Rev. Inst. Med. trop. São Paulo
10(4):219-228, julho-agôsto, 1968

UDC 616.995.122

THE REACTION OF *AUSTRALORBIS GLABRATUS (BIOMPHALARIA GLABRATA)* TO INFECTION WITH *SCHISTOSOMA MANSONI*

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SUMMARY

The effect of *S. mansoni* infection on growth, fecundity and mortality among laboratory reared *A. glabratus* was investigated. The infection induced alterations were studied in snails exposed individually either to a single male miracidium or to a single female miracidium.

From the analysis of data obtained it was seen that infection with *S. mansoni* retarded growth, suppressed the egg-laying capacity and decreased dramatically the life potential of the susceptible host. No apparent differences have been registred between physiological alterations in infected *A. glabratus* secured by exposure to a male miracidium and those in snails infected with a female miracidium. The comparison of data herein described with those previously reported by other investigators indicated that, there are no distinctive qualitative and quantitative differences in host response to infection with a single miracidium and in host reaction to infection with numerous miracidia secured by single or multiple exposures.

The decreased life potential and the lost egg-laying capacity indicate that *S. mansoni* infected *A. glabratus* have no significance in relation to population dynamics, since their eventual destruction may be assumed. Therefore, it does not seem out of place to suggest, tentatively, that a schistosome incapable to inflict damage to the final hosts but capable of a *S. mansoni*-like effect in the intermediate hosts may prove useful to their control.

INTRODUCTION

Effects of schistosoma infection on snail physiology have been frequently recorded. CORT [6] has shown that *Oncomelania nosophora* infected with *S. japonicum* were less resistant to desiccation. BRUMPT [3] found that this was true also of *A. glabratus* infected with *S. mansoni*. GORDON et al. [7] observed that *Planorbis pfeifferi* infected with *S. mansoni* had a higher death rate than uninfected snails. Likewise, OLIVIER et al, [10], BARBOSA et al. [1], BARRETTO [2], and COELHO et al. [5] have shown that *A. glabratus* infected with *S. mansoni* die in much greater number than uninfected snails, when the snails are removed from the water.

Other physiological changes which occurred in study snails and which appeared to be the effect of infection included also retarded growth and inhibition of the egg-laying capacity. COELHO [4] has, for example, observed that fecundity of *A. glabratus* decreased following infection with *S. mansoni* and PESIGAN et al. [15] reported that *Oncomelania quadrasi* infected with *S. japonicum* did not grow in size as rapidly as uninfected snails.

In the years following, notable contribution to the knowledge of physiological alterations in snails produced by schistosome infections were made by PAN [11, 12]. It would appear from the evidence presented by the Author that damage caused in snails by the penetrating miracidia is greater than previously thought. For example, the Author found that

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infection suppressed completely fecundity in snails, while others demonstrated that it was only partially inhibited by infection.

The reason for these differences has not been clearly elucidated but it has been ascribed by PAN [12], on a hypothetical basis, as being due to the varying intensity of infection. However, there is not direct evidence in the literature to support such a suggestion.

Insofar as physiological alterations developed in response to infection are concerned, most of the studies performed in the past have been directed towards understanding of the damage in the susceptible host, exposed singly or in mass to large numbers of miracidia. In other studies, infected snails have been secured by collections in natural habitats and the intensity of infection was not known. These studies prompted the present attempt to evaluate the degree of physiological alterations afforded by infection resulting from exposures to a uniform stimulus. It seemed reasonably certain that infection with a single miracidium, as compared with massive infections, warrants uniform data in evaluation of effects in the susceptible host.

Differences in the reactivity of the snail to a given sex of *S. mansoni* are also being recorded.

MATERIALS AND METHODS

The snail used was of a nonpigmented Brazilian/Puerto Rican cross. The procedure of securing snails of a uniform age follows; new polyethylene sheets were washed in warm tap water, rinsed in cold tap water and placed on the surface of cylindrical battery jars in which snails were maintained. The plastic sheets with the egg-masses deposited within 24 hours were removed from the jars and placed clutch site down in similar jars with fresh dechlorinated water. These clutches were held in the water for 8 days. At the end of this period the majority of eggs hatched, those unhatched, remaining on the plastic sheets were discarded. A group of 525 snails with a shell diameter of 4 mm, approximately, were used at the age of 30 days in exp. 1. Another group of 296 specimens 18 days old with a mean diameter of 2 mm, approximately, were used in exp. 2.

