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Biological, biochemical and histopathological features related to parasitic castration of *Biomphalaria glabrata* infected by *Schistosoma mansoni*

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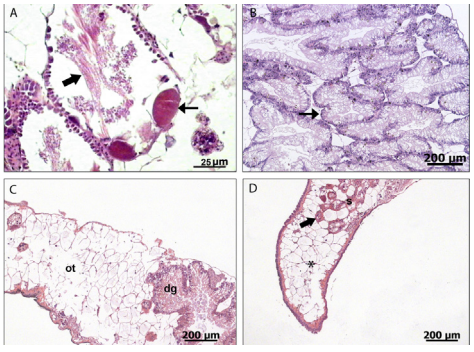
HIGHLIGHTS

- Snails experiencing cercarial shedding showed a reduction in fecundity and fertility.
- The haemolymph glucose levels showed an oscillating pattern.
- Histopathological examination showed that the ovotestis was highly atrophied.
- Sulphated carbohydrates were observed in albumen gland of infected animals.
- The process of parasitic castration was confirmed.

GRAPHICAL ABSTRACT



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ABSTRACT

Parasitic castration in the snail-trematode relationship can be understood as any change in the reproductive function of the snail that is due to interference by the developing larvae inside the snail that leads to the reduction or complete disruption of egg-laying activity. This study was designed to observe the parasitic castration of *Biomphalaria glabrata* infected with *Schistosoma mansoni* during both the pre-patent and patent periods. The effect of infection on snail fecundity and fertility, growth rate and survival was studied during the 62 days following miracidia exposure. An integrated approach was employed that used biochemical and histological tools over the same period.

To study the effect of infection on reproduction, we individually exposed 30 snails to 5 miracidia each and tracked their fertility and fecundity. For our histopathological studies, 50 snails were exposed to 20 miracidia each, and for our histochemical studies, 50 snails were exposed to 5 miracidia each. An equal number of uninfected snails were used as a control for each group. The *B. glabrata* exposed to the BH strain of *S. mansoni* showed 50% positivity for cercarial shedding. Both the experimental and control groups showed 100% survival. The pre-patent period lasted until 39 days after exposure to miracidia. Exposed snails that showed cercarial shedding exhibited higher growth rates than either exposed snails that did not demonstrate cercarial shedding or uninfected controls. Exposed snails without cercarial shedding and uninfected controls showed no differences in the reproductive parameters evaluated

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during the patent period; snails experiencing cercarial shedding showed a reduction in fecundity and fertility. These snails began to lay eggs only after the 50th day post miracidia exposure. The haemolymph glucose levels showed an oscillating pattern that decreased during periods of greater mobilisation of energy by the larvae and was accompanied by a depletion of glycogen in the cephalopodal mass and digestive gland. Histopathological examination at 55 days showed that the ovotestis was highly atrophied. There was almost complete disappearance of germ cells, and the supporting stroma formed a nearly empty net. At day 45, the infected digestive gland showed a high cylindrical epithelium with little preserved cytoplasm. The contents of the secretory granules of the albumen gland of infected animals stained with Alcian blue (AB), pH 1.0, indicating the presence of sulphated carbohydrates. Thus, parasitic castration in the *B. glabrata–S. mansoni* model may be regulated directly and indirectly by the developmental stage of the trematode and the biochemical and histopathological alterations during the patent period of infection.

1. Introduction

Schistosomiasis is one of the most widespread parasitic diseases in tropical countries; it is considered endemic in 74 countries around the world (WHO World Health Organization, 2011). In Brazil, the snail *Biomphalaria glabrata* Say, 1818 is the principal intermediate host of *Schistosoma mansoni* Sambon, 1917. This snail is widely distributed and is responsible for the maintenance of transmission in several states in Brazil (Barbosa et al., 2008).

It is widely accepted that trematode parasites adversely affect the fecundity of their intermediate snail host (Baudoin, 1975; Joosse and Van Elk, 1986, Tunholi et al., 2011). Infection by *S. mansoni* can alter the reproductive biology of *B. glabrata* (Etges and Gresso, 1965), which may be related to the severity of the infection (Pan, 1965). It has been demonstrated that *S. mansoni* parasitism is able to suppress the production of eggs by the host snail. The process of partial or definitive reduction or interruption of fecundity is called parasitic castration, and two mechanisms have been postulated to explain its outcomes: a direct process related to parasite-induced snail tissue damage that destroys the hermaphrodite gland or an indirect process that is associated with decreased energy reserves (Baudoin, 1975; Thornhill et al., 1986; Thompson and Lee, 1986; Pan, 1965; Tunholi-Alves et al., 2011). The carbohydrate reserves in snails comprise two polysaccharides, glycogen and galactogen (Lustrino et al., 2008). The latter is found in a large number of pulmonate snails in the albumen gland, and its presence is restricted to the reproductive biology of these animals. Glycogen exhibits a wider distribution in the body of snails and is metabolised as an energy source (Livingstone and Zwaan, 1983). Alterations in glucose and glycogen levels have been reported in various host-parasite models (Pinheiro and Amato, 1994; Crews and Yoshino, 1990). Alterations in the mobilisation of the galactogen reserves from the albumen gland have also been linked to the reproductive capacities of infected snails (Tunholi-Alves et al., 2011).

