

# Behavior in *Mus musculus* of *Schistosoma mansoni* from mollusks treated with hydrocortisone

## Comportamento em *Mus musculus* do *Schistosoma mansoni* oriundo de moluscos tratados com hidrocortisona

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### ABSTRACT

Twenty mice were exposed to cercariae from mollusks treated with hydrocortisone and another 20 mice received cercariae from non-treated mollusks. The behavior of the parasites from the two groups of mollusks was compared based on the ability of cercariae to penetrate mice, on the total number of worms recovered after eight weeks of infection, on the relationship between the number of penetrating cercariae and the number of recovered worms and on the number of eggs in the feces. Treating the mollusks with hydrocortisone did not alter the ability of cercariae to penetrate mice nor did it affect the total number of worms recovered. The number of female worms, the number of coupled worms and the number of eggs in the feces were greater in mice infected by cercariae from mollusks treated with hydrocortisone.

**Key-words:** *Schistosoma mansoni*. *Mus musculus*. Hydrocortisone. Cercariae. Eggs.

### RESUMO

Vinte camundongos foram expostos a cercárias oriundas de moluscos tratados com hidrocortisona e outros vinte receberam cercárias de moluscos não tratados. O comportamento dos parasitas dos dois grupos foi comparado com base na habilidade das cercárias em penetrar nos camundongos, no número total de vermes recuperados, após oito semanas de infecção, na relação entre o número de cercárias penetrantes e o número de vermes recuperados e o número de ovos nas fezes. O tratamento dos moluscos com hidrocortisona não alterou a habilidade das cercárias em penetrar nos camundongos nem afetou o número total de vermes recuperados. O número de vermes fêmeas, o número de vermes acasalados e o número de ovos nas fezes aumentaram em camundongos infectados por cercárias de moluscos tratados com hidrocortisona.

**Palavras-chaves:** *Schistosoma mansoni*. *Mus musculus*. Hidrocortisona. Cercária. Ovos.

The susceptibility of mollusks to infection by *Schistosoma mansoni* has a strong genetic component that determines the mollusks' metabolic and physiologic ability to interact with the parasite<sup>12,14</sup>. For each gene contributing to mollusk susceptibility there is a corresponding gene that determines parasite pathogenicity<sup>16</sup>. Cellular and humoral mechanisms are involved in the susceptibility of snails to trematode sporocysts<sup>14</sup>. In snails resistant to *S. mansoni* an intense hemocytic reaction occurs around the sporocysts and eventually leads to their death, whereas

in susceptible snails, the reaction is mild and most of the sporocysts remain viable<sup>2,3,5,12</sup>.

We have previously demonstrated that hydrocortisone increases the susceptibility of *Biomphalaria glabrata* to *Schistosoma mansoni*<sup>17,18</sup>. This increased susceptibility is associated with a reduction in number of hemocytes in the hemolymph and, consequently, little or no hemocytic reaction to *S. mansoni*. Susceptible snails carry a higher number of viable sporocysts which mature more rapidly, thereby increasing the infection rate through

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a greater increase of cercariae. Greater susceptibility of the snails means that more cercariae can penetrate the definitive host to produce a higher number of adult worms<sup>21</sup>. Consequently, we have examined the behavior in mice (*Mus musculus*) of *S. mansoni* obtained from *B. glabrata* treated with hydrocortisone compared to those from mollusks not treated with hydrocortisone.

## MATERIAL AND METHODS

*Biomphalaria glabrata* derived from specimens collected in Belo Horizonte (MG, Brazil) and housed in the Departamento de Parasitologia, Universidade de Campinas (UNICAMP), were used. Two experimental groups consisting of 52 mollusks each were studied: group A comprised infected mollusks not treated with hydrocortisone and group B infected mollusks treated with hydrocortisone<sup>17</sup>. The snails of both groups were infected on the second day of hydrocortisone treatment and in group A by exposing each snail to 10 miracidia of *S. mansoni* strain BH. Four weeks after exposure to miracidia, the snails were exposed to light and heat in order to obtain the cercariae needed to infect the mice. Mice were infected with *S. mansoni* cercariae from *B. glabrata* treated (Group I) or untreated (Group II) with hydrocortisone. Each group consisted of 20 female Swiss mice (~18g, 30 days old) obtained from the Central Animal House at UNICAMP. The mice were infected by exposing the tail of each mouse to 60 cercariae for 2h. After exposure of the mice to *S. mansoni* cercariae, the number of penetrating cercariae was determined as the difference between the number of cercariae in each mouse and the number of non-penetrating cercariae<sup>8</sup>.

