Effects of the Desiccation on *Biomphalaria tenagophila* (Orbigny, 1835) (Mollusca) Infected by *Schistosoma mansoni* Sambon, 1907

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Specimens of Biomphalaria tenagophila *exposed to miracidia of* Schistosoma mansoni *were submitted to different desiccation periods as follows: group I: 24 h after exposure, desiccated for 28 days; group II: after cercariae elimination, desiccated for 7 days; group III: 21 days after exposure, desiccated for 7 days; group IV: 14 days after exposure, desiccated for 14 days; group V: 7 days after exposure, desiccated for 21 days. From the obtained data it was verified that desiccation was not capable of interrupting the development of larvae of* S. mansoni *in mollusks. A delay in the development of* S. mansoni *larvae in groups I, III, IV and V was observed. A pause was verified in the development of* S. mansoni *larvae in groups II, III, IV and V. Some larvae, in groups I, III, IV and V, did not suffer as a result of desiccation and continued their development. Larvae in the cercariae stage were shown to be more sensitive to desiccation. It was possible to obtain clearing of mollusks infected by sporocysts II and cercariae using a period of 7 days of desiccation.*

Key words: *Biomphalaria tenagophila* - *Schistosoma mansoni* - desiccation

Biomphalaria tenagophila (Orbigny, 1835) is important in the propagation of the mansonic schistosomiasis in Brazil, mainly in the south and southeast areas. It is the intermediate host of *Schistosoma mansoni* Sambon, 1907 in Rio de Janeiro and Minas Gerais, besides being responsible for most of the autochthonous cases of schistosomiasis diagnosed in the State of São Paulo. Cases of transmission of the disease have been described in Santa Catarina and Rio Grande do Sul (Bernardini & Machado 1981, Paraense & Corrêa 1987, Espíndola et al. 1992, Souza & Clark-Lima 1997, Graeff-Teixeira et al. 1999, Katz & Dias 1999).

The susceptibility potential of mollusks to infection by *S. mansoni* can be modified through crossings among mollusks of different strains (Dias et al. 1987). Borda and Rea (1997) believe in the existence of variation in the degree of susceptibility of *B. tenagophila* to infection by *S. mansoni*, although there is a predominance of refractory populations in comparison to susceptible ones.

Studies on the resistance to desiccation were accomplished with *B. glabrata*, *B. straminea* and *B. pfeifferi* by Barbosa and Dobbin (1952), Olivier and Barbosa (1955, 1956), Olivier (1956), Jong-Brink (1973), Pieri et al. (1980), Vianey-Liaud and Lancastre (1986) and Vianey-Liaud and Dussart (1994) with healthy mollusks, and by Barbosa (1953), Barbosa and Coelho (1953, 1955), Barbosa and Barbosa (1958), Lancastre et al. (1987, 1989) and Badger and Oyerinde (1996) with mollusks infected by *S. mansoni*. Only one study on the resistance of *B. tenagophila* to desiccation is known. Specifically, Teles and Marques (1989) found specimens of healthy *B. tenagophila* in state of natural desiccation in Ubatuba and Conchas, in the State of São Paulo.

In spite of *B. tenagophila* being a species from areas with humid climate, such as the South and Southeast regions of Brazil, this does not signify that the species can not create conditions to adapt itself to areas of dry climate. Moreover, as it is the intermediate host of *S. mansoni* in the south and southeast of the country, it can become another species to transmit the disease in the area. The process of desiccation hinders the control of the schistosomiasis in areas subject to drought because it creates difficulties for the localization of mollusks, thus hindering their combat. Considering that the resistance of mollusks to desiccation facilitates recolonization and that their reproduction may be by self-fertilization, their viabilization is even

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greater. In cases where these mollusks are infected, the possibility of a new cycle of the disease exists. The study of the resistance of *B. tenagophila* to desiccation is of great value (under the aspect of its biology), mainly because it is a susceptible species to *S. mansoni*.

This work aimed to examine the development of larvae of *S. mansoni* in *B. tenagophila*, while submitted to desiccation.

MATERIALS AND METHODS

The mollusks used were *B. tenagophila* (selected parental generation for the susceptible and refractory characters) descend from specimens collected in 1992, in Florianópolis, Santa Catarina, Brazil. Crossings among specimens of parental melanic generation (susceptible to the infection for *S. mansoni*) and parental albino generation (refractory to the parasite) took place resulting in the melanic generation F_1. Starting from the melanic generation F_1, the melanic generation F_2 was obtained by self-fertilization. Melanic mollusks of the generation F_1 were obtained starting from eggs of the albino, which served as markers. The mollusks were maintained, individually, being separate from the parental generation during the period of sexual immaturity (around 30 days). Besides the generations F_1 and F_2, melanic mollusks of *B. tenagophila* derived from the non selected parental generation for the susceptible character, were exposed to the miracidia of *S. mansoni*.

The strain of *S. mansoni* was from São José of Campos (SJ), São Paulo and it is maintained by passage in hamsters.

The mollusks were exposed individually to 10 miracidia, for 4 h. The miracidia were obtained from eggs collected from hamsters' liver according to Pellegrino and Katz (1968).

The desiccation technique was performed according to Barbosa (1953, modified): 148 g of brown, sandy, sifted and sterilised earth (at 105ºC for 24 h) were placed, in open polietilene containers (7 cm diameter). A volume of 45 ml of dechlorinated water, enough to maintain the humidity of the earth, was added to each container, which was maintained inside a climatic chamber at 25ºC.

The relative humidity of the earth was measured by the weighing method. From each recipient, three earth samples were weighed on the first day and on the last day of desiccation. The mean weight of the three samples of the last day subtracted from the mean weight of the three samples of the first day gave as result the amount of evaporated water in the earth, in each recipient, during each desiccation period. Assuming the value of the weight of the earth in the first day, as being 100% humidity, by the percentage calculation, the humidity percentage of the earth in the last day of desiccation was obtained and also the evaporated water percentage during the desiccation period.

Five desiccation experiments with melanic mollusks of the F_1, F_2 and non selected parental generations were made (Fig. 1).

For the histological study, 20 mollusks of each group were used (groups I to V, of the non selected parental, F_1 and F_2 generations). The control group consisted of mollusks maintained in water after a exposure to the miracidia of *S. mansoni* for 24 h, 7 days, 14 days, 21 days, 28 days and 39 days – eliminating cercariae (20 mollusks per group, of the non selected parental, F_1 and F_2 generations). Sections of 5 to 7 µm thickness were prepared using an ultramicrotome. Sections were stained using the method of trichromy of gomori (Maia 1979).

