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QUANTITATIVE ASPECTS OF THE MIGRATION AND EVOLUTIVE ASYNCHRONISM OF SCHISTOSOMA MANSONI IN MICE

M. A. BARBOSA (1), J. PELLEGRINO (2)*, P. M. Z. COELHO (3) and I. B. M. SAMPAIO (4)

SUMMARY

Two groups of white mice (**Mus musculus**) were infected with 65 and 440 cercariae transcutaneously. Migration of **Schistosoma mansoni** from skin to the lungs and to the portal system thereafter was studied through fitting mathematical equations. Six evolutive stages previously defined were used to determine the asynchronic development of parasites in the portal system. Equations allow to predict the moment of maximum schistosomula recovery in skin and lungs. In the portal system the equations lead to different days of maximum recovery according to each stage. These differences measure quantitatively the asynchronism of **S. mansoni.**

INTRODUCTION

Since the early investigations by CORT [6] and FAUST & MELENEY [9] on the morphology and biology of **Schistosoma japonicum** in mammalian hosts, the phenomenon of asynchronism in the development of the Japanese blood-fluke has been recognized. Actually, it was pointed out by CORT [6], working with infected mice, that there is a "variation in time which is taken by the parasites in reaching their destination". In order to characterize as precisely as possible the morphological changes occurring during the evolution of the parasite, several stages have been recognized and defined. CORT [6] began his studies in mice, 40 hours after exposure to **S. japonicum** cercariae and, from this time on, described 18 different stages which were later extended to 24 by FAUST & MELENEY [9], who initiated their observations soon after cercarial penetration.

As far as **S. mansoni** is concerned, FAUST et al. [8], using experimentally infected rats, rabbits, and **Rhesus** monkeys, also observed the asynchronism in the development of this fluke. It was shown to occur as early as 3 days after exposure. The same stages previously described for **S. japonicum** were considered. These findings were later confirmed by several workers [1,27,28,31].

Attempts to study the asynchronism on a more specific basis were first undertaken by YOLLES et al. [32] and CLEGG [4]. The optimum development in mice was determined by selecting the **most fully developed** worms (**S. mansoni**) on each day after percutaneous ex-

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(1) Department of Parasitology, Julio de Mesquisa University, Faculty of Medical and Biological Sciences, Botucatu, São Paulo.
(2) Department of General Biology, Federal University of Minas Gerais.
* Deceased 9 February 1977.
(3) Department of Parasitology, Federal University of Minas Gerais.
(4) Department of Zootechny, Federal University of Minas Gerais.

Address for reprints: M. Aparecida Barbosa FCMBB), Distrito de Rubião Junior. C.P. 101, 18610 Botucatu, São Paulo, Brasil.

posure of the animals to 500 cercariae [4]. Despite the wide variation in the rate of development, the threshold was remarkably constant for each stage occurring on a particular day [4].

For the purpose of this work the term schistosomule was used to designate the evolutive stage of **Schistosoma mansoni** while in skin, after cercarial penetration, and in the lungs.

To study the evolution of this parasite in mice, mathematic equations were defined, from the relative percentage of the evolutive stages established throughout the experiments. Furthermore, it was determined, using the mathematic models, some quantitative aspects of the dynamics of migration of schistosomes from the skin to the lungs and, thereafter, to the portal system.

In the present study, the asynchronism that occurs in the development of **S. mansoni** was investigated quantitatively by determining the relative percentages of 6 morphologically-characterized stages, starting from the schistosomular phase, in the skin, to the mature worms within the portal system. Some aspects involved in optimal conditions for infection and rate of migration will be also considered.

MATERIALS AND METHODS

The LE strain of **S. mansoni** (Belo Horizonte, Brazil), shed by laboratory-reared and infected **Biomphalaria glabrata** was used in the present study. Large numbers of cercariae were obtained from at least 50 infected snails, which were then concentrated in sintered-glass crucibles as described by PELLEGRINO & MACEDO [17].

Two experimental groups were defined using 65 and 440 cercariae, respectively, during exposure.

White Swiss mice (GIDE), weighing 18 to 20 g, where anesthetized with Veterinary Nembutal (65 mg/kg). The lower abdomen was carefully shaved and moistened with water before exposure. A plastic ring of 1.9 cm inside diameter and 0.3 mm height was firmly held to the abdomen with strips of scotch tape. The cercarial suspension was gently stirred and a volume of 0.3 ml, containing the desired number of cercariae was pipetted (Cornwall BD automatic pipette) into the ring.

Schistosomula recovery from the skin

After the mice were sacrificed by cervical fracture the skin area limited by the plastic ring was removed and chopped into small fragments using a sharp point scissors in a small Petri disk containing 0.2 ml of Hanks balanced salt solution (HBSS) with pH from 7.2 to 7.6. The moistened fragments were then transferred to a 2 cm diameter cylindrical vessel closed at its lower end by a stainless steel screen of mesh 0.09 mm and supported by three 1 cm long plastic legs. This vessel was put into a 50 ml beaker and heparinized HBSS with 7.2 to 7.6 at 37°C was poured in up to the screen level so that it moistened the fragments of screen tissue. The young parasites migrated from the tissue to the warm and might be collected after four hour incubation.