The present study investigates parasitic castration in the *B. glabrata–S. mansoni* model with a focus on the reproductive capacities and the biochemical and histopathological features of the snails during the pre-patent and patent periods of infection.

2. Materials and methods

2.1. Maintenance of the snails in the laboratory

The snails were raised under laboratory conditions and were descended from snails collected in the Ressaca District, Belo Horizonte, MG, Brazil (19° 86′ 42″ S and 43° 99′ 67″ W). They were kept in 2-L glass aquariums with chlorine-free water at 25 ± 1 °C, on a layer of clay mixed with calcium carbonate (10:1) in the Malacology Laboratory of Oswaldo Cruz Institute (FIOCRUZ) in Rio de Janeiro, Brazil. The snails were fed *ad libitum* with fresh lettuce leaves (*Lactuca sativa* L.) according to Paraense and Corrêa (1989).

The *S. mansoni* strain (BH) was obtained from human faeces, and it has been maintained in the same laboratory by passage in mice (Fernandez, 1997). The procedures for collecting the faeces of infected mice and for later exposure of the snails to miracidia were also those described by Paraense and Corrêa (1989). Approval of this study was obtained from the Oswaldo Cruz Foundation Ethics Committee on Animal Use (CEUA Number LW031/10).

2.2. Experimental design

We used 360 sexually mature snails (approximately 72 days old) with shell diameters of 8–10 mm. They were divided into three groups: (i) 30 specimens individually exposed to 5 miracidia and used to measure reproductive parameters; (ii) 50 specimens exposed to 20 miracidia for histopathological procedures to ensure full visualisation of tissue reactions; and (iii) 50 snails exposed to 5 miracidia for histochemical and 50 snails for biochemical studies. An equal number of uninfected control snails were used with each group. After exposure, the infected and uninfected control snails were kept under the same conditions described above.

2.3. Reproductive parameters

The fecundity (number of egg masses per snail, number of eggs per egg mass and number of eggs per snail) and fertility (rate of eggs hatched per snail) were measured weekly as previously described (Costa et al., 2004). Beginning on day 30, the snails were individually exposed to light (60-watt incandescent bulb for 4 h) to look for the release of cercariae. Snails that tested positive were kept in separate aquaria. The survival and growth rates (shell diameter) were measured weekly until 62 days after infection.

2.4. Histological analysis of ovotestis and digestive gland

The exposed snails that released cercariae 30, 35, 40, 45, 50, 55 and 62 days after exposure were separated and placed in an aqueous solution of 1% sodium pentobarbital (Hypnol®, an anaesthetic) for 4 h. Next, the viscera were removed and placed in Carson's Millonig formalin for 24 h. Then, the material was dehydrated with increasing concentrations of ethanol, cleared with xylol and embedded in liquid paraffin at 60 °C (Tolosa et al., 2003). Inclusion was performed to allow for the observation of longitudinal sections of the snails body structure on the histological slides. Serial 5-μm-thick slices were obtained using a microtome Leica RM2125. The material was stained with hematoxylin-eosin (HE). The histological and histochemical slides were observed under an Observer Z1 Zeiss light microscope, and images were acquired

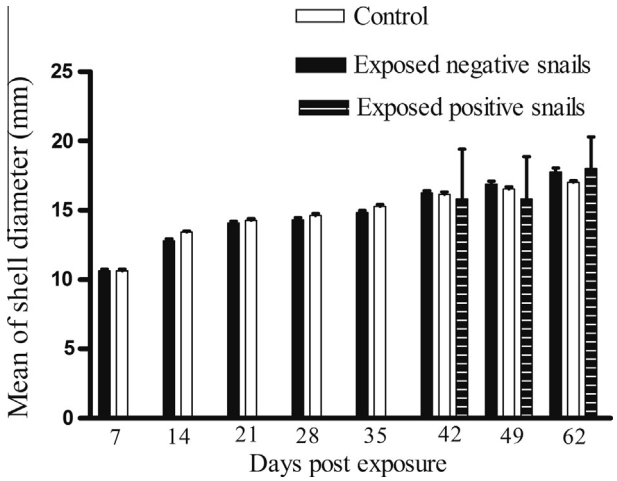

Fig. 1. Weekly growth of the shell diameter (mm, mean ± standard deviation) of *B. glabrata* post exposure to miracidia of *S. mansoni*. The means were significantly diferent ($p < 0.0001$).

using an Axio Cam HRc Zeiss camera and processed by the Axio Vision Rel. 4.7 software.

