 147-153, 1965.
19. ROWAN, W. B. & GRAM, A. L. — Relation of water velocity to *Schistosoma mansoni* infection in mice. *Amer. J. Trop. Med. Hyg.* 8:630-634, 1959.
20. WEBBE, G. — The effect of water velocities on the infection of animals exposed to *Schistosoma mansoni* cercariae. *Ann. Trop. Med. Parasit.* 60:78-84, 1966.
21. WEBBE, G. — The effect of water velocities on the infection of *Biomphalaria sudanica tanganyicensis* exposed to different numbers of *Schistosoma mansoni* miracidia. *Ann. Trop. Med. Parasit.* 60:85-89, 1966.

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