Schistosomula recovery from the lungs

Mice were sacrificed and rapidly opened. The thoraxic aorta was cut close to the heart and 10 ml of Hanks-Balanced Salt solution (HBSS) (pH 7.2 to 7.6 temperature of about 37°C), containing 25 units of heparin, was injected into the right ventricle to perfuse the lungs. This organ, free of blood, is greatly expanded, and the schistosomula, firmly held inside the lung capillaries, are not perfused out.

The perfused lungs were excised and chopped into fragments as small as possible with fine scissors and introduced into a small Petri disk containing 0.2 ml of Hanks solution. The moistened fragments were then transferred to a 2 cm diameter cylindrical glass vessel closed at the lower end by a stainless steel screen of mesh 0.09 mm, which was supported by three (perspix) legs inside a 50 ml beaker, such that the screen was 1 cm above the bottom. The beaker was filled with heparinized HBSS, pH 7.2 — 7.6 at 37°C, up to the level of the screen, so that it moistened the fragments of lung tissue. The young worms migrate from the tissue into the warm HBSS and may be collected after 4 hours incubation.

Schistosomula recovery from the portal system

Shortly after the lungs were detached perfusion of the portal system was performed according to PELLEGRINO & SIQUEIRA [11] with light modifications. The perfusion device was the Brewer pipetting machine model 40. The portal vein was cut and the retum simply tied. A BD 30/10 needle of the machine was introduced into the thoraxic aorta and then injected 250 ml of heparinizeted salt solution (0.85% NaCl) for complete perfusion.

In some animals, especially in highly injected ones, perfusion was not enough to recover all worms. Adult worms would obstruct tiny vessels and should be removed through nippers. The perfusion liquid was collected in 250 ml bottles, rested for 15 minutes and then eliminated all supernatant through vacuum trump until 35 ml was left. This operation was repeated three more times, for each final product was diluted up to 200 ml in salt solution eliminating blood residuals. The last 35 ml was centrifugated at 1000 rpm for 2 minutes and only the bottom 2 ml were placed on the counting disk and all parasites were counted and classified under the stereomicroscope (40 times magnification).

Portal system worm classification

Morphological criteria were used to classify the parasites in relation to their stage. For technical facilities the gastric caecum shape was chosen, according to FAUST et al.[3] (Fig. 1):

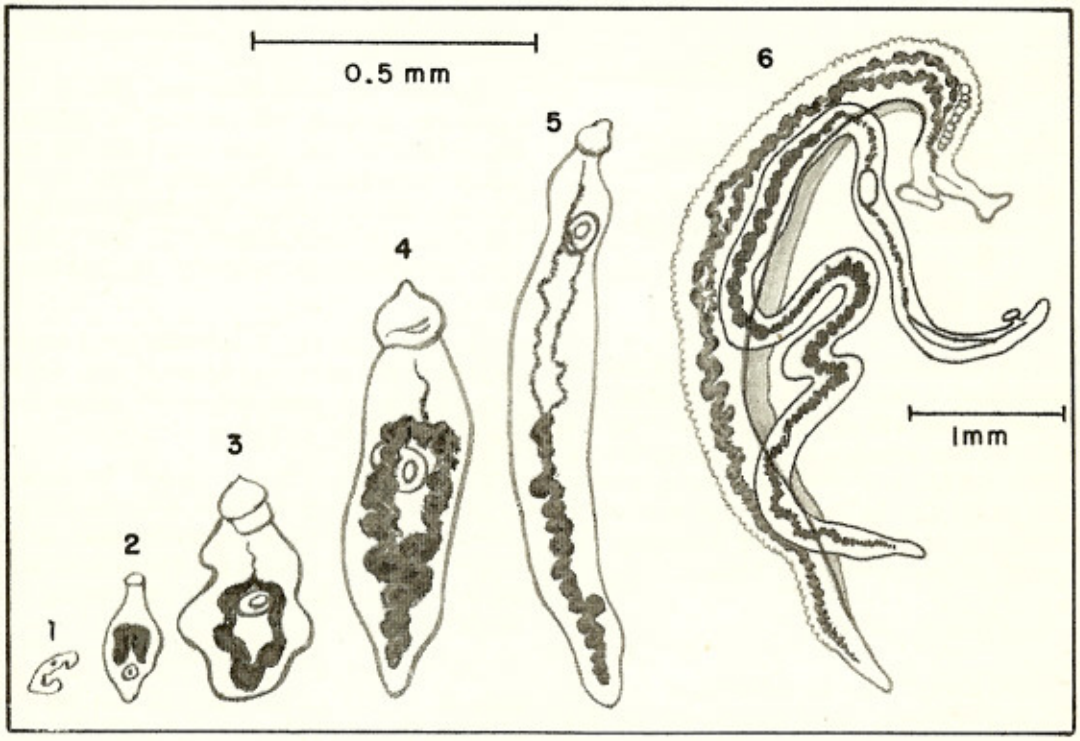


Fig. 1 — Evolutive stages of **S. mansoni** considered in the **schistogram**

Stage 1. Newcomers from the lung presenting only a light stain which stands for the beginning of caecum. This stage has a short lifespan.