The schistosoma eggs were obtained from Albino Swiss mice exposed to *S. mansoni* cercariae of Puerto Rican origin, 3 months previously. From 3 to 5 livers were removed and minced in a blender containing 0.85% saline. The eggs obtained were hatched in tap water and used immediately. Active miracidia were picked up individually by a capillary pipette and deposited in droplets of water on the bottom of glass dishes, 25 x 8 mm. The dishes were examined to assure the presence of only one viable miracidium. Single snails were placed near the droplets and immediately flooded with water. Series of dishes, each containing one snail, one miracidium and 1.5 ml of water, were covered with glass lids to reduce evaporation and prevent snails from crawling out during the 60 min of exposure. Losses of water, due to evaporation, were corrected when necessary.

After exposure snails were placed in groups of 15, approximately, in battery jars containing 5 liters of dechlorinated water. The snails were held at room temp of 23-25°C and fed fresh Romaine lettuce which was changed daily. The water was changed at weekly intervals and calcium carbonate was added. Forty days after exposure, the interval necessary to assure emergence of cercariae, the snails were washed thoroughly, removed individually to beakers 20 ml capacity and examined for cercarial emergence. Snails failing to shed cercariae were held for additional 3 days and reexamined.

In the group of 525 snails exposed at the age of 30 days, 60 died during the interval from exposure to cercarial emergence and of the remaining 465, only 230 proved infected. In the group of 296 specimens, exposed at the age of 18 days, 46 died and of the remaining 250, 83 were found to shed cercariae.

Each infected snail was placed in a numbered beaker containing 600 ml of dechlorinated water and a scrap of lettuce. Three days later snails were transferred to small beakers containing 20 ml of tap water and exposed to artificial light for 90 min to force the emergence of cercariae. Groups of 3 Albino Swiss female mice, weighing 17-20 g, were exposed by tail immersion to cercariae yielded by individual snails, by the method of OLIVIER & STIREWALT [9]. After exposure

each group of 3 mice was placed in a numbered wired cage.

All mice were perfused 45 days later by the method of YOLLES et al. [17], and the sex of infection determined. In exp. 1, 5 groups of mice were found to harbor female and male worms, 120 groups carried male worms and 105 groups yielded female worms. Thus, of the total number of 230 snails infected at the age of 30 days, 5 were found to be infected with more than one miracidium, and were discarded. Of the remaining 225, 120 specimens were shedding male cercariae and 105, female cercariae. In exp. 2, of a total of 83 snails, infected at the age of 18 days, 41 yielded male cercariae and 42 had a female infection.

Each infection will be dealt separately and its relative effect on the snail physiology assessed. The 2 experiments involved 4 groups of infected snails. Snails, shedding male cercariae and those shedding female cercariae, were separated in subgroups of 10 specimens, approximately, in battery jars containing 5 liters of dechlorinated water, scraps of lettuce and calcium carbonate. Two control groups of uninfected snails of corresponding ages were given identical care throughout the experiments.

EXPERIMENTS

Growth — Growth of snails was determined by measuring the shell diameter to the nearest 0.1 mm at the end of the 2nd and 3rd month, prior to sex determination of infection (Fig. 1), and at the end of the 4th, 5th and 6th month after sex differenciation (Fig. 1 and Table I).

As judged by data on the life potential (Table III) of infected snails, it is evident that there exists a considerable reactivity variation to the standardized infection with a single miracidium, though, the snails were uniform in size and age at exposure. The number of living snails decreased progressively throughout the observations. Moreover, it was evident that specimens, showing most advanced growth retardation, died earlier than the faster growing. In order to assess the actual pattern of growth among infected snails, it seemed advisable to include in calculation of mean diameters those, which succumbed within each month between subsequent measurements of surviving snails. Therefore, the numbers of snails seen in Table I are greater than those in Table III.