2.5. Biochemical measurements

The amount of glycogen in the digestive gland and cephalopodal mass and the haemolymph glucose concentration were measured 1, 7, 14, 21, 28, 35, 42, 49, 55 and 62 days after infection. The shell was perforated using a scalpel, and aliquots of haemolymph were harvested directly from the pericardial cavity of the snails using a 1-mL syringe and immediately placed in microtubes. After the haemolymph was harvested, the snails were dissected to remove the digestive gland and the cephalopodal mass. During collection, all of the biological materials were kept in an ice bath. The tissues were weighed and stored at −10 °C until analysis. The glucose oxidase method (Trinder, 1969) was used to determine the glucose contents in the haemolymph, and its concentration is expressed as mg/dL. The measurements of the amount of glycogen extracted from the digestive gland and the cephalopodal mass were made according to Pinheiro and Gomes (1994) and are expressed as mg glucose/g tissue, wet weight.

2.6. Histochemistry of the albumen gland

Similar methods as described above for histopathology were used to prepare the histochemical slides, and the same kinetics as described for the biochemical determinations was applied to our observations. The following stains were used to stain for the indicated substances: (i) PAS (Periodic acid-Schiff) stain for glycogen and mucoproteins and (ii) Alcian Blue, pH 1.0, for sulphated glycoproteins. The procedures used in the above staining protocols followed Tolosa et al. (2003).

2.7. Statistical analysis

The results of the fecundity and fertility measurements of the snails and the biochemical observations were evaluated using ANOVA with the Tukey–Kramer test for comparison of means ($p < 0.05$). The mean fecundity and fertility of the snails exposed to *S. mansoni* in the pre-patent and patent periods and the control group were compared between the groups and within each group during the experiment using Student's *t*-test for unpaired data ($p < 0.05$). Pearson's chi-square test was used to compare the percentage of relative growth of the snails in the control, exposed and infected groups between days 42 and 62.

3. Results and discussion

Of all of the *B. glabrata* specimens exposed to the BH strain of *S. mansoni*, 50% released cercariae. After exposure to 5 miracidia, the pre-patent period lasted 39 days. The survival rate at the end of the