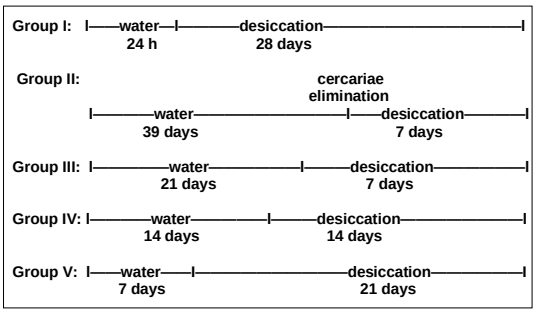


Fig. 1: definition of the experimental groups of *Biomphalaria tenagophila* maintained in water after exposure to miracidia of *Schistosoma mansoni* and submitted to desiccation.

RESULTS

B. tenagophila maintained in water during the period of incubation with larvae of S. mansoni - There was no difference in the development of *S. mansoni* larvae among the mollusks of *B. tenagophila*, of the non selected parental, F_1 and F_2 generations.

Twenty four hours after the exposure to larvae of the parasite - Miracidia were found in the anterior area of the body of mollusks (exposed parts of the body of the mollusks, where the penetration probably occurred). Inside of the larvae it was possible to observe several germinative elements (Fig. 2). Miracidia with cellular reaction provoked by hemocytes, were found in the anterior area of the body of mollusks. In all the cellular reactions, the hemocytes formed concentric layers (granuloma type) around the miracidia. Miracidia partially entire enclosed by granulomas, were found in the tentacles. In the posterior area of these miracidia, germinative elements could be observed. In some miracidia the nervous spot could also be identified (Fig. 3). Partially degenerated larvae were observed

in the tentacles. The germinative structures inside the larvae were not well identified. Degenerated larvae were found in the mantle, in the tentacles, close to the ocular area and in the foot. The larvae were reduced to granules or to a mass. Some hemocytes had invaded the interior of the degenerated larvae.

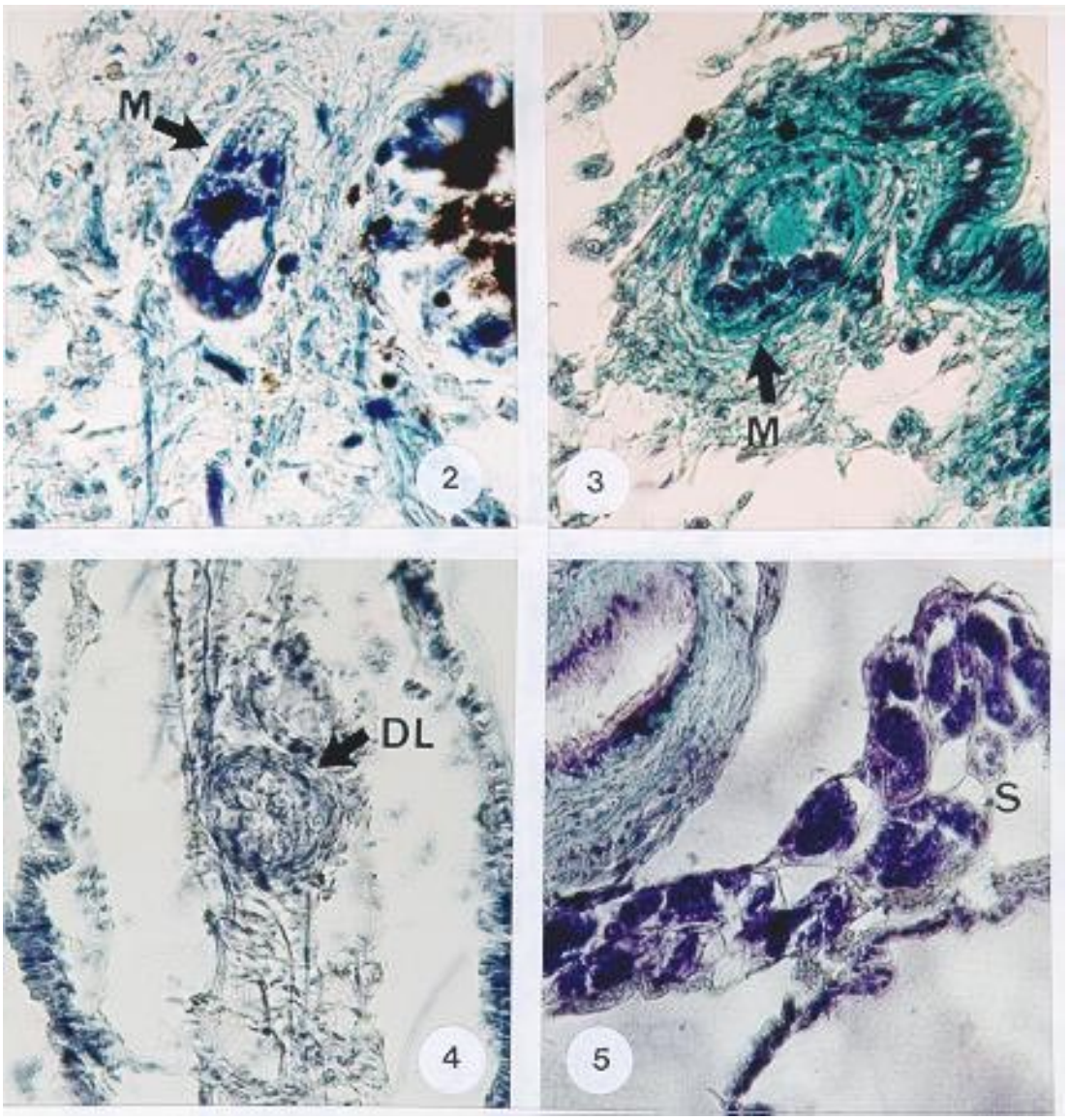
Seven days after the exposure to the larvae of the parasite - Sporocysts I in the anterior area of the mollusks body were observed. Usually the sporocysts I showed an elongated form and were involved, externally, by an epithelium. Internally, in the wall of the sporocysts, there were individual germinative cells. Fibro-muscular deposition was not observed around the wall of the sporocysts I. Some sporocysts I possessed individual germinative cells, individual germinative cells and agglomerated germinative cells with and without covering membrane or only agglomerated germinative cells with and without covering membrane. The individual germinative cells can form cellular agglomerates that, in turn, can compose the future sporocysts II. Sporocyst partially entire enclosed by granuloma were observed in the labial palps. Due to the fact that the cellular reaction occurred at an initial stage, part of the integrity of the larvae was preserved. Partially degenerated larvae were observed enclosed by granulomas, in the foot. It was possible to identify some germinative cells inside these larvae. Degenerated larvae were found enclosed by the granulomas, in the tentacles and in the mantle. Some hemocytes invaded the interior of larvae. It was not possible to identify the structure of the larvae (Fig. 4).