The general shape of the growth curves for infected snails (Fig. 1) was similar to that for uninfected specimens, although there were quantitative differences. As judged by data recorded within the second month after infection, the tested snails did not grow in size as rapidly as control specimens. Growth damage observed at the beginning progressed during the following months. Thus, by month 4 after exposure the mean diameter of infected snails ranged from 17.3 to 18.7 mm, while that of uninfected snails varied from 19.7 to 20.2 mm. The difference is further emphasized when it was found to be of the order of 3.2 and 3.5 mm within the 6th month after infection of snails in exp. 1. Although the differences in shell diameter between infected and control snails were somewhat lower in exp. 2, they were also found to be statistically significant (Table I).

Separate evaluation of growth was made to assess possible differences in snail reactivity to infections caused by each sex of the same *S. mansoni*. Although the mean diameter was somewhat lower among snails infected with male miracidia than in those infected with female miracidia in exp. 1, the differences were not striking. Nevertheless, the differences of — 0.8 mm and of — 0.9 mm by month 4 and 5 after infection were found to be statistically significant. However, such was not the case for snails tested in exp. 2. There was either a very small or, practically, no difference in growth between male and female infected snails. Furthermore, while the male infection was more effective in depressing growth than the female infection in snails tested in exp. 1, the contrary occurred among snails in exp. 2. No apparent logic underlies the disparity of data recorded in 2 subsequent experiments. Nevertheless, tentative explanation of this follows. On the other hand, for differences of a relatively low order, ranging from 0.1 to 0.4 mm, such results are not peculiar. By month 6 after infection the differences in growth between male and female infected snails tended to disappear completely.

TABLE I

Mean shell diameter of uninfected *A. glabratus* ($\overline{X}_1$), of infected with one male miracidium ($\overline{X}_2$) and of infected with one female miracidium ($\overline{X}_3$) of *S. mansoni*

Month post infection	Mean shell diameter (mm) of snails			Difference between means *			$\frac{\overline{X}_1-\overline{X}_2}{\sigma_1}$	$\frac{\overline{X}_1-\overline{X}_3}{\sigma_2}$	$\frac{\overline{X}_2-\overline{X}_3}{\sigma_3}$
	uninfected $\overline{X}_1 \pm SD$	infected (♂) $\overline{X}_2 \pm SD$	infected (♀) $\overline{X}_3 \pm SD$	$(\overline{X}_1 - \overline{X}_2) \pm \sigma_1$ **	$(\overline{X}_1 - \overline{X}_3) \pm \sigma_2$	$(\overline{X}_2 - \overline{X}_3) \pm \sigma_3$			
				Experiment 1					
4	(90)19.7 ± 0.2	(68)17.3 ± 0.2	(52)18.1 ± 0.2	2.4 ± 0.31	1.6 ± 0.35	−0.8 ± 0.37	7.74	4.57	2.16
5	(90)20.7 ± 0.2	(68)17.5 ± 0.3	(52)18.4 ± 0.3	3.2 ± 0.35	2.4 ± 0.36	−0.9 ± 0.41	9.14	6.67	2.19
6	(72)22.7 ± 0.3	(27)19.2 ± 0.4	(19)19.5 ± 0.5	3.5 ± 0.52	3.2 ± 0.54	−0.3 ± 0.67	6.81	5.92	0.45
				Experiment 2					
4	(32)20.2 ± 0.3	(31)18.7 ± 0.4	(36)18.3 ± 0.3	1.4 ± 0.51	1.8 ± 0.44	+0.4 ± 0.52	2.74	4.09	0.77
5	(31)21.4 ± 0.4	(24)19.7 ± 0.5	(22)19.5 ± 0.5	1.7 ± 0.61	1.9 ± 0.64	+0.2 ± 0.72	2.79	2.97	0.28
6	(29)23.2 ± 0.4	(16)20.8 ± 0.5	(17)20.9 ± 0.4	2.4 ± 0.64	2.3 ± 0.56	−0.1 ± 0.69	3.75	4.11	0.14

Numbers in parenthesis represent the number of snails tested
* 95% confidence limits for the difference between means
** Standard deviation of difference between the means

