SHORT COMMUNICATION

A New Method for Fixing *Biomphalaria glabrata* for Histologic Studies, Using Shell Perforation

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A new technique for fixation of Biomphalaria glabrata *for histologic studies is described. It consists in performing several external holes in the shell, before placing the entire snail into the fixative. It is a very practical and quick procedure that showed excellent results when compared to the usual techniques.*

Key words: *Biomphalaria glabrata* - snail fixation - histology

Since the pioneer studies related to the anatomy of the soft parts of planorbida, authors have been searching for a practical and rapid fixation method that would preserve the anatomical position of the mollusk inside the shell, as well as the anatomical structures of internal organs. Up to now, the methodology used in histology of planorbida utilizes menthol (Pan 1958) or urethane aqueous solution (Michelson 1958) aiming at obtaining muscular relaxation and exposition of the snail soft part. Anesthetized animals, with soft part exposed, are killed in warm water at 70°C (Borges et al. 1998). After the animal death in warm water, the columellar muscle is sectioned at the end of the spiral shell, the soft part removed and put into the fixative.

Nowadays, the methodology used is time consuming (it is necessary several hours in menthol or urethane for muscular relaxation), the temperature of 70°C of warm water may denature snail proteins and damages the more sensitive anatomical structures. When the snails are infected with parasites, parasitic structures could be altered as well by the water temperature. In the present study a technique was developed in which anesthesia and warm water are not necessary for the process of fixation of *Biomphalaria glabrata.* The mollusk may be either infected or not with *Schistosoma mansoni.*

Using a mini electric drilling machine (model 1-Dremel MFG Co., Racine WFS, USA) and 1 mm drill, small holes were made on the superior and inferior parts of the spiral shell and also in its lateral exposed parts. For a snail with 1 cm of shell diameter, it was necessary to perforate about 15 holes. It is important not to damage the internal membrane, to avoid bleeding and consequent shrinkage of the tissues. The procedure is also possible to be performed in snails smaller than 1 cm of shell diameter, such as *B. straminea* and juveniles of *B. tenagophila* or *B. glabrata*. However, in this case smaller drill should be used to avoid internal membrane damage.

To test these new technique, normal or *S. mansoni*-infected *B. glabrata*, were anesthetized with pentobarbital [4 mg/l for 8 h, Martins-Souza et al. (2001)] to expose the soft parts of the snail, and then the shell holes were performed. The shell-perforated snails were placed into the fixative, i.e. phosphate-buffered 10% formalin for 24 h or Bouin´s fluid for 5 h, followed by washing in 70% alcohol. For comparison of the quality of fixation, normal and infected *B. glabrata* were anesthetized and fixed as described, but no shell holes were performed.

Trying to obtain a faster and efficient snail fixation, another group of either normal or *S. mansoni*-infected *B. glabrata* was submitted to shell perforation and fixation without anesthesia. As a control, *B. glabrata* directly placed into the fixative was examined.

After fixation, the snail shells were carefully broken and removed, and the soft tissues were dehydrated in graded alcohol, cleared in xylol, and embedded in paraffin. The 5-µm-thick sections obtained were stained with hematoxylin and eosin.

The results showed that the snails fixed after shell perforation yielded better preservation of the anatomical structures than those treated by classical procedures. Shell perforation clearly improved the quality of histologic details, as especially noted in the ovotestis, the digestive, albumen, and nidamental glands, as well as in renal tubules. (Fig. 1). The multiplying forms of *S. mansoni* were similarly better preserved (Fig. 1C). Fixation with Bouin's fluid, which better preserves epithelial tissues than formalin, can be used. Classical way of fixation yielded inferior histologic results (Fig. 1C). Previous anesthesia followed by shell perforation did not improve histological quality and thus can be discarded.