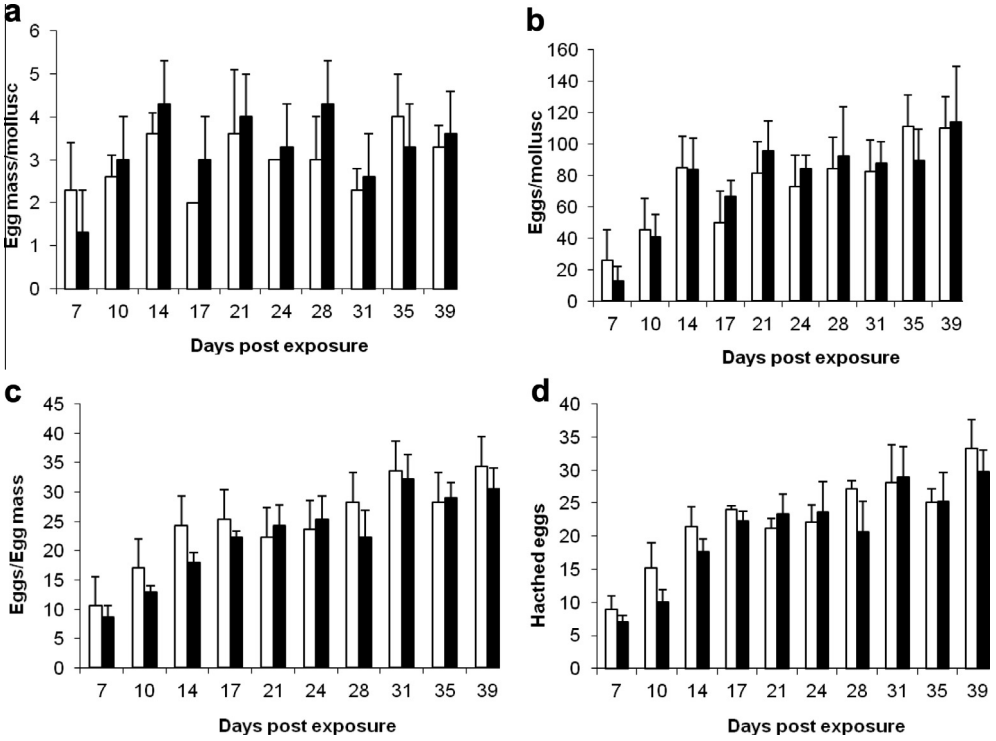

Fig. 2. Mean ± standard deviation of number of egg mass per mollusc (a), eggs per mollusc (b), eggs per egg mass (c) and hatched eggs (d) of *B.glabrata* exposed negative ($n = 30$) or normal control ($n = 30$) to *S. mansoni* during the pre-patent period of infection. (ANOVA test: $p < 0.05$) □ Control group ■ Exposed negative group.

experiment was 100% in the exposed negative snails ($n = 15$) and the unexposed control snails ($n = 30$). However, the exposed positive snails ($n = 13$) presented an 86.6% survival rate. This difference has been attributed either to the parasite strain or to the susceptibility profile, age and size of the mollusc population, in addition to the time elapsed since exposure (Sorensen and Minchella, 2001; Fernandez and Pieri, 2001). These results might be associated with the finding that the development of the larvae is slower in infected snails that do not release cercariae, leading to gradual consumption of the energy reserves. This gradual loss allowed the host to use compensatory resources, which resulted in less damage and a higher rate of survival compared to the group that released cercariae.

Fig. 1 shows the growth pattern of the control group, exposed negative snails and exposed positive snails in the pre-patent and patent periods. The data depicted were subjected to Pearson's chi-square test to compare the percentage of relative growth of the snails in the control, exposed and infected groups between days 42 and 62. The average shell diameter increased from 10.6 to 17.0 mm for the control group during the experimental period, representing a growth of 60.3%, and from 10.6 to 17.7 mm for exposed negative snails, an increase of 66.9%. In the exposed positive snails that released cercariae from the 42nd day onward, the shell diameters increased from 15.8 to 18.0 mm on average, representing an increase of 13.9% during this interval. Over the same period, the average shell diameters of the exposed negative snails and unexposed controls snails increased 9.2% and 5.6%, respectively. When the growth in this period (42nd day post-exposure onward) is considered, the infected snails that released cercariae grew 148% more than other groups ($p < 0.0001$).

Changes in the growth rates of infected snails in the *S. mansoni*–*B. glabrata* model have been reported in the literature, although the effects of larval trematode-induced changes have been quite variable (Sturrock and Sturrock, 1970; Cheng and Lee, 1971; Minchella, 1985; Joosse and Van Elk, 1986; Crews and Yoshino, 1989; Sorensen and Minchella, 2001).

Several reports have documented an increase in growth rate (Sturrock and Sturrock, 1970; Cheng and Lee, 1971; Joosse and Van Elk, 1986) or growth inhibition in infected snails (Pan, 1965; Looker and Etges, 1979; Meier and Meier-Brook, 1981). During the patent period in this study, a 148% ($p < 0.0001$) increase in the growth of exposed snails that released cercariae was observed over the course of infection compared to the non-infected controls. These results agree with Minchella (1985) and Sorensen and Minchella (2001), who attributed such a significant increase in size to the phenomenon of gigantism. Some authors found greater growth in snails infected by Schistosomatidae (Minchella, 1985; Sorensen and Minchella, 2001). However, there are few data on whether the exacerbation of growth is related to alterations in the amount of specific agents produced by the parasites or to hormonal mechanisms (Joosse and Van Elk, 1986; Becker, 1980).

In regard to fecundity, the exposed-positive snails laid eggs only on day 50 after exposure. The average number of egg masses per snails was 0.9, the average number of eggs per egg mass was 5.3,