Fourteen days after the exposure to the larvae of the parasite - Sporocysts II were seen in the anterior area of the body of mollusks in proximity to the kidney area. The sporocysts II possessed an elongated form and their body wall was formed by an epithelium covered with muscular fibres. Internally, in the wall of the sporocysts II, there were individual germinative cells. The larvae may present themselves full of individual germinative cells or of individual germinative cells and agglomerated germinative cells with and without covering membrane (Fig. 5). Sporocysts partially entire enclosed by granulomas were observed in the foot and in the pseudobranchia. It was possible to identify the germinative cells inside the sporocysts. Partially degenerated larvae were found, enclosed by granulomas, in the cephalic area, in the mantle, in the pseudobranchia, in the kidney and in the foot. Inside the larvae, some germinative cells were observed. Some hemocytes were observed near the larvae. Degenerated larvae were found, enclosed by granulomas, in the cephalic area, in the tentacles and in the mantle. Due to the advanced stage of degeneration of these larvae it was not possible to identify the germinative cells. Enclosed by granulomas there were larvae reduced to granules or to a mass. Some hemocytes invaded the interior of the larvae.

Twenty one days after the exposure to the larvae of the parasite - Sporocysts II were seen distributed throughout the body of mollusks. Inside the sporocysts II we observed agglomerated germinative cells with and without covering membrane and individual germinative cells, as well as individual or agglomerated germinative cells with covering membrane (Figs 6, 7). In some sporocysts II it could be observed that the cellular agglomerates began to elongate in the posterior area, initiating the formation of the tail of the future cercariae. In another cellular agglomerate a prolongation was observed in the anterior extremity of the larvae that would give rise to the buccal cavity and the oral sucker of the cercariae (Fig. 7). Internally, in the wall of the sporocysts II, individual germinative cells are observed. After 21 days of infection the formation of the cercariae is verified. An asynchrony exists in the development inside of the same sporocyst II. Partially entire sporocysts were observed in the oesophagus and in the stomach. The larvae were found enclosed by granulomas. Partially degenerated larvae enclosed by granulomas, were found in the foot, in the oesophagus, close to the gut, in the pseudobranchias and among the lobes of the digestive gland. The germinative cells of the larvae possess piknotic nucleus. Some hemocytes invaded the interior of the larvae (Fig. 8). Degenerated larvae, enclosed by granulomas, were found in the tentacles, in the foot, in the kidney and in the pseudobranchias. Inside the larvae, hemocytes were also observed with eosinophil and brown granules in the cytoplasm, indicating a possible phagocytosis. In the cephalic area, a larvae with cellular reaction could be observed in a more advanced stage. In this case the germinative cells of the larvae were not identifiable because they were transformed in granules. At this point the hemocytes did not form a granuloma around the larval residues, they remained dispersed.

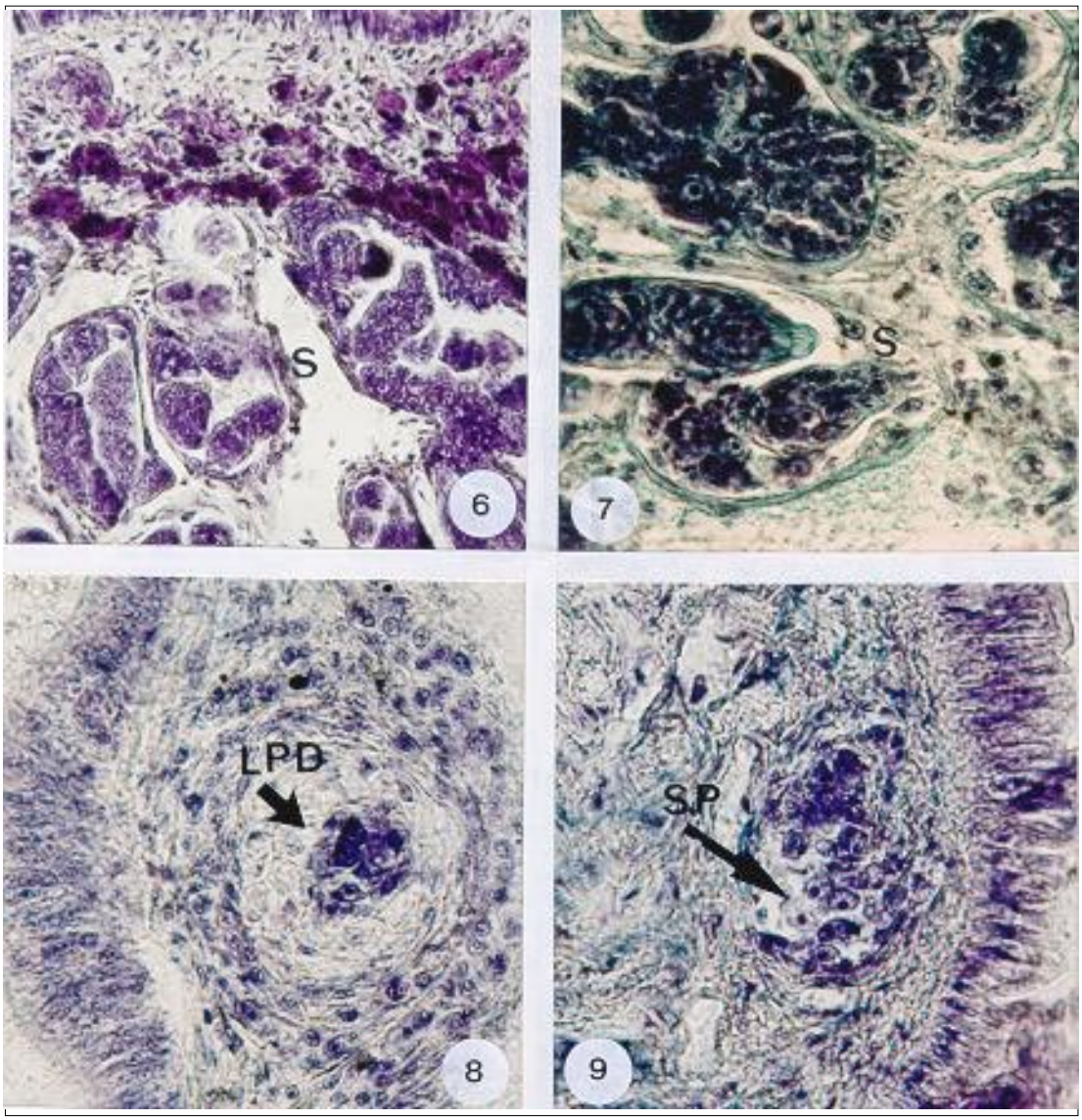
Twenty eight days after the exposure to the larvae of the parasite (mollusks not eliminating cercariae) - Sporocysts II were seen distributed in the body of mollusks. Within the sporocysts II there were agglomerates of germinative cells with covering membrane, individual germinative cells and agglomerated germinative cells with and without covering membrane, agglomerated germinative cells with covering membrane and cercariae in different developmental phases, as well as only cercariae at different stages. Internally, in the wall of the larvae, individual germinative cells are ob-