Stage 2. A darker stain now bifurcating but not bypassing the acetabulum.

Stage 3. The dark bifurcated stain bypasses the acetabulum linking themselves later on.

Stage 4. The dark bifurcated stain after reconnection grows to the parasite end, but not longer than the bifurcated caecum.

Stage 5. The final linked caecum grows longer than its bifurcated part, but shorter than three times of it.

Stage 6. Young and mature adults. Their linked caecum grows 3 times longer than their bifurcated caecum.

Statistical analysis

Mathematic model

For every stage after penetration a forth degree predicting equation was estimated:

$$\hat{y} = a + b_1 X + b_2 X^2 + b_3 X^3 + b_4 X^4$$

where

$\hat{y}$ = estimated recovery percentage on a certain day X

a = linear coefficient of equation

b_i = regression coefficients for linear, quadratic, cubic and quartic effect of day (i = 1 to 4)

x = days after infection.

When variation of the recovery percentages suggested a curve with two maxima, the complete model was maintained, otherwise a more simplified model was tried through data selection. Within this procedure the quadratic models came out to be the best predicting equations in most cases, easily detecting the day when recovery was the highest.

Data selection

There always was an interval of time in which recovery was more conspicous, as can be seen from the histograms. Outside of each interval either the schistosomules had not come yet or after it, just a few of them were eventually recovered. Later reduction of these intervals was performed so that the selected equation for it had the highest multiple correlation coefficient, lowest coefficient of variation and had no cubic or higher effects.

For some stages the complete model was imposed for any attempt to subdivide the corresponding interval led to poor sampling.

RESULTS

As can be seen in Table I, the decrease of parasites recovery, according to our method, is related with the period of time after cercaria penetration. No significant statistical differences were detected between the two groups.

TABLE I

Recovery of Schistosomules (**S. mansoni**) from the skin 24 hours after infection

Number of cercaria	% of recovery after infection		
	1/2 hour	2 hours	24 hours
65	64.0	45.5	26.0
440	59.0	41.6	25.0

It can be concluded (see Fig. 2) that, using our method, the recovery of schistosomules from 4th day on is very poor. No significant statistical differences were detected between the two groups. As can be seen in Fig. 2, there is a clear decrease of the mean of skin schistosomula recovery in relation to time.

As stated in Fig. 3, the statistical analysis of data show that group infected with 440 cercariae reach the peak of recovery before group infected with 65 cercariae.

It is shown (Fig. 4) through the analysis of data, that it is possible to note the morphogenesis of **S. mansoni (schistogram)** in the portal system, as well as the dynamic of arriving of parasites to portal system coming from the lungs.

As summarized in Figs. 5 and 6, it is possible to have a global view of the dynamics of parasite migration, from skin to lungs, as well as from lungs to the portal system. It is important to note that a residual percentage of parasites remains in the lungs until the end of the period of observation in both infections.

Table II and III contents allow, through mathematic equations deduced by statistical analysis of experimental data, to evaluate the

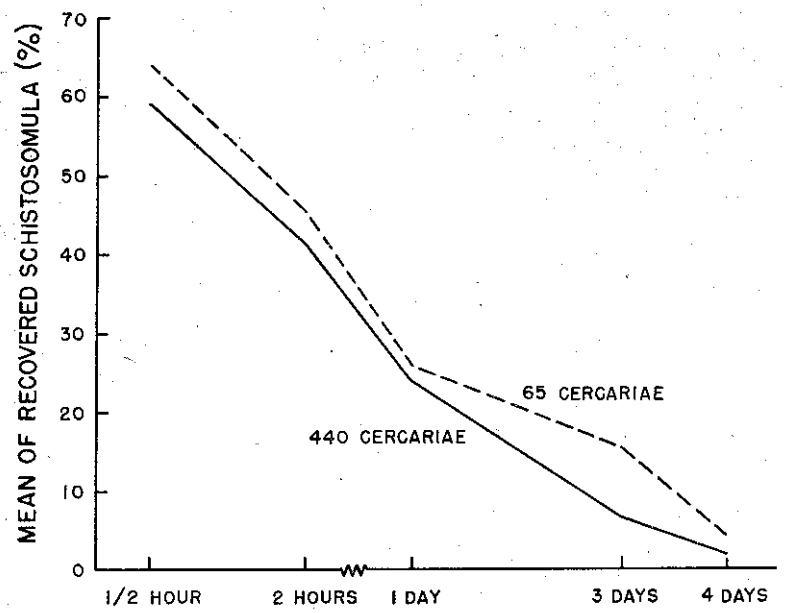


Fig. 2 — Mean of percentual recovery of schistosomules from the skin of 5 mice infected with 65 and 440 **S. mansoni** cercariae, 1/2 and 2 hours, and 1, 3, and 4 days after infection, respectively, by transcutaneous route.