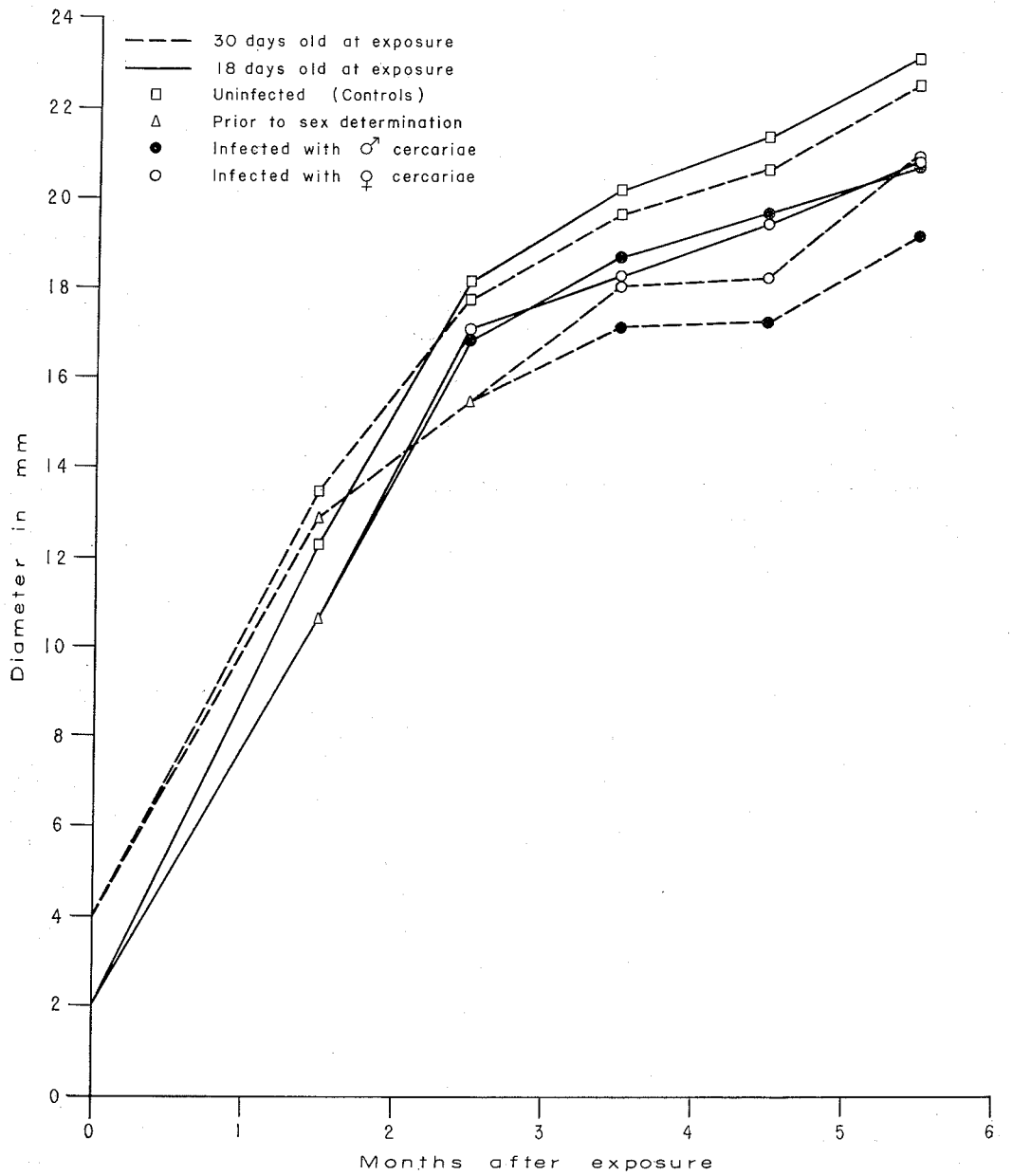

Fig. 1 — Mean shell diameter of infected and uninfected *A. glabratus*

Fecundity — Observation on the effect of infection upon the reproductive mechanism of snails involved two main functions, egg production and fecundity, as determined by the number of living embryos. In addition to these effects, onset of sexual maturity, as determined by the appearance of depositions among snails, has also been recorded.