served. The sporocysts II containing agglomerates of germinative cells and cercariae, as well as those presenting only cercariae were more commonly to be found in the seminal vesicle, among the lobules of the digestive gland and of the ovitestis. In addition to sporocysts II, mature cercariae were seen distributed along the body of mollusks. Sporocysts partially entire enclosed by granulomas, were found in the stomach, in the wall of the intestine and among the lobules of the digestive gland. It was possible to identify the germinative cells inside of the larvae. Considering the time of infection and the location, they were most likely sporocysts II (Fig. 9). Partially degenerated larvae, enclosed by granulomas, were found in the foot, in the mantle, in the cephalic area and among the lobules of the digestive gland. The germinative cells of the larvae could be identified. Degenerated larvae enclosed by granulomas were seen, in the mantle, in the pseudobranchias and in the gland of the albumen. In the mantle, the larva was reduced to a mass. In the gland of the albumen and in the pseudobranchias, the larvae were reduced to granules. Some hemocytes invaded the interior of the larvae.



Biomphalaria tenagophila (Orbigny, 1835) - Figs 2-3: group 24 h in water – 2: miracidium close to the ocular area, 549x; 3: miracidium partially entire in the tentacle, 549x. Fig. 4: group 7 days in water – degenerated larvae in the tentacle, 275x. Fig. 5: group 14 days in water – sporocysts II in the cephalic area, 275x. DL: degenerated larvae; M: miracidium; S: sporocyst II

Eliminating cercariae (mollusks with 39 days of infection) - Sporocysts II were seen distributed in the body of mollusks. Within the sporocysts we observed agglomerated germinative cells with covering membrane, agglomerated germinative cells with and without covering membrane, individual germinative and agglomerated germinative cells with and without covering membrane, agglomerated germinative cells with covering membrane and cercariae at different phases of development or just cercariae at different stages. Internally, in the wall of the sporocysts, individual germinative cells were observed. The sporocysts II containing agglomerated germinative cells and cercariae, as well as those containing cercariae were more frequently found close to the seminal vesicle, among the lobules of the digestive gland and of the ovitestis. This occurrence does not impede the presence in other organs of the mollusks. Mature cercariae were also seen distributed in the body of mollusks (Fig. 10). In sporocyst and cercariae stages, partially entire larvae were found, enclosed by granulomas, in the cephalic area, in the kidney and among the lobules of the digestive gland. Partially degenerated lar-



Biomphalaria tenagophila (Orbigny, 1835) - Figs 6-8: 21 days group in water – 6: sporocysts II in the foot, 275x; 7: sporocysts II in the tentacle, 549x; 8: partially degenerated larvae close to the intestine, 549x. Fig. 9: 28 days group in water – partially entire sporocysts in the wall of the stomach, 549x. LPD: partially degenerated larvae; S: sporocyst II; SP: partially entire sporocyst

vae are observed enclosed by granulomas, among the lobules of the digestive gland and of the ovitestis. Only a few germinative cells with piknotic nucleus could be identified in the interior of the larvae. Degenerate larvae were noted in the cephalic area, in the mantle, in the tentacles, among the lobules of the digestive gland and of the ovitestis (Fig. 11). In the cephalic area larvae reduced to granules could be observed. Granuloma formation was no longer observed. The hemocytes were dispersed close to the larval remains. There were hemocytes around one degenerated larvae, in the tentacle. In the mantle, among the lobules of the digestive gland and of the ovitestis the degenerated larvae were transformed to granules, enclosed by granulomas. Some hemocytes were observed enclosed by granulomas close to the larval remains.

B. tenagophila exposed to the larvae of S. mansoni and submitted to desiccation - Differences in the development of the larvae of *S. mansoni* in not selected parental F_1 and F_2 generations of *B. tenagophila* were not observed, with the exception of group III, where infected larvae were not found in the mollusks of the F_2 generation.