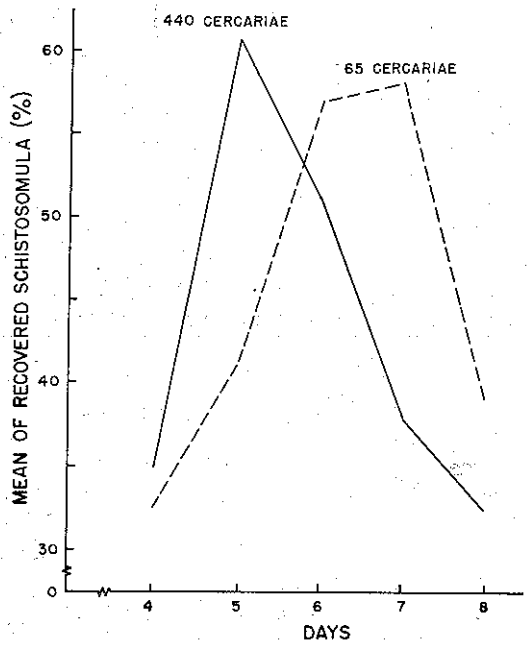


Fig. 3 — Mean of percentual recovery of schistosomula from the lungs of 5 mice infected with 65 and 440 **S. mansoni** cercariae, 4, 5, 6, 7, and 8 days after infection, by transcutaneous route.

percentual of recovery of parasites obtained from skin, lungs, and portal system in a specific day "D", in white mice infected with **S. mansoni** (LE strain), and using the methodology described in this paper.

Figure 7 shows a 4th stage **S. mansoni** schistosomulum in the lungs.

DISCUSSION

In interpreting the results of the recovery of schistosomules from the skin 30 minutes, 2 hours, and 3 and 4 days after exposure (Fig. 2), it is important to recall that, in both groups of infected mice, cercariae were applied to the skin in a very small volume of tap water (0.2 to 0.3 ml). Therefore, the number of parasites per skin area, especially in the second group of mice (440 cercariae), was quite high. Statistical analysis showed no differences between the data expressing the decrease in the percentages of schistosomula recovered 30 minutes, 2 hours, and 1 day for mice exposed to 65 and 440 cercariae.

Considering the mean of schistosomules recovered from the skin and lungs of mice exposed to 65 and 440 cercariae, a careful inspection of Figs. 5 and 6 clearly shows the existence of a gap in schistosomule recovery between the first and the fourth day after