After the pretest period during which attention was focused on the appearance of depositions, those found were recorded and discarded, water was changed in the battery jars containing 10 snails, approximately, and egg-masses, deposited the last 7 days of each month after infection, were used for fecundity determination. The number of embryonated

eggs in clutches, deposited on the walls of the jars, was counted with the aid of a 10 X magnifier. The egg-masses, deposited on snail shells and on lettuce, were removed with a razor blade and the number of embryonated eggs was counted under a dissecting microscope.

The first observable effect of infection was a general retardation of maturation, as indicated by a varying delay in the appearance of egg depositions. Two months after infection egg-masses were seen only occasionally among infected snails, whereas the mean number of depositions per control snail per day was 1.5 (Table II). Furthermore, the early depositions among infected snails were either empty or revealed the presence of very few eggs, abnormal in shape with dead embryos or without embryos. Thus, by month 2 after infection the number of viable eggs per infected snail was 1, while it was 66 per control snail per day. A gradual improvement in fecundity occurred during the following month, the number of eggs per infected snail per day ranged from 7 to 12. However, fecundity decreased rapidly during the following month and was practically abolished by month 5 after infection.

No apparent differences in fecundity have been registered between infected snails, secured by exposure to female miracidia and those exposed to male miracidia, at the age of 18 days (Table II).

Mortality — The snails were checked daily and those found dead recorded.

From data presented in Table III it is apparent that infection decreased considerably the life potential of the susceptible host. Four months after exposure the cumulative deaths among infected snails ranged from 37% to 50%, while it varied from 4% to 14% among control snails. From data seen in Table III it is obvious that, practically, all infected snails were dead by month 7 after exposure, while from 67% to 75% of control snails were living.

Of a total of 308 infected snails only one lived as long as 9 months and it was found to shed cercariae until it died.

It is also obvious from data summarized in Table III, that the sex of infection did not affect the pattern of death caused by infection.

DISCUSSION

That *A. glabratus* showed definite signs of damage in respect to growth, fecundity and longevity, following massive infection with *S. mansoni* miracidia, has been described in the literature.

The present work, to our knowledge, is the first in which single miracidia were used separately to evaluate the effects upon several aspects of snail physiology.

In judging the value of alterations, produced by infection in the susceptible host,

TABLE II

Effect of infection on fecundity of *A. glabratus*

Month post infection	Infected snails (♂)			Infected snails (♀)			Uninfected snails (Control)		
	Number tested	em/s/d*	e/s/d**	Number tested	em/s/d	e/s/d	Number tested	em/s/d	e/s/d
2	83 ***	0.4	1	—	—	—	36	1.5	66
3	28	0.6	12	30	0.3	7	32	0.9	60
4	21	0.1	2	19	0.2	7	31	0.5	27
5	16	0.1	1	17	0.1	1	28	1.1	77

* em/s/d, egg-mass per snail per day
** e/s/d, egg per snail per day
*** Counted prior to sex determination

TABLE III

Mortality of *S. mansoni* infected and uninfected *A. glabratus*

Exp. no.	1					2				
Age at exposure	30 days					18 days				
Sex of infection	Number of snails tested	Cumulative mortality (%)				Number of snails tested	Cumulative mortality (%)			
		4 months	5 months	6 months	7 months		3 months	4 months	5 months	6 months
Male	110	37	75	96	100	41	24	42	61	90
Female	105	50	81	96	99	41	12	46	60	100
Uninfected	94	4	23	33	35	36	11	14	22	25

it should be borne in mind that physiological demonstration of infection by a single miracidium is a response to the lightest infection that may occur, while alterations produced by massive exposure is thought to be a response to varying infections. Nevertheless, quantitative and qualitative results herein described have a provocative similarity to those reported in the literature.

The pattern of growth of infected snails paralleled closely that described by PAN [12] except, that we did not observe that the infected snails grew faster than uninfected during the first 6 weeks after exposure; possibly because our first determination of shell diameter took place as late as 2 months after infection.

