

# MORPHOLOGY OF *BIOMPHALARIA GLABRATA* HEMOCYTES AND THEIR INTERACTION WITH MIRACIDIUM OF *SHISTOSOMA MANSONI*

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**Abstract-** Schistosomiasis is the second most common parasitic cause of death, after malaria. *Biomphalaria glabrata* is a fresh water snail with medical importance since it is the intermediate host of *Schistosoma mansoni*, an agent of schistosomiasis. The internal defense system of *B. glabrata* snails is mostly represented by circulating elements of the hemolymph, hemocytes, which are important factors in fighting against infections in snails. The purpose of this study was morphological study of *B. glabrata* hemocytes and their interaction with miracidia of *Schistosoma mansoni*. *B. glabrata* hemolymph was collected by heart puncture and a differential count of hemocytes was done in dyed preparations. Dyeing with Giemsa revealed two cell types: type 1, hemocytes with basophilic nucleus, little cytoplasm and sub-spherical shape and type 2, nucleated hemocytes, uniformly basophilic and spherical shape. Hemocytes showed cytoadherence and encapsulation after 1 h of miracidium-hemocyte incubation. These results could be of concern in the control programs of schistosomiasis.

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**Key words:** Hemocytes, *Biomphalaria glabrata*, *Schistosoma mansoni*

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## INTRODUCTION

Schistosomiasis affects 200-300 million people in 76 countries and is the second most common parasitic cause of death after malaria. *Schistosoma mansoni*, a trematode parasite that inhabits the blood stream of the infected person, is a cause of schistosomiasis. It is likely that immunological phenomena dictate the success or failure of trematode larva in its intermediate host (snails). Studies about the molecular and cellular basis of the susceptibility or resistance of *B. glabrata* to *S. mansoni* indicate that hemocytes and humoral factors are determinants for the success of the infection (1-3). The purpose of this work was morphological study of *B. glabrata*

hemocytes and their interaction with miracidia of *S. mansoni*.

## MATERIALS AND METHODS

### Obtaining Snail hemolymph

*B. Glabrata* snails were kept in glass containers in chlorine free water, having adequate aeration and fed with lettuce. Seven juvenile (7-10 mm diameters) *B. glabrata* were selected and each snail was cleaned externally and transferred to a container with distilled water. To obtain the hemolymph, a puncture in the heart was performed. The hemolymph was kept in an ice bath during its collection.

### Obtaining *S. Mansoni* miracidia

Eggs were obtained by crushing the infected liver of mice. The purified eggs were placed in distilled water, under direct light. After liberation of miracidia, they were collected and cold concentrated.

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### Hemocyte dyeing

Biomphalaria hemolymphs were placed on 22 × 22 mm slides and were dried at room temperature, fixed with methanol and dyed with May-Grünwald Giemsa. Different types of hemocytes were differentiated on the bases of morphology and dyeing affinity of the nucleus and cytoplasm of cells present in the hemolymph obtained from *B. glabrata*. Observation was done by immersion into a Leitz photomicroscope.

### Hemocyte adherence to glass and interaction with miracidia

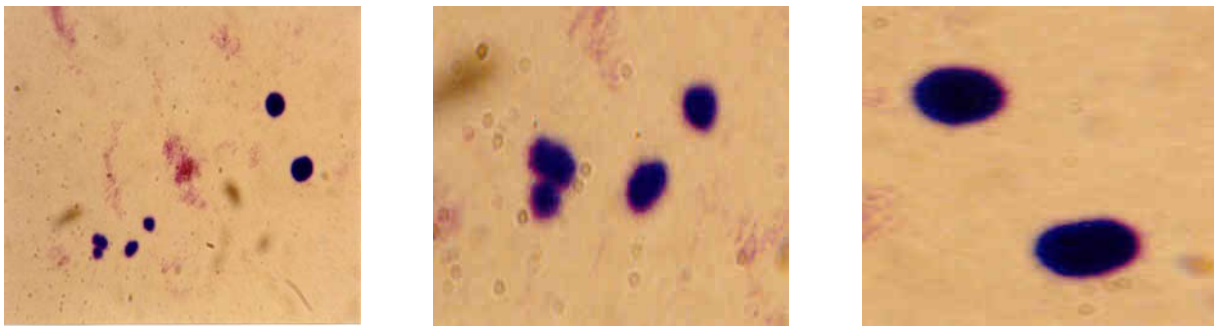
10 µl of hemolymph obtained through heart puncture out of 7 and were placed on 22 × 22 mm slides in a humid chamber for 2 h at 27°C. The supernatant containing hemocytes that did not adhere to the glass was discarded. The adhered population

was exposed to 20 *S. mansoni* miracidia at a volume of 10 µl water for 1h at 27°C. The adherence of the hemocytes and their interaction with the miracidia was evaluated by an inverted Leitz microscope (4).

## RESULTS

Dyeing of hemocytes with Giemsa showed the following cell types (Fig. 1): type 1, hemocytes with basophilic nucleus, little cytoplasm and sub-spherical shape, and type 2, a nucleated hemocyte, uniformly basophilic and spherical shape.

The adherent hemocytes showed an interaction pattern with the miracidia of *S. mansoni* after one hour of incubation. Phagocytic activity of these cells could be observed (Fig. 2). These cells showed the ability to attack and kill the miracidium of schistosoma mansoni *in vitro*.



**Fig. 1.** Cell types of hemocytes of *Biomphalaria glabrata* hemolymph (Fig. 1.A): type I, small- sized (6.6-13.2 µm), nucleated, little cytoplasm and uniformly basophilic (Fig. 1.B); type II, large-sized (19-22 µm) spherical, a nucleated and uniformly basophilic (Fig 1.C).



**Fig. 2.** Interaction pattern of hemocytes with *Schistosoma mansoni* miracidium: inverted microscope picture of engulfing (Fig.2.A-B); Light microscope picture of engulfing (Fig. 2.C).

## DISCUSSION

The internal defense system of snails consists of both cellular and humoral components. Circulating hemocytes are the principle line of cellular defense. They can be bound to and kill trematode larva by phagocytosing the syncytial tegument or releasing cytotoxic compounds or both (5). The susceptibility of fresh water snails of genus *Biomphalaria* to infection by *S. mansoni* is linked to the hemocytes present in the hemolymph (6). These cells present morphological and biochemical heterogeneity that make their characterization based on simple criteria difficult (7). The present work showed that *B. glabrata* is resistant to *S. mansoni*, and also showed variability in the type of circulating hemocytes. Other authors have pointed out that the number of hemocytes in the hemolymph is influenced by the snail's age, physiological condition and by the method of obtaining (1, 8). Hemocytes are not easily stained and a better contrast is obtained with May-Grünwald Giemsa. Four types of hemocytes have been reported in *Biomphalaria* isolates (4). The adherence to glass assays showed that hemocytes had engulfing property and the ability to encapsulate *S. mansoni* miracidia in 1 h of contact. Results of similar studies suggest that detection of role of hemocytes, although likely, may require different assays, possibly of a more prolonged nature (9). Other studies conclude that the encapsulation process and the production of toxic radicals against the parasite define resistance (10).

Briefly, the interaction of *Biomphalaria* hemocyte with *Schistosoma* larvae (miracidium) could be of concern for evaluation of cellular immunity of this snail against infections and could affect schistosomiasis control programs.

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