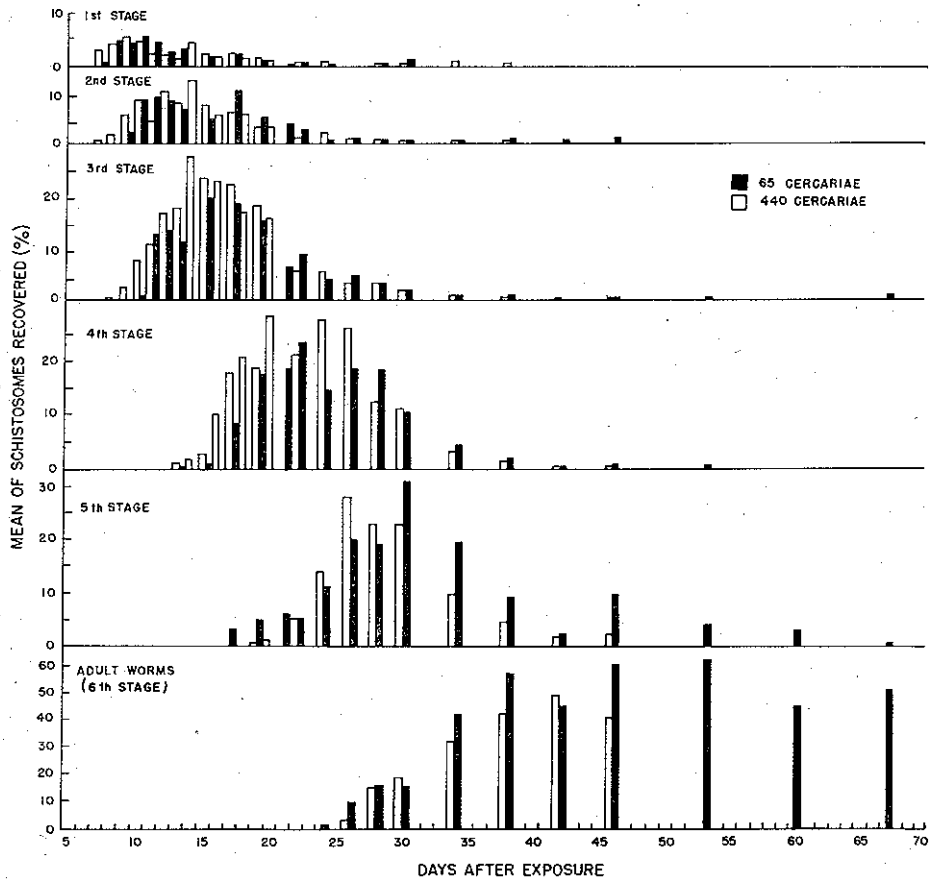
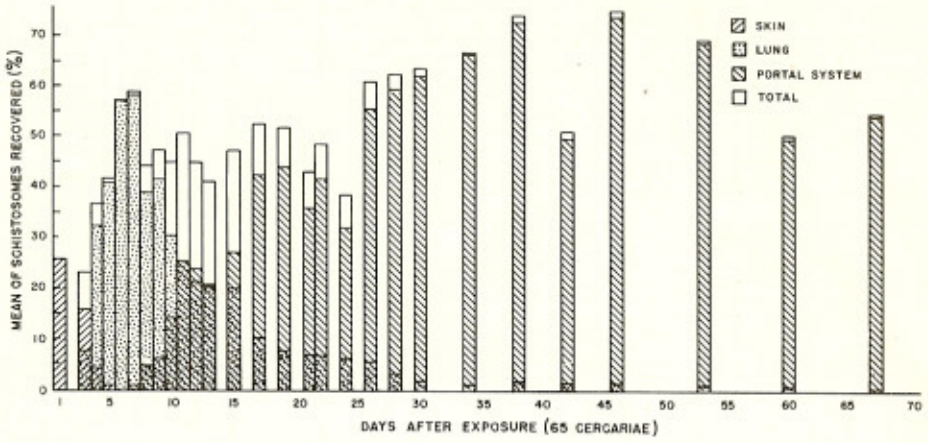
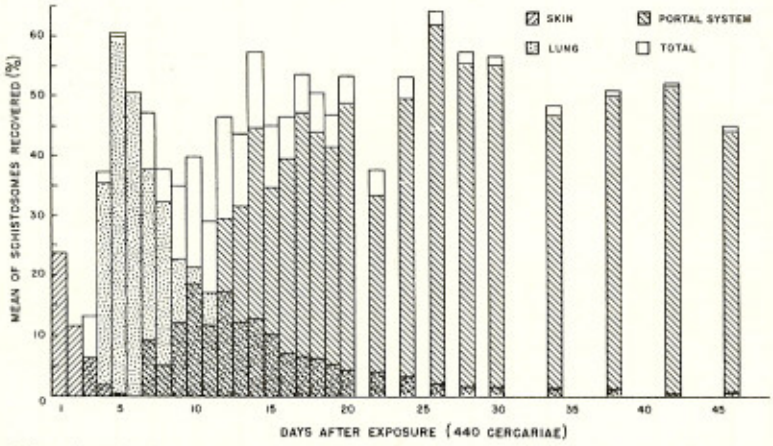


Fig. 4 — Mean of percentual recovery of parasites from the portal system of 5 mice classified according 6 stages (**schistogram**), and infected with 65 and 440 cercariae, respectively, by transcutaneous route.

exposure. This finding can be explained assuming that the deeper the migration through the skin and the firm lodging inside blood vessels, the more difficult the recovery of parasites. It is well known that migrating schistosomules can be found at different deepness of the skin and subcutaneous layers for one week or more, depending on the host and the site used for skin exposure. Serial skin biopsies (up to 2 days after infection) of exposed skin areas of the ear and tail of mice, conducted by STIREWALT [26], showed that no schistosomes could be found in the portal system, 6 weeks later. For 3 days one there was a progressive increase in worm burdens, indicating that migration to the lungs have occurred by that time. These findings have been confirmed in our laboratories by serial biopsies (1, 2, 3, 6, 22, 29, and 77 hours) performed on the whole exposed (250 cercariae of **S. mansoni**) skin areas of the lower abdomen of mice.

CLEGG & SMITHERS [5] demonstrated that the skin of some laboratory animals constitutes a major barrier to invading cercariae of **S. mansoni.** In rats, as many as half of the cercariae which enters the abdominal skin die during the early stages of penetration: in mice, about one-third, and, in hamsters, one-tenth of the cercariae die while in the skin. A rapid recovery of schistosomules from mouse abdominal skin at intervals of 10, 20, and 30 minutes after infection, proved that most of the death in the skin occurs within the first 10 minutes after penetration [5], apparently during the passage through the Malpighian layer. According to GHANDOUR & WEBBE [10],





Figs. 5 and 6 — Mean of percentual recovery of schistosomula from the skin, lungs and portal system of 5 mice infected with 65 (figure 5) and 440 (figure 6) **S. mansoni** cercariae followed up during the hole period of experiments.