The remarkable thing about growth retardation in snails infected with one miracidium is not its actual presence, but the fact that it has been found to be more striking than in snails exposed to numerous miracidia. For example, the difference of shell diameter between infected and uninfected snails was of the order of 2.4-3.5 mm by month 6 after infection, while that recorded by Pan was 0.9 mm.

The mechanism, whereby infection with one miracidium induces more damage to growth than exposure to numerous miracidia, remains obscure. Possible explanation of this would include the following: It seems reasonably certain that retardation of growth among our infected snails would have been less striking, had the calculations of mean shell diameter been based on data recorded for snails living at the end of each month (Table III). However, since the appreciable number of snails, succumbed between subsequent measurements, was found to demonstrate the most advanced growth retardation, it seemed advisable to include them in calculating of mean shell diameters. It is believed that this markedly lowered the mean diameter of infected specimens and consequently increased the difference in growth between control and infected snails.

It should also be remembered that the effect of age of snails at exposure, on growth after exposure, may be of importance in relation to the degree of growth retardation. PAN [12] investigated the effect of infection upon adolescent snails with a mean shell diameter of 7.3 mm and upon young and old adults with a mean shell diameter, ranging from 10.3 mm to 15.6 mm. We tested juvenile snails at the age of 18 and 30 days with an average shell diameter of 2 and 4 mm. If infection induced degenerative changes, related to the growth mechanism, are similar to those induced by radiation, previously described by the Author [13], infection will be more effective in depressing growth in younger snails than in older specimens. This hypothesis gets some support from the find-

ings by Pan that *S. mansoni* did not affect the growth of snails which were relatively old at exposure.

In this connection, one may question the present results on eventual stunting among older juveniles, exposed at the age of 30 days, and among those, infected at the age of 18 days. If increased age was a factor in decreasing the sensitivity of snails to infection induced growth damage, one would expect the 30 days old juveniles to show less growth retardation than the 18 days old specimens. However, such was not the case. Infection was more effective in depressing growth in the former than in the latter specimens. The difference of mean shell diameters, between uninfected and infected snails in exp. 1, was of the order of 3.2-3.5 mm by month 6 after exposure vs. 2.3-2.4 mm, between uninfected and infected specimens in exp. 2 (Table I).

This brings up another factor involved, namely the growth capability of snails utilized in the 2 experiments. In analysing growth among control snails, the conclusion was reached that the growth potential of the 18 days old snails was greater than that of the 30 days old specimens. Although the mean shell diameter of the former was 2 mm vs. 4 mm of the latter at the start of the experiments, they exceeded in size the older specimens throughout the observations (Table I). Apparently the greater growth capability in the younger juveniles was adequate to repair partially the infection induced damage to growth. This, of course, does not rule out the possibility that infection induced injury is age dependent and decreases with the increase of age.

The concept of increased sensitivity to infection induced damage, in individuals with a relative low growth potential, finds some support in the information concerning the differences in snail reactivity to infection, caused by different sexes of the same *S. mansoni*. It will be remembered that the male infection was more effective in depressing growth than the female infection in the older juveniles (Table I, exp. 1). Though the differences recorded disappeared by month 6 after infection, they were statistically significant early after infection. Such was not the case among the younger juveniles (Table I, exp. 2). The growth mechanism of these specimens was not capable to distinguish injury induced by a male infection from that induced by a female infection, possibly because they were less sensitive to infection induced damage, due to their natural greater growth capability.

It would appear from evidence cited that, although infection induces growth retardation, this is not striking and would seem a less logical indication of infection in the susceptible host.

Effects of infection upon fecundity and longevity of snails seem far out of proportion to the effect on growth. Moreover, fecundity appears to be the most important site of physiological alterations induced, although, according to PAN [12], "the ovotestis remains relatively intact structurally even among snails infected for long periods".

Changes in the reproductive mechanism, leading to reduced and abnormal depositions following infection, were progressive. It would appear from data summarized in Table II that a 5 month lasting infection was sufficient to suppress egg production. This confirms the observation by Pan who also reported cessation in egg production among snails exposed to numerous *S. mansoni* miracidia. However, the present results do not support the suggestion by the Author that partial inhibition of egg-laying, in field collected *S. mansoni* infected *A. glabratus* [4] and in *S. haematobium* infected *Bulinus truncatus* [8], might be due to "light" infection.