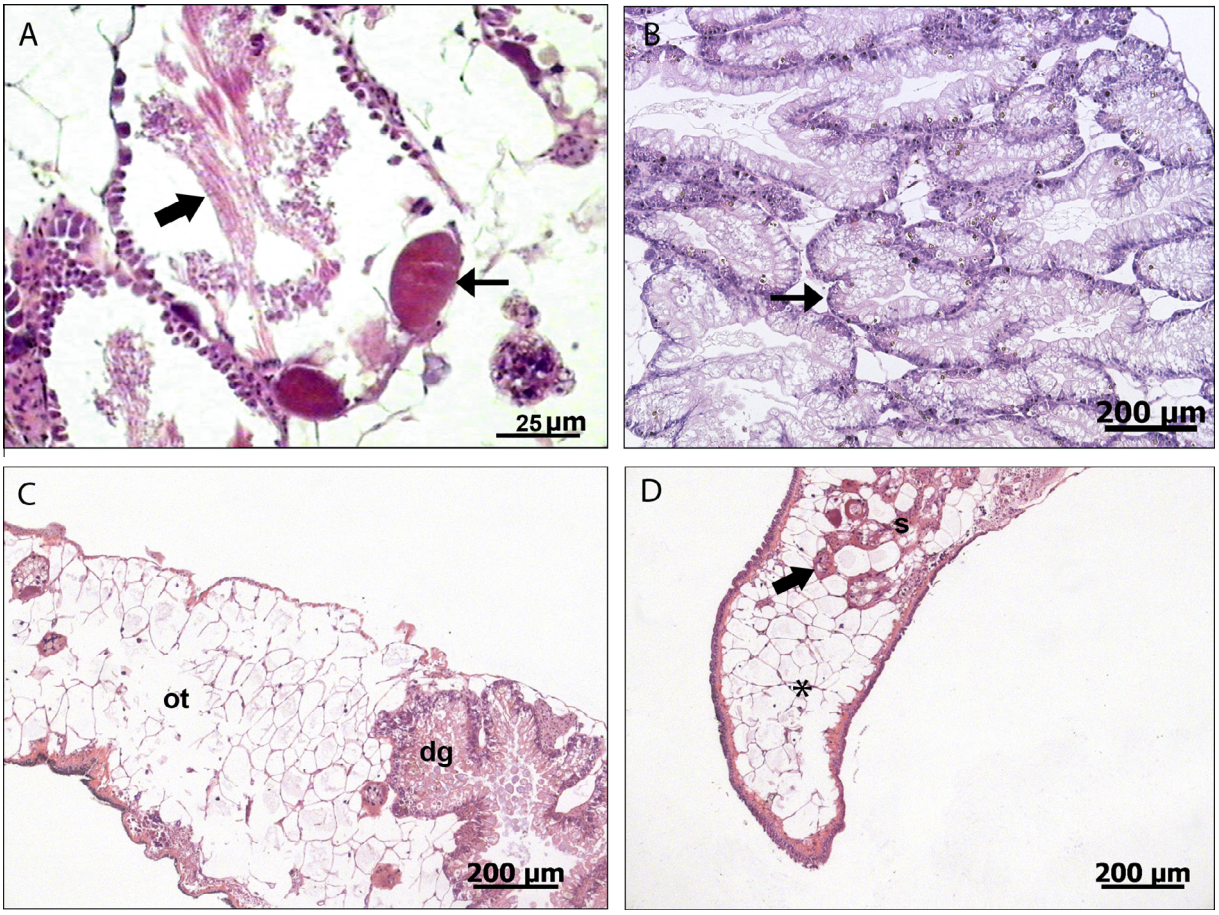

Fig. 3. Light microscopy examination of reproductive and visceral features. (A,B) Non-infected control *B. glabrata*: (A) Ovotestis with spermatozoa ➡ and ovules →. (B) Digestive gland exhibiting mucous-type high cylindrical epithelium with nuclei in the basal position →. (C,D) *B. glabrata* infected by *S. mansoni*: (C) Area of the digestive gland exhibiting high cylindrical epithelium and poorly preserved cytoplasm on day 45 (dg). Area of the ovotestis with remarkable atrophy on day 45 (ot). (D) Ovotestis with some sporocysts (➡ and s) and atrophy of the original tissue on day 55 (*).

and the average number of eggs per snails 4.6; however, the eggs were not viable. In comparing the positive exposed group with the unexposed group in the period from 42 to 62 days after the start of the experiment, the number of eggs/egg mass, egg masses/snail and eggs/snail were all significantly different ($p < 0.001$). These findings suggest a reduction in the resources involved in reproduction, leading to interruption of oviposition during the patent period of infection. This outcome characterises the biological phenomenon of parasitic castration. Cooper et al. (1994) found total or partial reduction of gamete production in *B. glabrata* infected by *S. mansoni*.

In the pre-patent period, the unexposed control group ($n = 30$) exhibited a significantly increased ($p < 0.05$) number of eggs per snails and number of eggs per egg mass (Fig. 2b and c). In contrast, although the number of egg masses per snails in the group negative exposed ($n = 30$) to *S. mansoni* was significantly lower on day 7 of infection compared to days 14 and 28 ($p < 0.05$) (Fig. 2a), the number of eggs per snails exhibited a significant increase from day 10 onwards, except on day 17 (Fig. 2b). In addition, the number of eggs per egg mass was significantly increased from day 10 to day 28, followed by stabilisation of the values between days 31 and 39 (Fig. 2c). Therefore, the present study corroborates the results of Minchella and Loverde (1981) and Thornhill et al. (1986), who found significant alterations in the reproductive parameters between exposed and control groups during this period.