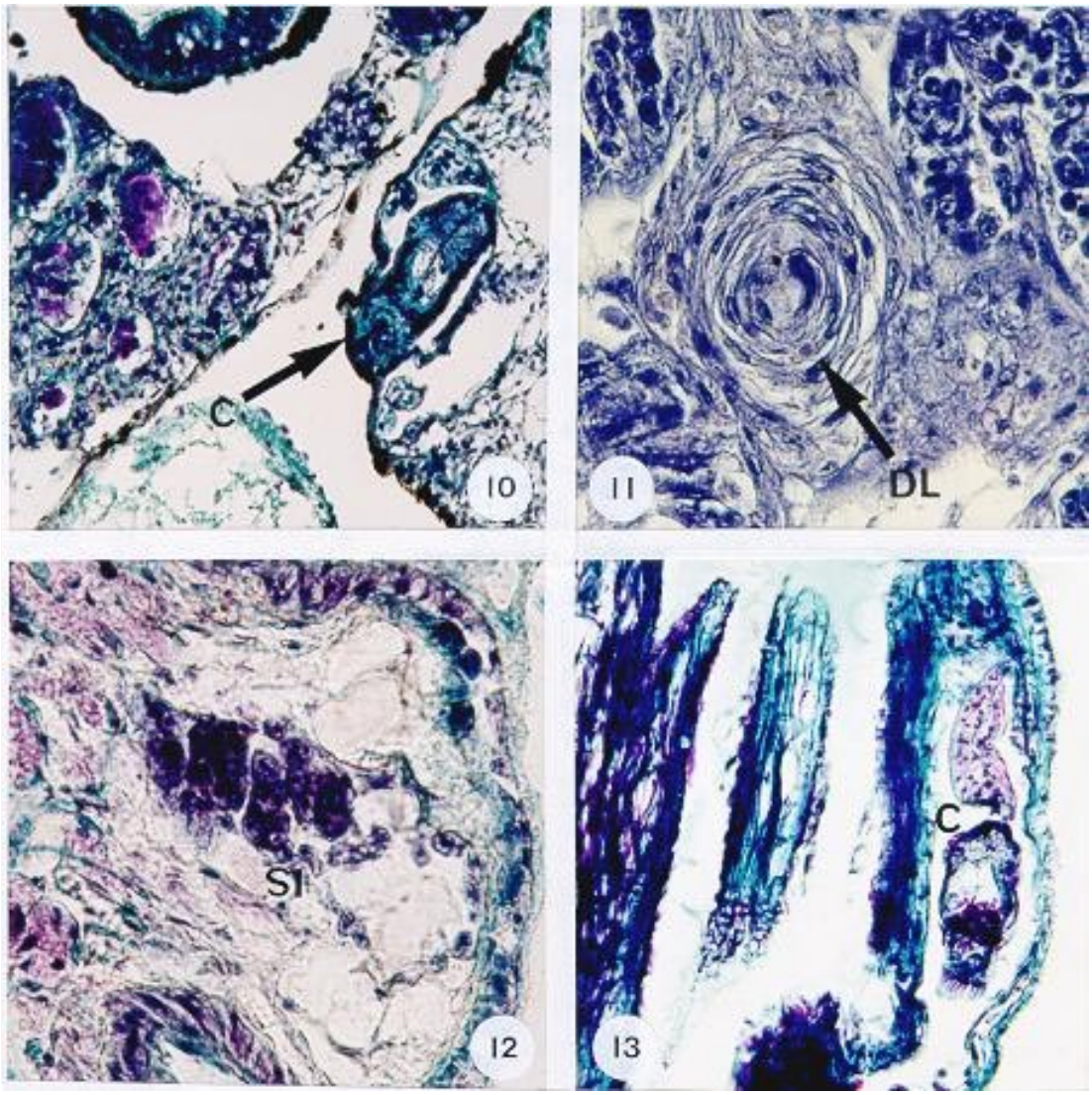
Group I: mollusks maintained in the water during 24 h after the exposure of the larvae to the parasite and desiccated for 28 days - Up to 24 h post exposure to the larvae of the parasite, the mollusks became infected by larvae in the miracidium stage. After being submitted to 28 days of desiccation, some larvae developed to the stage of sporocysts I and others to the sporocyst II. The sporocysts I were found in the anterior area of the mollusks. The larvae possessed agglomerated germinative cells with and without covering membrane, individual germinative cells and agglomerated germinative cells with and without covering membrane or only individual germinative cells. Internally, in the wall of the sporocysts I, individual germinative cells were observed (Fig. 12). The sporocysts II were seen distributed in the body of mollusks. Inside the sporocysts II there were individual germinative cells and agglomerated germinative cells with and without covering membrane, only agglomerated germinative cells with and without covering membrane or just agglomerated germinative cells with covering membrane. Internally, in the wall of the larvae, individual germinative cells could be observed. Partially entire sporocysts, enclosed by granulomas, were found in the tentacles. In the larvae it was possible to observe germinative cells. Partially degenerated larvae were found enclosed by granulomas, in the tentacles, in the mantle and in the foot; inside these larvae some germinative cells could be identified. In the cephalic area, in the mantle, in the foot, in the pseudobranchias and among the lobules of the ovitestis there were degenerated larvae enclosed by granulomas, transformed in granules. Some hemocytes invaded the interior of the larvae. In the foot and in the cephalic area there was one degenerated larvae reduced to a mass, enclosed by granulomas.

Group II: mollusks maintained in the water until the cercariae elimination (with 39 days of infection) and desiccated for 7 days - There are sporocysts II distributed in the body of mollusks. The sporocysts II possessed agglomerated germinative cells with covering membrane, agglomerated germinative cells with and without covering membrane, agglomerated germinative cells with covering membrane and cercariae at different stages of development. Sporocysts II containing agglomerated germinative cells and cercariae or only cercariae were more frequently found among the lobules of the digestive gland and of the ovitestis. Internally, in the wall of the larvae, individual germinative cells existed. Besides sporocysts II, mature cercariae were seen distributed in the body of mollusks (Fig. 13). Some mollusks of the not selected parental generation (5%) did not possess infected larvae in their tissue after desiccation for 7 days. Partially degenerated cercariae were found, enclosed by granulomas, in the cephalic area, in the mantle, in the tentacles, in the wall of the palial cavity and in the pseudobranchias. It was possible to identify the general structure of the larvae in spite of the stage of degeneration of the cercariae. Degenerated larvae, transformed in granules and enclosed by granulomas, were seen in the cephalic area, in the tentacles, in the mantle, in the wall of the palial cavity, in the pseudobranchias and among the lobules of the ovitestis. Hemocytes were observed near the remains of larvae. Again in the pseudobranchias, degenerated larvae reduced to granules were observed, not enclosed by granulomas. The hemocytes were dispersed close to the larval remains.

Group III: mollusks maintained in the water until 21 days after the exposure to the larvae of the parasite and desiccated for 7 days - In this group were seen larvae of the not selected parental and F_1 generations. In the F_2 generation, larvae were not observed infecting the organs of the mollusks. Sporocysts II are seen distributed in the body of mollusks. The sporocysts II possessed individual germinative cells and agglomerated germinative cells with and without covering membrane, only agglomerated germinative cells with and without covering membrane or just agglomerated germinative cells with covering membrane. In the cephalic area, in the mantle, near to the ocular area and in the tentacles, sporocysts II were also observed full of individual germinative cells. Among the lobules of the digestive

gland and of the ovitestis, sporocysts II containing cercariae at different stages or agglomerated germinative cells with covering membrane and cercariae at different development stages were seen (Fig. 14). Internally, in the wall of the larvae, individual germinative cells existed. Partially entire sporocysts and partially degenerated larvae, enclosed by granuloma, were found in the mantle. It was possible to identify some germinative cells inside the larvae. Hemocytes were found close to larvae. Degenerated larvae were found enclosed by granuloma, in the wall of the palial cavity, among the lobules of the digestive gland and of the ovitestis. The degenerated larvae were reduced to granules.