It is evident from our experiments that even the lightest infection secured by exposure to a single miracidium was sufficient to prevent the reestablishment of a progeny generation.

The cessation of egg production in our snails did not coincide in time with that reported by PAN [12]. This was perhaps due to differences in the developmental stage between snails utilized in exposures. Pan infected either snails approaching sexual maturity with a mean shell diameter of 7.3 mm, or young and old adults, ranging in size from 10.3 to 15.6 mm. Our 18 days old snails (Table II), with a mean shell diameter of 2 mm at exposure, had still a long way to go to reach sexual maturity. There is no question but that retarded maturation caused by infection, as determined

by the long preoviposition period, contributed to the fact of depositions occurring later in life of snails.

It is also possible that the fully developed reproductive mechanism in the older snails, utilized by Pan, was more sensitive and responded promptly and with intensity to the stimulus provided by the miracidia. This hypothesis gets some support from other findings previously reported by the Author [14], that the hazards of inducing irreparable damage to the reproductive mechanism by radiation are reduced when radiation is given to juvenile snails. Nevertheless, the effects of infection on fecundity even in juvenile snails were impressive, all losing their egg-laying capacity 5 months after infection.

Mortalities among infected snails herein described (Table III) coincided in time with those reported by PAN[12]. Six months after infection the cumulative deaths of snails ranged from 90% to 100% and those described by Pan varied from 89.3% to 100% by week 24 and 30 after infection.

It is believed that lower mortality rate of 71% among infected snails described by RITCHIE et al. [16], was perhaps due to the formula fed to snails, which differs from the standard diet consisting of fresh Romaine lettuce.

Only one of the 308 snails infected lived as long as 9 months and it was found to shed cercariae until it died. This confirms the observation by Pan who reported emergence of cercariae from a few longer living snails for as long as 35 weeks.

The comparison of our data with those of experiments previously reported disclosed that there are no distinctive qualitative and quantitative differences in host response to infection, secured by a single exposure to one miracidium, and in host reaction to a single or multiple exposures to numerous miracidia.

It would, therefore, appear that even the lightest infection with *S. mansoni*, as demonstrated by the exposure to a single miracidium, might have appreciable impact on the population of the susceptible host.

If so, it seems appropriate here to mention that schistosomes, that have the potentiality to produce irreparable damage in the intermediate host but are incapable to induce injury to the final host, are not to be ignored in the search for means that may be of certain value in controlling snails.

RESUMO

Reação do Australorbis glabratus (Biomphalaria glabrata) *à infecção com* Schistosoma mansoni

Foram investigados os efeitos da infecção provocada pelo *S. mansoni* com relação à fisiologia do caramujo, notadamente ao seu crescimento, fecundidade e longevidade. As alterações causadas pela infecção foram estudadas nos caramujos expostos à infecção de um miracídio macho e de um miracídio fêmea, separadamente.

Da análise dos dados obtidos verificou-se que a infecção com o *S. mansoni* retarda o crescimento, suprime a oviposição e diminue sensìvelmente a longevidade do hospedeiro intermediário.

Não foram registradas diferenças aparentes entre as alterações fisiológicas nos *A. glabratus* infetados pelo miracídio macho e aquelas nos infetados com miracídio fêmea.

A comparação dos dados obtidos com os relatados por outros investigadores indica que não há diferença quantitativa nem qualitativa na reação do hospedeiro à infecção com um só, ou com numerosos miracídios em uma ou múltiplas exposições.

A perda da capacidade reprodutora e o índice de alta mortalidade de caramujos infetados pelo *S. mansoni* sugerem que pode ocorrer um contrôle biológico do *A. glabratus* infetado em criadouros naturais. Assim sendo, não parece fora de propósito a sugestão de uma tentativa de ser provocada no caramujo uma infecção com um *Schistosoma* inócuo para o homem, mas capaz de produzir no caramujo um efeito similar ao produzido pelo *S. mansoni*, fato êste que poderá ser útil para o contrôle biológico do hospedeiro intermediário da esquistossomose.

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Recebido para publicação em 7/2/1968.