The number of eggs in the feces of infected mice was determined weekly starting from the fourth week of infection using Kato-Katz method<sup>6</sup>.

After eight weeks of infection, the surviving mice were sacrificed and the worms present in the mesenterium were collected by perfusion of the portal-mesenteric system<sup>19</sup> and crushing the liver between glass plates. The worms were classified according to sex and the number of coupled worms was recorded.

Statistical analysis was realized through *linear* generalized models.

## RESULTS

Treatment of the mollusks with hydrocortisone did not alter the ability of cercariae to penetrate the host ( $p = 0.0737$ ) (Table 1).

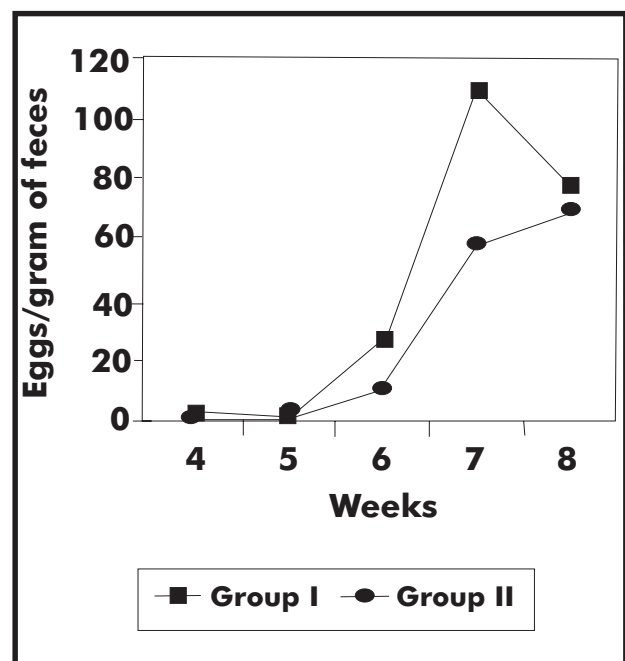
There was no significant difference ( $p = 0.1332$ ) in the number of penetrating cercariae between groups, or in the number of adult worms recovered from mice of both groups after eight weeks of infection (Table 1). However, the number of mated worms ( $p < 0.0001$ ) and the total number of females were greater in mice infected by cercariae from snails treated with hydrocortisone, whereas the number of male worms was lower in this group ( $p < 0.001$ ) (Table 1).

**Table 1 - Number of penetrating cercariae and recovered worms in Swiss mice exposed to 60 cercariae of *Schistosoma mansoni* from *Biomphalaria glabrata* treated (Group I) or untreated with hydrocortisone (Group II).**

	Group I*	Group II*
Penetrating cercariae	54±6	55±3
Worms		
female	12±6	4±1
male	12±10	27±12
mated	8±4	6±4
total	41±14	43±12
recovered (%)	75±24	77±21

\* Mean ±SD

Mice infected with cercariae from mollusks treated with hydrocortisone (Group I) were the first to release *S. mansoni* eggs in their feces (fourth week of infection). These rodents shed a greater number of eggs/gram of feces each week ( $p < 0.001$ ) (Figure 1). At the end of the experiment, no difference was observed between the two experimental groups in relation to the ratio of number of eggs per gram of feces and the number of couples ( $p = 0.1736$  and the survival of the mice ( $p = 0.0909$ ).