During the pre-patent period, the fertility (the number of hatched eggs per egg mass) was significantly increased ($p < 0.05$) in both the exposed negative and control groups at almost all points of observation (Fig. 2d). A comparison of fecundity and fertility during the patent period (days 42–62) between the exposed negative group and the unexposed control group did not reveal any significant differences, as was the case over the course of the full experiment.

Therefore, our study suggests that the parasitic load used was not sufficient to deplete the energy resources needed to maintain the reproductive condition of the exposed group. However, the snails that released cercariae exhibited a clear reduction in their reproductive capacity as a function of the infection, a condition that characterises parasitic castration.

Analysis by optical microscopy showed that the ovotestis of non-infected control snails exhibited a typical appearance of loose connective tissue composed of spermatozoa, ovules, oviducts and germ cells (Fig. 3A). In the digestive gland, mucous-type high cylindrical epithelia, nuclei in the basal position and hepatic ducts were observed (Fig. 3B).

In the infected animals, the epithelium of the digestive gland was poorly preserved 45 days after exposure to miracidia, and partial atrophy of the original tissue was observed (Fig. 3C). After 55 days of infection, the atrophy was more severe, and the germ cells had almost completely disappeared from the ovotestis, leaving empty spaces delimitated by thin septa of connective tissue that formed a supportive network with the remnants of parasites frequently enveloped by haemocytes (Fig. 3D). Pan (1965) related the presence of sporocysts with reduction of the parenchyma, where abnormal congestion develops in the haemolymphatic sinuses of the cavity below the tunic of the digestive gland and the ovotestis after 4–6 weeks of infection. These histopathological observations indicate that infection alters the host's gonadal tissue and directly affects the reproductive system, leading to a reduction in reproductive capacity.

Histochemical analysis showed that the contents of the granules secreted by the albumen gland in the control group could be stained with HE. Individual granules could be visualised, thus demonstrating the basophilic and eosinophilic character of the content of the granules (Fig. 4E). Alcian Blue (AB) staining, pH 1.0, indicated the presence of sulphate carbohydrates in the infected snails on day 42 (Fig. 4G). This study represents the first demonstration of the expression of carbohydrates other than galactogen, which is mostly produced by the albumen gland.

The duct contents in the infected group stained with PAS, thus denoting the polysaccharide nature of the secreted material.