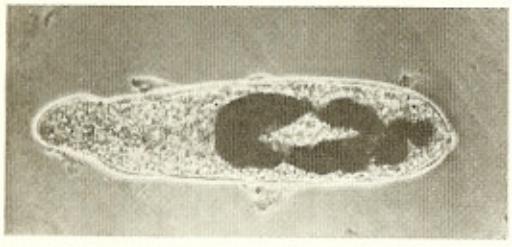


Fig. 7 — Schistosome that reached the fourth stage of development in the lung

30 to 38% of **S. mansoni** cercariae die while penetrating the skin of mice.

In our experiments the recovery rates of schistosomules from the skin at 30 minutes and the peaks in the lungs, for both infections, were about 60% (Figs. 5 and 6), confirming the findings reported by previous investigators [5,10]. It was also shown that practically all the cercariae that were able to overcome the skin barrier reached the lungs.

Figure 3 shows that the peak of schistosomules recovered from the lungs of mice was reached on day 5 (60.2%) in the group of animals exposed to 440 cercariae, 2 days before than that of mice exposed to 65 cercariae (58.2%). This finding suggests that the migration rate was faster in the first group of mice (440 cercariae). Statistical analysis showed these curves to be significantly different

TABLE II

Evaluation of the days of maximum recovery and respective mean worm recovery in the skin, lungs, and portal system of mice infected with 65 S. **mansoni** cercariae, transcutaneously

Local and/or stage		Proposed intervals (days)	Day of maximum recovery	Highest percentage recovery	Multiple correlation coefficient	Coefficient of variation	Best predicting equation for percentage recovery on a certain «D» day
Skin		1 to 5	1	26.40	79.60	66.37	$Y = 32.94942 - 6.54066\ D$
Lungs		3 to 10	6.92	53.62	78.00	31.07	$Y = -81.57607 + 39.05583\ D - 2.82066\ D^2$
Portal system	1st	7 to 13	9.62	6.05	24.40	121.45	$Y = -28.03438 + 7.08441\ D - 0.36808\ D^2$
	2nd	9 to 19	12.00	7.59	32.80	64.14	$Y = 254.24257 + 73.10686\ D - 7.5496\ D^2 + \frac{341.86377\ D^3}{1000} - \frac{5.74331\ D^4}{1000}$
	3rd	10 to 21	15.85	19.10	68.80	49.86	$Y = -98.52733 + 14.84459\ D - 0.46833\ D^3$
	4th	17 to 30	23.76	20.29	48.90	40.59	$Y = -104.14433 + 10.47363\ D - 0.22038\ D^2$
	5th	24 to 38	30.66	26.19	63.50	43.49	$Y = -287.81372 + 20.47746\ D - 0.33385\ D^2$
	6th	24 to 46	50.91	61.73	89.10	35.84	$Y = -160.01390 + 8.71063\ D - 0.08554\ D^2$

TABLE III

Evaluation of the days of maximum recovery and respective mean worm recovery in the skin, lungs, and portal system of mice infected with 440 S. **mansoni** cercariae, transcutaneously

Local and/or stage		Proposed intervals (days)	Days of maximum recovery	Highest percentage recovery	Multiple correlation coefficient	Coefficient of variation	Best predicting equation for percentage recovery on a certain «D» day
Skin		1 to 4	1	21.63	83.00	50.71	$Y = 28.70453 - 7.06818\ D$
Lungs		3 to 7	5.46	56.75	92.20	20.99	$Y = -194.26852 + 91.85309\ D - 8.40258\ D^2$
Portal system	1st	7 to 13	9.23	4.56	56.20	53.55	$Y = -18.40010 + 4.97341\ D - 0.26930\ D^2$
	2nd	8 to 17	12.95	9.32	56.20	45.91	$Y = 36.75504 + 7.11248\ D - 0.27447\ D^2$
	3rd	8 to 20	15.70	22.11	73.80	46.22	$Y = -80.24042 + 13.03828\ D - 0.41521\ D^2$
	4th	16 to 28	22.41	27.43	62.20	33.69	$Y = -173.1046 + 17.89307\ D - 0.39913\ D^2$
	5th	24 to 34	28.30	25.48	58.50	45.18	$Y = -371.97778 + 28.0874\ D - 0.49621\ D^2$
	6th	26 to 42	42(*)	52.56	84.60	37.50	$Y = -64.87993 + 2.79618\ D$

(*) The linear effect was observed and, therefore, theorically, the maximum day would be the day in which it was possible to obtain 100% of recovery

($P < 0.05$). It is important to note that the peak of percentages obtained in the present study (about 60%) were remarkably higher than those reported for mice in the literature: 16.0 [22,4,23,20] to 45.0% [31].