**Figure 1 - Average weekly number of *Schistosoma mansoni* eggs expelled in the feces of mice infected with cercariae from *Biomphalaria glabrata* treated (Group I) or untreated (Group II) with hydrocortisone.**

## DISCUSSION

Cercariae of the BH strain penetrate mouse skin with more facility than those of the SJ strain, perhaps due to susceptibility of the intermediate host<sup>7 10</sup>. Although the hemocytic reaction around sporocysts is modest or absent in mollusks treated with hydrocortisone, and therefore similar to mollusks genetically selected for their susceptibility, the ability of cercariae produced in these mollusks to penetrate the tegument of mice was not

greater than that of cercariae from non-treated mollusks. A greater ability to penetrate for cercariae from *B. glabrata* genetically selected for their susceptibility has been reported<sup>21</sup>. In schistosomiasis, the adult parasitic load is less important since the pathogenesis is more related to the presence of granulomatous reactions around the parasite eggs. Thus, BH strain is more pathogenic than SJ strain because of the greater number of hepatic granulomas caused, though the number of worms produced by both strains is the same<sup>9 10 11</sup>. In a study of the development of various geographical strains of *S. mansoni*, it was verified that the Egyptian strain was less pathogenic, despite the large number of worms produced in mice<sup>15</sup>. In our experiments, the total number of worms in infected mice was not related to the susceptibility of mollusks in which the cercariae developed, since mice infected by cercariae from mollusks treated with hydrocortisone (more susceptible) did not present a greater number of worms. However, cercariae from more susceptible mollusks (mollusks treated with hydrocortisone) produced a greater number of female schistosomes and of coupled worms. The smaller number of male worms offset the increase in females to yield similar total numbers of worms in the two experimental groups. In *S. mansoni* infections, males frequently outnumber females.

Predominance of male worms may be related to some evolutionary singularity of the mollusk's larval stages, or to the greater vulnerability of female cercariae to destruction by the vertebrate host's defense mechanisms<sup>13</sup>. In the first of these hypotheses, the production of cercariae that result in female worms would be less intense than that of larvae that yield male worms. This difference could result from a slower release by the mollusks of cercariae for female worms such that over a given period a smaller number of females emerge compared to males. In this case, the infection of mollusks by larvae that will develop into females will last longer than infections by larvae that will give rise to males<sup>13</sup>. Our results confirmed the first hypothesis. In previous experiments<sup>17 18</sup>, we observed that mollusks treated with hydrocortisone were the first to eliminate cercariae. Since cercariae that gave rise to females emerged from mollusks treated with hydrocortisone more rapidly than non-treated mollusks, the percentage of female cercariae available to infect rodents would be greatest with the former group of mollusks.

Mating worms were observed mainly in the mesenteric vein, regarding the worms' displacement in preparation for mating and oviposition<sup>20</sup>. The greater number of coupled worms reflected the equal proportions between sexes and was not observed in mice infected with cercariae from mollusks untreated with hydrocortisone. This finding is important to the biology of schistosomes, since the somatic and germinative development of *S. mansoni* is dependent on the presence of both sexes and contact of the female with the male reproductive canal during mating is fundamental to development of the females. Hence, the females generally return to the male reproductive canal after oviposition<sup>20</sup>. The greater number of mating worms in the infections by cercariae from mollusks treated with hydrocortisone is an important factor in pathogenesis since the eggs produced by worms are responsible for unchaining the illness.

Treated mollusks showed a greater rate of infection and a greater release of cercariae. Since there was no difference in the number of penetrating cercariae, we concluded that the greater ease with which these larvae developed<sup>17 18</sup> did not interfere with their ability to penetrate. Mice infected by cercariae from treated mollusks had a greater number of female worms and coupled worms and a smaller number of male worms, although the total number of worms was similar in both groups of mice. The mice infected by cercariae from treated mollusks released a greater number of eggs/gram of feces ( $p < 0.001$ ), probably because of the larger number of mated worms ( $p < 0.0001$ ), although the ratio of the number of eggs/gram of feces from coupled worms was not significantly different between the experimental groups ( $p = 0.1736$ ). These findings corroborate those of other workers who have demonstrated that the conditions under which *S. mansoni* larvae develop within the mollusks affect the behavior of adult worms in the definitive host<sup>15 21</sup>.

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