Group IV: mollusks maintained in the water during 14 days after the exposure to the larvae of the parasite and desiccated for 14 days - Sporocysts II were seen distributed in body of mollusks. The sporocysts II possessed individual germinative cells and agglomerated germinative cells with and without covering membrane or only agglomerated germinative cells with and without covering membrane. Sporocysts II containing individual germinative cells were seen in the cephalic area, in the mantle, in the foot, in the tentacles and in the wall of the palial cavity. Sporocysts II containing agglomerated germinative cells with covering membrane and cercariae in different development



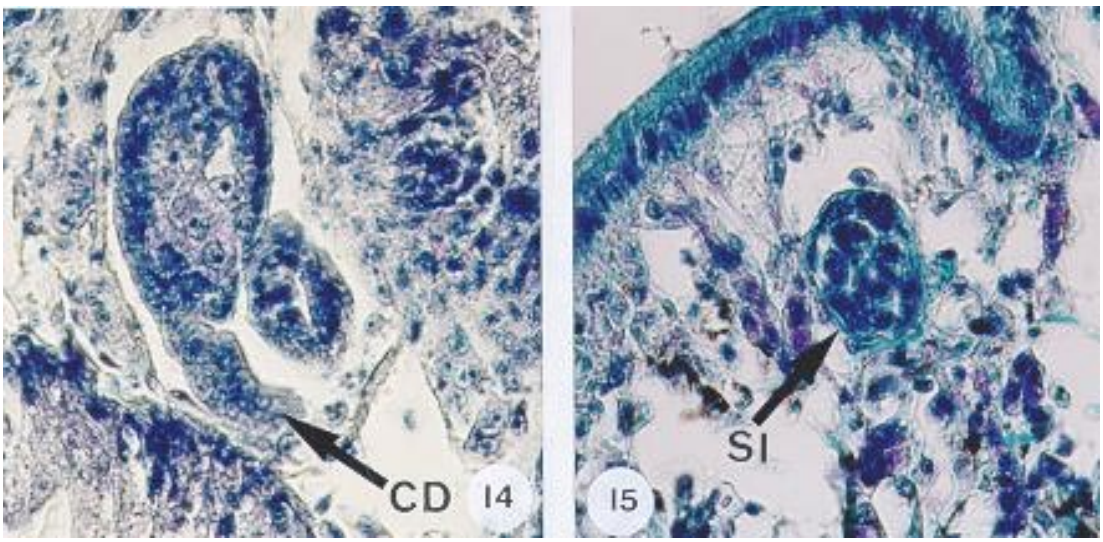
Biomphalaria tenagophila (Orbigny, 1835) - Figs 10-11: eliminating cercariae group (39 days in the water) – 10: cercariae in kidney, 275x; 11: degenerated larvae among the lobes of the digestive gland, 549x. Fig. 12: group I – sporocysts I in the foot, 549x. Fig. 13: group II –cercariae in the foot, 275x. C: cercariae; DL: degenerated larvae; SI: sporocyst I

stages were found close to the oesophagus, to the stomach, to the intestine, in the gland of the albumen, in the seminal vesicle, and among the lobules of the digestive gland and of the ovitestis. Internally, in the wall of the larvae, individual germinative cells can be found. Partially degenerated larvae enclosed by granuloma, could be observed in the tentacles and in the mantle. Some germinative cells inside of the larvae were identified. Degenerated larvae enclosed by granuloma, were found in the cephalic area, in the tentacles, in the foot, in the mantle, in the kidney and among the lobules of the digestive gland. Some hemocytes were found inside the larvae. It was not possible to identify the germinative cells of the larvae which were reduced to granules. In the foot, one degenerated larvae reduced to granules was found. In this case, the hemocytes no longer formed a granuloma, they were dispersed close to the larval remains.

Group V: mollusks maintained in the water during 7 days after the exposure to the larvae of the parasite and desiccated for 21 days - Sporocysts I were seen in the anterior area in the body of the mollusks. The sporocysts I possessed individual germinative cells and agglomerated germinative cells with and without covering membrane. Internally, in the wall of the larvae, individual germinative cells could be seen (Fig. 15). Sporocysts II were seen distributed in the body of the mollusks. The larvae possessed individual germinative cells and agglomerated germinative cells with and without covering membrane. Internally, in the wall of the larvae, individual germinative cells were observed. Partially entire sporocysts could be seen enclosed by granulomas, in the foot. Partially degenerated larvae enclosed by granulomas, were observed in the foot, among the lobules of the digestive gland and of the ovitestis. Some germinative cells, inside the larvae, were identified. Degenerated larvae were found enclosed by granulomas, in the cephalic area, in the labial palps, in the ocular area and among the lobules of the ovitestis. The larvae were reduced to a mass. Some hemocytes invaded the interior of the larvae. Many eosinophil granules were seen in the cytoplasm of the hemocytes, probability remains of larvae.

DISCUSSION

In group I, the stage of development of the larvae of the parasite, before 28 days of desiccation, was the same as that of the larvae in the mollusks of the 24 h control group, that is, they were at the miracidium stage. The miracidia were found in the exposed parts of the body of the mollusks, where probably, the penetration occurred. Coelho (1957) observed that in *B. glabrata* the miracidia can travel through lymphatic spaces, lodging in deeper areas such as the buccal bulb and the foreskin. According to Maldonado and Acosta-Matienzo (1947) the course accomplished by the miracidia depends on their available energy reserve after the penetration. After 28 days of desiccation, some miracidia were developed to the stage of sporocysts I and others to sporocysts II.



Biomphalaria tenagophila (Orbigny, 1835) - Fig. 14: group III – sporocysts II among the lobes of the digestive gland, containing cercariae in development, 549x. Fig. 15: group V – sporocysts I in the mantle, 549x. CD: cercariae development; SI: sporocysts I

The sporocysts I were located in the anterior area of the body of the mollusks, possibly in the places where the penetration of the miracidia occurred. The absence of the fibro-muscular deposition in the sporocysts I justifies that these larvae do not move about (Guaraldo et al. 1981), with development taking places where the penetration of the miracidia occurred.