Figures 5 and 6 show that schistosomules begin to appear in the lungs only 3 days after exposure, which agrees with the observation of other workers [22,14,32]. Thereafter, a rapid decline was observed in both groups of exposed mice. After 15 days, the percentages of recoveries were 20.0 and 10.3 (animals exposed to 65 and 440 cercariae, respectively). Although in small numbers, schistosomules could be recovered from the lungs as long as 67 and 46 days (65 and 440 cercariae; Figs. 5 and 6), which corresponded to the entire period of observation. The long stay of schistosomula in the lungs was also reported by other workers [4,8,31,14,32]. In this connection, it was recently mentioned by WAKSMAN & COOK [18] that, in mice experimentally infected with **S. mansoni**, an IG_2 antibody appears to slow the passage of schistosomules through the lungs, without killing the parasite. It is generally assumed that, while in the lungs, schistosomules do not feed on blood. Only a small proportion — about 4% — shows signs of feeding on red blood cells, which results in the appearance of black pigment in the gut [9,7] (black or brown-gutted schistosomula). In the present study, black or brown pigmented larvae were regularly found (more than 20%), starting 7 to 10 days after exposure. Two schistosomes reached the fourth stage of development (Fig. 7).

It is known, since the early investigations undertaken by FAUST et al. [8] on the mammalian phase of the life cycle of **S. mansoni** that, 6 days after cercarial exposure of laboratory animals, "worms are found plentiful in the liver". In white mice, schistosomes were found to appear in liver perfusates from the sixth [7] to the eighth day [9] after infection. In the present study, the first schistosomes collected from the liver by perfusion of this organ appeared 7 days after exposure in both groups of infected mice (Figs. 4, 5 and 6).

Considering the peaks of maximum recovery of the several stages of schistosomes collected by perfusion of the liver and mesenteric vessels of infected mice (Fig. 4), it is clear that somatic development of the worms is very rapid since they reach the portal system.

The relative percentages of each evolutive stage in the portal system were calculated in order to define the **schistogram.** The small size of stages 1 and 2 rendered difficult their recovery even after a carefully perfusion. Stage 1 in the liver is continuously supplied by schistosomules coming from the lungs, and apparently it grows rapidly reaching the following stage soon after ingestion of blood. This probably accounted for the relative small percentages of these stages recovered from the portal system (Fig. 4). Peaks corresponding stages 3, 4, and 5 were reached after 15 (19.4%), 22 (24.3%) and 30 (31.4%) days in the group of mice exposed to 65 cercariae and after 14 (26.8%), 20 (28.0%) days in mice exposed to 440 cercariae. The peaks of maximum recovery of adult worms were observed 53 (63.4%) and 42 (49.7%) days for the groups of mice exposed to 65 and 440 cercariae, respectively. These data refer to the experimental results obtained in the present study. Equations have been established for the determination of the optimal day to recover any of the developmental stages of schistosomes in the portal system (Tables II and III).

Besides the phenomenon of asynchronism, which is clearly shown in Fig. 4, it can be assumed that the faster rate of migration of schistosomula through the skin of mice exposed to 440 cercariae was responsible for the appearance of the peaks slightly before than that in mice exposed to 65 cercariae. This also applies to stages 1 to 4. Considering stages 5 and 6 (adults), the opposite was observed. This can be explained by an early death occurring in mice harboring higher worm burdens in the group infected with 440 cercariae. Actually, the death rates observed in mice exposed to 65 and 440 cercariae were 6.5% (155 animals infected) and 30.0% (206 animals infected), their life span being 67 and 46 days, respectively.

Figures 5 and 6 summarize the results obtained throughout the experiments. Besides the means of schistosome recoveries, it was calculated the total mean recovery (first to

the last day of observation) for both groups of infected mice: 50.80% ± 12.65 and 45.06% ± 12.89 (animals exposed to 65 and 440 cercariae, respectively). Considering only the stage 6 (adult worms) the recovery rates were 60.47% ± 11.0 and 53.76% ± 6.32 for the animals infected with 65 and 440 cercariae. This apparent discrepancy is explained by the greater chance of death amongst mice harboring high worm burden (exposure to 440 cercariae).

It is interesting to note that, in the present study, the recovery rates of adults worms from the portal system were remarkably higher than those reported by other workers in mice infected with **S. mansoni:** BRENER [2], 20.0%; CLEGG [4], 20.0%; GRIMALDO & KERSHAW [12], 34.3%; PELLEGRINO & KATZ [16], 22.3%; SAOUD [21], 28.3 to 39.9%; SMITHERS & TERRY [24], 24.4 to 48.2%; STANDEN [25], 20.1%; WARREN & PETERS [30], 36.9 to 39.5%.

The degree of asynchronism phenomenon can be evaluated by the difference between the percentages of adult worms recovered in relation to the total of parasites (= 100%, cincluding all stages) for each day of observation.

The peak of schistosomes recovered in the portal system (again about 60%) led to speculate that mortality, if any, is remarkably small after the parasites cross the skin barrier.