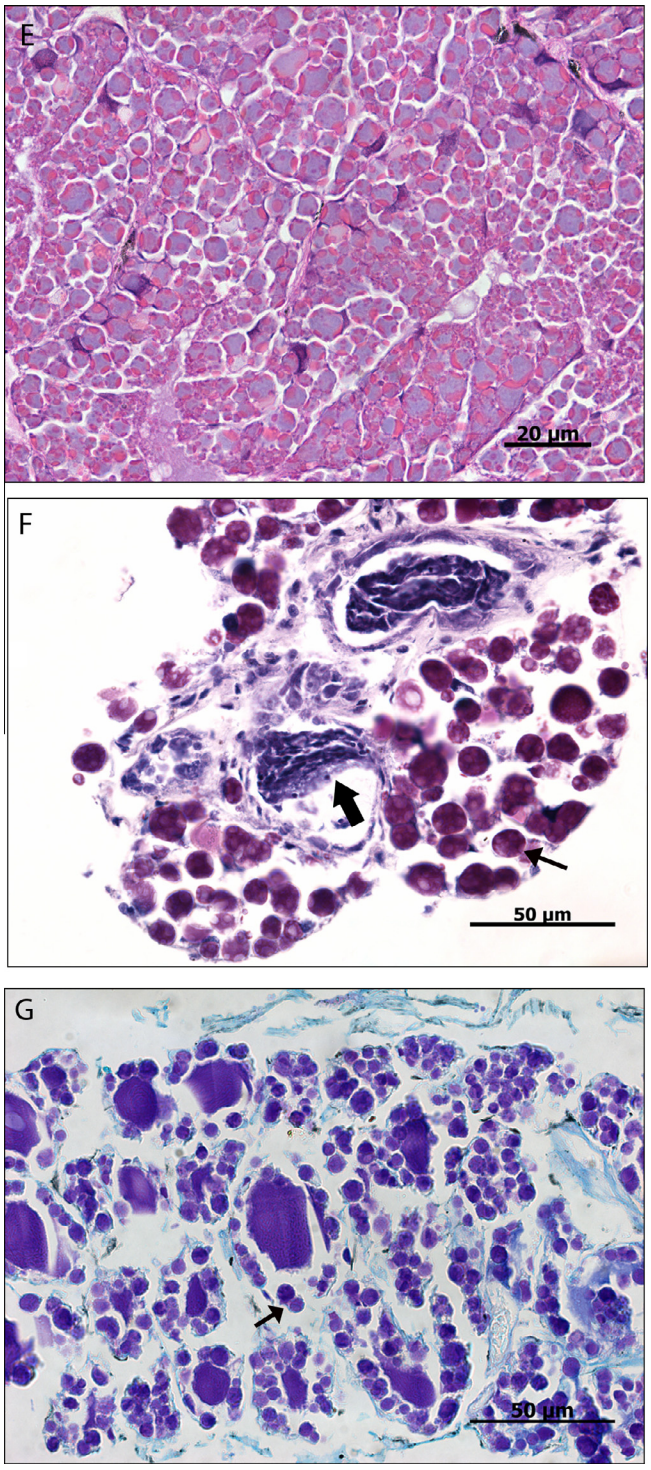

Fig. 4. Histochemical examination of visceral features. Albumen gland of non-infected control *B. glabrata*: (E) Secretory granules stained with HE denoting the basophilic and eosinophilic character of the contents of the granules; (F–G) *B. glabrata* infected by *S. mansoni*: (F) Reduction in acinar cells and secretory granules stained by PAS (→) and sporocysts (➡) on day 42. (G) Secretory granules of the albumen gland stained by AB indicating the presence of sulphated carbohydrates on day 42 (→).

(Fig. 4F).This finding confirms the literature reports of the reduction of galactogen as observed by ultraviolet/visible spectrophotometry (Tunholi-Alves et al., 2011).

The major alterations to the albumen gland caused by infection with *S. mansoni* manifested from day 35 onwards with the appearance of clear areas due to decreased cell density, leading to spacing between the cells by day 42 of infection (Fig. 4F). In the controls at the same time point, the cells were closer to each other. Such modifications are related to the development and multiplication of secondary sporocysts that establish themselves in this organ. Thus, changes in the galactogen content in this site are reflected in the formation of eggs and the viability of the embryos because these polysaccharides form part of the perivitellinic fluid that is used as a nutrient source by the developing embryos in their eggs and after hatching. Thus, reduction in the galactogen content results in a low hatching rate, which characterises the nutritional process in parasitic castration. Alcian blue stain (pH 1.0) colored the sulphated mucins and proteoglycans, in the last case due to the presence of sulphated glycosaminoglycans associated with the protein filaments. To date there have been no reports in the literature of the presence of proteoglycans or sulphated glycosaminoglycans in the albumin gland of *B. glabrata*. The perivitelline fluid produced by this gland is composed of proteins and galactogen (Looker and Etges, 1979). Although galactogen is the carbohydrate found in the greatest quantity in snail albumen glands, glycosylated proteins have also been identified. Hathaway et al. (2010), in describing the proteomic profile of the albumen gland and perivitelline fluid of *B. glabrata*, identified various glycoproteins related to immunoprotection of the offspring, some of them with disulfide bonds. Although the nutritional role exercised by galactogen is stressed because of its high concentration in the perivitelline fluid, the biological role of proteins and glycoproteins, sulphated or not, is no less important. More sensitive techniques can help in the molecular and functional identification of other components of the perivitelline fluid, as well as the assessment of possible changes in these components during infection by *S. mansoni*.

The haemolymph glucose concentration in *B. glabrata* infected with *S. mansoni* varied between 5.3 mg/dl and 70.0 mg/dl; significant alterations were observed between days 21 and 35. Between days 49 and 62, the haemolymph glucose concentration was significantly increased compared to the rest of the investigated period ($p < 0.05$) (Table 1). The results of the present study demonstrated that the glucose levels of the infected snails were stable from day 1 to 14 of infection. Thompson and Lee (1986) named this process glycaemic homeostasis, which is maintained and regulated by *B. glabrata* when it is exposed to any environmental stress and can result in the triggering of compensatory mechanisms to maintain homeostasis as a function of survival. To develop within the snail host, *S. mansoni* larvae mainly need glucose and arginine. The amount of glucose in the haemolymph is regulated by precise mechanisms according to Thompson and Lee (1986). However, for the cercariae to mature in the secondary sporocysts, the parasite needs arginine and glucose. The larvae located in the interfollicular tissues of the digestive gland continuously deplete this carbohydrate. This phenomenon leads to mobilization of the energy reserves in the tissues to the haemolymph, promoting glucose homeostasis (Bezerra et al., 1999). Similar physiological alterations are observed in snails submitted to other physiological stresses such as severe starvation, aestivation (Becker, 1980), exposure to natural molluscicides (Mello-Silva et al., 2010) and association of infection with molluscicides (Mello-Silva et al., 2011).