The development of some miracidia was not interrupted during the 28 days desiccation period, being verified only a delay in the development of those that were developed to the sporocysts I stage. Considering the amount of time since the exposure to the end of the desiccation (24 h in the water, added of 28 days of desiccation), the larvae should have developed, at least, to the stage of sporocyst II.

The stage of development of the sporocysts II, in the group I, was the same as that of some sporocysts II observed in the 28 days control group. Similarity, they were found dispersed throughout the organs of the mollusks. The muscular layer that covers the wall of the larval body, allows the sporocysts II to migrate through the organs of the mollusks (Coelho 1995). In this case, apparently, the desiccation did not affect the development of these larvae. In the 28 days control group, besides sporocysts II as in the group I, sporocysts II in more advanced stages were observed as well as mature cercariae dispersed throughout the organs of the mollusks. Although there were already mature cercariae distributed throughout the organs, these mollusks are not yet at the phase of elimination of the larvae. That should probably be about to happen. Some sporocysts II of the group I did not reach the stage of development of certain sporocysts II observed in the 28 days control group, demonstrating a delay in their development.

Barbosa and Coelho (1953) did not observe, in *B. glabrata*, entire larvae nor cellular reactions in the tissues of mollusks desiccated, soon after the exposure for the *S. mansoni*, for more than 22 days. According to the authors after the period of 22 days of desiccation it is possible to cure the infected mollusks.

In *B. tenagophila* entire larvae and larvae with cellular reaction were observed after 28 days of desiccation (desiccated commenced 24 h post exposure). However, these results can not be compared to those obtained by Barbosa and Coelho (1953), due to the differences in the methodology used in the two studies. *B. glabrata* was desiccated on sand, in mud vases. It is believed that due to the fact of being transferred from water and placed on the earth, the mollusks suffer a state of sudden stress, leading to increated parasite and mollusk death rates. In contrast to what happens with *B. tenagophila*, the survival chances are increased when the mollusks are buried in humid sand, entering gradually in desiccation (as the water is being evaporated from the earth).

In group II differences were not observed in the development of most of the larvae (after the 7days of desiccation to which they were submitted), in relation to what was observed in the eliminating cercariae control group (39 days).

It is believed that a pause can occur in the development of some of these sporocysts II and cercariae, when the mollusks were submitted to the desiccation. After their return to water, many of these larvae succeeded in following their natural development.

In *B. glabrata* eliminating cercariae, Barbosa and Coelho (1955) observed that desiccation (from 21 to 60 days) interrupts the development of the sporocysts II and of the cercariae, frequently causing the death of the mollusk. They also showed that mollusks resistant to desiccation became cured of the infection. In our experiments, 5% of mollusks from the non selected parental generation of the group II, were observed free of infected larvae in their tissues after desiccation so, they may be considered cured of the infection.

In group III differences as to the location of the sporocysts II were not observed, in relation to what was seen in the 21 days control group and in the 28 days control group. In general, most sporocysts II had already migrated to the organs of mollusks, when they were desiccated. In this group larvae of the generation F_2 were not found infecting the mollusks. But this fact does not mean that this generation was unable to develop. The larvae with 21 days were also found to be more sensitive to the desiccation in the non selected parental and F_1 generations. It is worth pointing out that in the other desiccation groups differences are not observed in the development of the larvae among mollusks of the three generations. Some sporocysts II (containing agglomerated germinative cells and individual germinative cells as well as only agglomerated germinative cells) were found, after the period of 7 days of desiccation, in the same stage of development as the sporocysts II observed in the 21 days control group. In these cases, a pause may have occurred in the development of the sporocysts, during the desiccation. Sporocysts II that apparently did not suffer the action of the desiccation were observed (they contained agglomerates of germinative cells and cercariae or only cercariae). Sporocysts II in these two development phases were also found in the mollusks of the 28 days control group. Mature cercariae, present in the 28 days control group, were not found in the mollusks of group III, this fact allows the interpre-

tation that a delay in the development of some larvae took place.

In group IV, some sporocysts II (containing individual germinative cells), at the end of the 14 days of desiccation, remained at the same development stage that they supposedly presented at the end of the 14 days in the water (14 days control group). Indeed, they were located in the anterior area of the body of the mollusks, the same form found in the 14 days control group. This indicates that they may not have been migrating and that a pause in the development may have occurred. However, other sporocysts II apparently did not suffer from the effects of desiccation and continued their development (containing individual germinative cells and agglomerated germinative cells, only agglomerated germinative cells or then agglomerated germinative cells and cercariae). Sporocysts II, at these same stages, were observed in the mollusks of the 28 days control group. The frequent presence of sporocysts II, containing agglomerated germinative cells and cercariae, near the oesophagus, the stomach and the intestine, may indicate that the desiccation, although having not impeded the development of the larvae, had hindered their migration. In general, the formation of cercariae inside the sporocysts II occurs when they reach the digestive gland and the ovitestis. It is in these two organs that the sporocysts II usually matures, liberating the cercariae. This is not a general rule as in many cases, sporocysts II also occurs, at this same stage reaching maturity in organs of the anterior area of mollusks. Such phenomena were observed in the mollusks of the 28 days control and eliminating cercariae groups. In these cases, when the digestive gland and the ovitestis (organs rich in nutrients) are hyperinfected, the larvae are forced to find space and nutrients in another organs. A competition for nutrient reserves produced by mollusks may exist among the larvae. In spite of having identified sporocysts II containing agglomerated germinative cells and cercariae, it was not possible to observe sporocysts II containing only cercariae, as was observed in the 28 days control group, which demonstrates a delay in development.

In group V, some larvae developed to the stage of sporocyst I, the same stage 7 for larvae in the 7 days control group. The desiccation, in spite of not having impeded, probably caused a pause in the development of the sporocysts I found in group V. The sporocysts I, of the group V and of the 7 days control group, were located in the anterior area of the mollusks. Experiments with desiccation in mollusks of *B. glabrata*, containing larvae of sporocyst I in the initial stage and of sporocyst I showing in their interior sporocysts II in formation, were accomplished by Barbosa and Coelho (1955) and Lancastre et al. (1987). Theses authors also observed a pause in the development of the larvae. In the present study, it was not observed in the group V sporocysts II containing agglomerated germinative cells and cercariae or only mature cercariae, as found in the mollusks of the 28 days control group. In these cases, there was a delay in the development of the sporocysts II.