The proposed new technique has two main advantages: (a) the rapid contact and diffusion of the fixative within the snail tissues, allow for good and uniform tissue preservation, even with fixative of slow grade of diffu-

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Received 23 April 2001
Accepted 5 November 2001

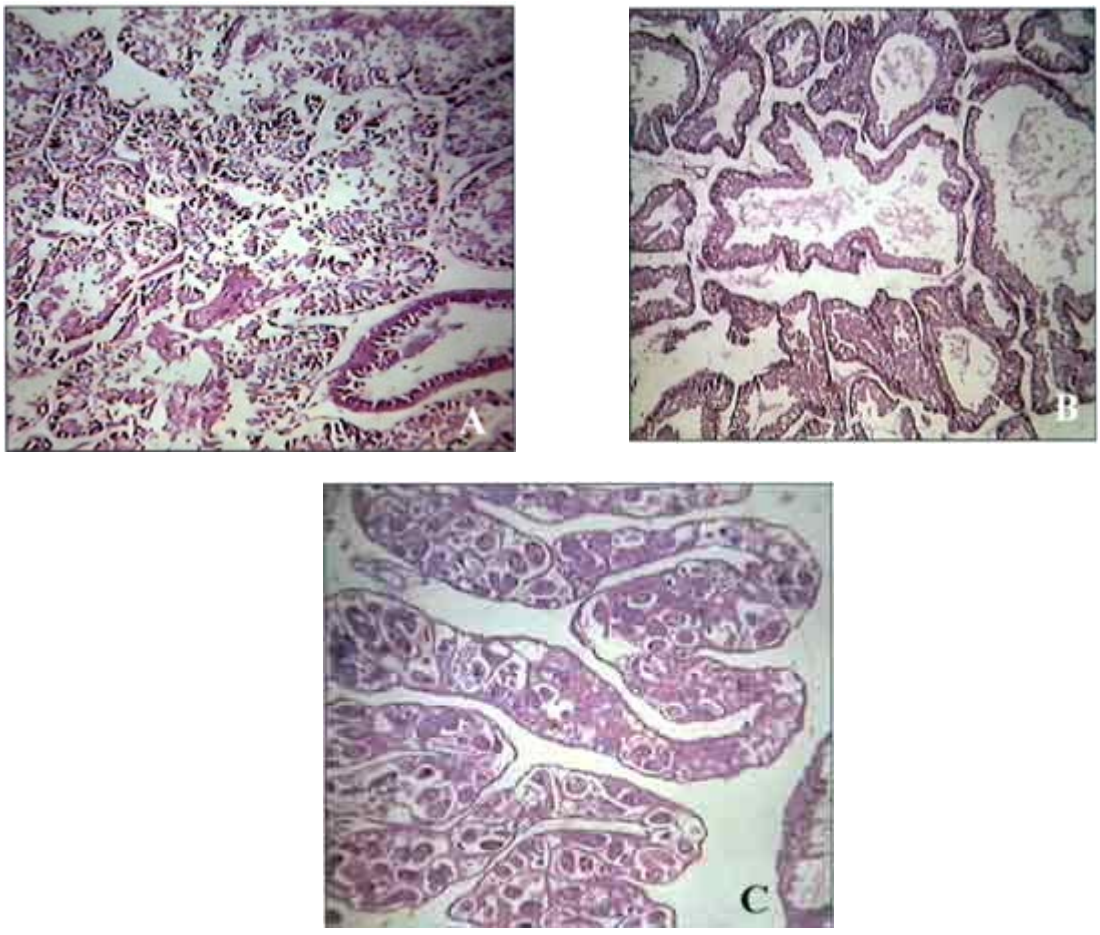

Fig 1-A: the entire mollusk was fixed in 10% formalin while still inside the shell. Note poor preservation of histological structures; with previous shell perforation excellent preservation of the structures can be appreciated, both in normal (B) and infected tissues (C). Hematoxylin and eosin, 120X

sion, such as the Bouin's fluid; (b) the maintenance of the fixed snail in its normal position inside the shell, thus permitting sections at a same plan through the entire snail body, a great advantage when stepped or serial sections are contemplated.

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