Table 1
The average concentration of glucose (mg/ml) in the haemolymph and average concentration of glycogen (mg of glucose/g of tissue fresh weight) in the digestive gland and cephalopodal mass of *B. glabrata* infected and not infected by *S. mansoni*.

Time of infection (days)	Concentration of glucose in haemolymph (mg/dl)	Concentration of glycogen (mg of glucose/g of tissue)	
		Cephalopodal mass	Digestive gland
(control)	16.3 ± 2.8[a,c,d]	5.0 ± 1.0[a,d]	39.0 ± 15.8[a]
1	15.6 ± 2.3[a,d]	17.0 ± 4.3[b]	12.0 ± 1.0[b]
7	11.6 ± 1.1[a,d]	8.0 ± 1.0[a,c]	8.6 ± 0.5[b]
14	10.0 ± 2.0[a,d]	6.0 ± 0.0[a,c,d]	5.3 ± 0.5[b]
21	19.0 ± 2.6[d]	4.0 ± 0.0[a,d]	5.0 ± 0.0[b]
28	5.6 ± 0.5[a]	4.0 ± 1.0[a,d]	14.4 ± 1.1[b]
35	5.3 ± 1.1[a]	5.0 ± 1.0[a,d]	5.9 ± 0.1[b]
42	29.3 ± 5.8[c]	9.6 ± 1.1[c,e]	6.1 ± 0.1[b]
49	70.0 ± 9.1[b]	5.0 ± 1.0[a,d]	9.4 ± 1.3[b]
55	59.3 ± 7.5[b]	2.6 ± 0.5[d,e]	4.4 ± 1.0[b]
62	69.6 ± 4.5[b]	5.3 ± 0.5[a,e]	3.6 ± 1.0[b]

[a–e] Different letters denote means that were significantly different (ANOVA test; $p < 0.05$).

The cephalopodal mass exhibited variations in its glycogen content over the investigated periods. Between days 21 and 35, the glycogen content in the exposed snails did not exhibit significant differences compared to the controls; however, between days 49 and 62, the glycogen content decreased in this area in preparation for the production of cercariae. Thus, a significant difference was observed on day 55 compared to the control ($p < 0.05$) (Table 1). A similar finding was also noted by Mello-Silva et al. (2010).

In regard to the digestive gland, we observed that from day 28 of infection onwards, homeostasis was disrupted. The mobilisation of glycogen from this gland was insufficient to maintain the glycaemic levels of the snails when the haemolymph glucose concentration became markedly reduced, and there were oscillations in the glycogen content of the digestive gland. From day 42 to day 62 of infection, we observed increases in the haemolymph glucose content and oscillations in the glycogen content of the digestive gland. During this period, the cercariae required only a low supply of energy because they had already formed and only their migration and emergence remained. According to Becker (1980), snails infected by the larvae of trematodes exhibit variations in the circulating glucose levels because glucose molecules are mobilised by the breakdown of glycogen from the reserve tissues of snails after being consumed by the larvae. Therefore, when the level of circulating glucose increased, the levels of glycogen in the tissues decreased, particularly in the digestive gland, thus suggesting that the stress caused by infection triggered compensatory processes to produce energy. Consequently, the pressure of infection was more significant on the glycogen content of the digestive gland, which thus decreased over the course of infection.

The results of the histological analysis in the present study showed significant destruction of the ovotestis tissues in response to infection by *S. mansoni*. This organ is responsible for the production of both male and female gametes. Therefore, the destruction of acini and parenchyma due to infection interferes directly with the reproductive process of the host. Indeed, Baudoin (1975) defined parasitic castration as a process that involves the destruction or alteration of the gonadal tissue or the reproductive behaviour or other modifications that result in reduced reproductive capacity. The results of the histochemical analysis of galactogen in the albumen gland suggest that production of eggs is associated with the reserve products in this gland. The amount of material secreted by the gland was reduced by the direct action of the developing larvae that affected this organ. Depletion of the energy substrates resulted in reduced concentrations of glycogen and free glucose, thus decreasing the levels of glucose that could be activated to form uridine diphosphate (UDP)-glucose and later converted in UDP-galactose, which is the initial step in the synthesis of

galactogen. Galactogen is an essential component of the perivitelline fluid, which nourishes the embryo during its development inside the egg and during the initial periods after hatching. These results show for the first time that the process of parasitic castration mentioned in the literature and reviewed in the present study results from a combination of direct processes (destruction of the gonadal tissue) and indirect processes (starvation of the snails caused by the parasitic action of the developing larvae). This combination results in a decrease in available nutrients for basic metabolic processes and reproduction in the infected snails.

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