Badger and Oyerinde (1996) performed desiccation experiments with infected *B. pfeifferi*, which were very similar to those presented in this work. The authors submitted the mollusks to 7, 14, 21 or 28 days of desiccation, after 24 h, 7 days, 14 days, 21 days and 28 days of exposure to larvae of *S. mansoni*. Among the experiments with *B. pfeifferi* that correspond to those undertaken with *B. tenagophila*, only the group of mollusks maintained in the water during 21 days and desiccated for 7 days survival rate was obtained. However, it was not possible for those authors to observe the development of the larvae, because the mollusks died soon after the end of desiccation. In other experiments, Badger and Oyerinde (1996) observed that the development of the cercariae was delayed because of the prolonged desiccation period of the mollusks. The same authors affirmed that the development of the parasite in *B. pfeifferi* occurs usually until the 7th day of desiccation where up on it ceases, to resume only by the introduction of the mollusk to water again. In *B. tenagophila*, with 7 days of desiccation, we observed larvae that did not suffer from the action of desiccation, showing normal development (group III), and larvae that delayed their development during the period when the mollusks were desiccating (II and III groups).

Some larvae of *S. mansoni* entered a state of dormancy and interrupted their development temporarily inside the mollusks, when submitted to desiccation.

According to Rey (1991) and Jourdane et al. (1980), in addition to cercariae, the sporocysts II are able to generate other sporocysts generations (III, IV...). In this case, the sporocyst I would form the sporocysts II, that would give rise to the cercariae and sporocysts III, which would give rise to new cercariae and sporocysts IV, and so on.

Jourdane et al. (1980) stated that the reproduction of sporocysts II, III, IV,... is not a natural mechanism in the biological cycle of parasite, but rather an exceptional one.

We can suppose that this reproduction mechanism would explain the observation that some mollusks continue to eliminate cercariae for prolonged period (we observed mollusks eliminating cercariae for 81 days). It was possible to observe, in these mollusks, a pause in the elimination of the

cercariae, returning to eliminate again a few later days. Perhaps this pause may have occurred during the period of maturation of possible sporocysts III. And after their matured, they eliminated a new cercariae generation.

This hypothesis is in agreemet with the ideas of Maldonado and Acosta-Matienzo (1947) who stated that the initial stage of formation of cercariae inside a sporocyst is very similar to the stage of formation of new sporocysts, with it being difficult to distinguish the two forms.

Stages of cellular reactions in larvae subject to the action of hemocytes - The presence of the larvae in the tissue of the mollusks can stimulate the concentration of hemocytes around them. When the hemocytes concentrate around the larvae they form granuloma type structures (Borges et al. 1998). The hemocytes are small phagocytic cells with basophil cytoplasm and varied forms, due to pseudopodia emission. The hemocytes are responsible for the ingestion and destruction of particles or foreign micro organisms in the bodies of the mollusks (Coelho 1957).

Three stages of cellular reactions were observed: (1) Initial stage: in this phase the larva is partially entire. The germinative cells can be identified. The integrity of the larva is preserved as the cellular reaction is at its initial stage; (2) Intermediary stage: at this stage the larva is considered partially degenerated, because in most cases it is not possible to identify, with certainty, its developmental stage. In general, some germinative cells with piknotic nuclei are observed in the larvae. There are cases when the hemocytes invade the interior of larvae. According to studies performed by Coelho (1957), in *B. tenagophila*, in this stage of the cellular reaction the first histolysis signs of are observed, beginning the phagocytic process; (3) Developed stage: at this point the larvae are degenerated. Due to the advanced stage of degeneration, it is not possible to identify the phase of the development of the larvae. The larvae can be transformed in granules or in a mass. In general, hemocytes close to the larvae are observed. In more advanced stages of degeneration, larvae reduced to granules are observed, around which hemocytes do no longer exist. In these cases, the hemocytes generally no longer form granulomas, staying dispersed close to the larval remains. The hemocytes are observed in full phagocytic process. Following this process, the hemocytes return to the blood stream and leave no traces of a possible cellular reaction in the tissue of the mollusks (Coelho 1957).

Miracidia with cellular reaction were not found in mollusks of the group I and of the 28 days control group, only in the 24 h control group. The absence of miracidia with cellular reaction in the mollusks of the first two groups is due to the period that covered the exposure of the larvae of parasite to the end of the desiccation in the case of the group I, as well as in the 28 days when the mollusks stayed in the water, in the case of the 28 days control group. Probably, in case that there were miracidia with cellular reaction in these two groups, after 28 days of desiccation (group I) or after 28 days in the water (28 days control group), the miracidia would be already totally degenerated.

In the mollusks of the group II and of the eliminating cercariae control group cellular reaction in the cercariae stage was more commonly found. These cercariae demonstrate an increased sensitivity, mainly in the mollusks submitted to desiccation. Therefore, the amount of cercariae with cellular reaction was larger in the group II than in the eliminating cercariae control group.

In all the desiccated groups (I to V) the larvae with cellular reaction located in the anterior area of mollusks surpassal the levels found in deeper organs. Guaraldo et al. (1981) in *B. tenagophila*, Maldonado and Acosta-Matienzo (1947) and Olivier and Mao (1949) in *B. glabrata* also observed larvae with degenerative processes at greater frequency in the anterior area of the body of mollusks. Guaraldo et al. (1981) also observed, with more than 49 days of infection, cellular reactions in sporocysts I and also in sporocysts II, in the location occupied by the same sporocysts I that originated sporocysts II.

The survival of the parasite larvae, in the tissues of mollusks, is influenced by on its genetic constitution, that can or can not be compatible with that of the mollusks, and on its adaptive capacity inside the mollusks (Paraense & Corrêa 1963, Basch 1975, Guaraldo et al. 1981, Richards et al. 1992).

From the data recorded in this study it was verified that desiccation was not capable of interrupting the development of larvae of *S. mansoni* in mollusks. We observed a delay in the development of larvae of *S. mansoni* in groups I, III, IV and V. A pause was verified in the development of larvae of *S. mansoni* in groups II, III, IV and V. Some larvae, in groups I, III, IV and V, did not suffer as a result of desiccation and continued their development. Larvae in the cercariae stage were found to be more sensitive to desiccation. It was possible to obtain curing of mollusks infected by sporocysts II and cercariae after a period of 7 days of desiccation.

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