Although it is true that penetration of **S. mansoni** cercariae into the skin of mice, and doubtless infection, takes place under a wide range of environmental conditions — even those which would seem to be very unfavourable — it is important to realize that, in order to obtain reproducible and consistent results, penetration must occur, as far as possible, under precisely defined condition. Even so, un-explained variability in penetration and maturation of **S. mansoni** in mice, under apparently adequate experimental conditions, has been a common experience.

As demonstrated by STIREWALT & FREGEAU [27], the type of water is very important when establishing the exposure medium of choice for experimental infections. The exposure media, ranked in descending order of percentage of total penetrating cercariae were: dechlorinated tap water, 92%; creek water, 86%; single-distilled water, 76%; and triple-distilled water, 68%. Furthermore, variability in experimental groups among individual samples of cercarial suspensions was least in decholorinated tap water [27].

In the experiments of STIREWALT & FREGEAU [27] it was surprising that penetration occurred from 7.° to 45°C and that average penetration was good over a broad range of temperature: from 16°C through 35°C. These findings were also confirmed by DEWITT [7] but, notwithstanding this very wide temperature tolerance, there was a single optimal temperature level for all criteria: 27° to 28°C.

The cercarial post-emergence age is also of utmost importance. The decline in infectivity of **S. mansoni** cercariae was clearly demonstrated by OLIVIER [15] and STIREWALT & FREGEAU [28]. After having passed the optimal post-emergence age of 1 to 3 hours, most cercariae undergo slight loss of penetrating power but no diminution of maturation ability within the following 5 hours.

In our experiments care was taken to follow, as closely as possible, the ideal conditions reported in the literature for the infection of mice. Actually, dechlorinated tap water kept at 27°C, a post-emergence period for cercariae from snails to penetration of host within 3 hours, and an exposure time of 60 minutes [3,15,7] were used. For technical reasons, it was not possible to control the patency of snail infection as a factor influencing infectivity of cercariae and percentage of maturation. As shown by STIREWALT & FREGEAU [28], a consistent decrease is observed within 41 to 46 days of patency, which probably reflects some kind of crisis in snail physiology.

The relation of cercarial concentration per unit of skin to penetration and further development is still a matter of controversy. It has been first assumed by STIREWALT [26] that the greater the cercarial concentration, the easier the penetration. According to GRIFFITHS [11], massive invasion by cercariae tends to alter the pattern of penetration and to increase the rate of migratory behavior. Actually, the cercariae become markedly gregarious in

their attack upon the epidermis, modifying the pattern of migration. Later on, STIREWALT & FREGEAU [27], exposing mice from 25 to 500 cercariae by tail immersion, concluded that they early assumption that greater numbers of cercariae allow higher percentages of cercarial penetration or maturation is not warrented any more.

It is worthwhile to mention that, for immunologic studies in schistosomiasis, especially for the evaluation of the degree of resistance to challenge, SHER et al. [22] and PEREZ et al. [20] suggested to measure the survival of schistosomules in the lungs (first week of infection), instead of allowing the worms to mature (four to six weeks), as used by most Authors. The technique here described for recovering lung schistosomules is particularly helpful for this purpose.

The **schistogram** (relative percentages of developmental stages found in the portal system) affords an interesting model for studies on the activity of antischistosomal agents on developing forms of schistosomes. It is well known that the majority of drugs acts preferently on mature schistosomes, SN 10275 (laboratory animals) and oxamniquine (laboratory animals and humans) being exceptions [19]. Furthermore, the mathematical model here described as far as the **schistogram** is concerned, could be used to investigate which evolutive stages are more sensitive to the immune response of the host. This model is now being evaluated in studies of possible differences of **S. mansoni** strains in mice, and, eventually, in other laboratory animals.

It could be speculated that the asynchronism development of **S. mansoni** in the definitive host is related to individual migratory capacities of the parasite. Actually, the observation by MICHALICK [13] showed that a similar pattern of asynchronism also occurs in **in vitro** culture of **S. mansoni**, starting from a same cercarial population. These considerations suggest that, although the migratory capacity of **S. mansoni** interfers in the asynchronism development of this trematode, genetic factors certainly play a decisive role in governing this phenomenon.

RESUMO

Aspectos quantitativos da migração e do assincronismo evolutivo do Schistosoma mansoni **em camundongos**

Dois grupos de camundongos (**Mus musculus**) foram infectados com 65 e 440 cercárias, transcutaneamente. A migração do **S. mansoni** da pele para o pulmão e para o sistema porta foi estudada através de equações matemáticas adequadas. Seis estágios evolutivos previamente definidos foram usados para determinar o desenvolvimento assincrônico dos parasitos no sistema porta. As equações permitem predizer o momento da recuperação máxima de esquistossomos na pele e no pulmão. No sistema porta as equações conduzem a dias diferentes de recuperação máxima, de acordo com cada estágio. Estas diferenças medem quantitativamente o assincronismo evolutivo do **S. mansoni.**

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