LOCALIZATION AND CHARACTERIZATION OF HEMATOPOIETIC TISSUES OF TWO SPECIES OF STYLOMMATOPHORA GASTROPODS

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RESUMO
O tecido hematopoietico é o local de formação dos hemócitos. É conhecido em algumas espécies de gastrópodes, principalmente em espécies com importância na parasitologia médica. Esse é o primeiro relato do tecido hematopoietico em M. abbreviatus e B. similaris. Em ambas espécies, o tecido hematopoietico ocupa a mesma região, dentro do saco pericárdico, próximo a âtrio e ao ventrículo, com poucas diferenças morfológicas e de espessura (M. abbreviatus a espessura foi de 70 a 138 μm, e em B. similaris foi cerca de 70 a 162 μm). Foi observado também que pode haver outros locais de formação de hemócitos, como dentro do ventrículo e do átrio.
Palavras-chave: Megalobulimus abbreviatus; Bradybaena similaris; Hematopoiese.

ABSTRACT
The hematopoietic tissue is known as the site of formation of hemocytes. It has been described in few species of gastropods, mostly in especially in species with importance in medical parasitology. This is the first report about hematopoietic tissue in M.
abbreviatus and B. similaris. In both species it is located within the pericardial sac, near the atrium and the ventricle, with few morphological differences and thickness (M. abbreviatus thickness was 70-138 µm and B. similaris was about 70-162 µm). It was also observed that there may be other sites formation of hemocytes, inside the ventricle and atrium.

Keywords: Megalobulimus abbreviatus; Bradybaena similaris; Hematopoiesis.

INTRODUCTION

In 1932 BAECKER stated that the site formation of hemocytes in gastropods was unknown, but HAUGHTON (1934) indicated the ‘blood’ spaces as the center of origin of these cells. According to PAN (1958), possible sites of hemocytes production would be the blood sinusoids and the wall of the saccular portion of the kidney, which form the vesicular portion of the pericardium. Such could be composed by lymphoid tissue, and the author considered it as a possible hematopoietic tissue. Thus, depending on the gastropod species, specific areas of tissue were considered as hemocyte formation points and, hence, they were called hematopoietic organ or hematopoietic tissue. The hematopoietic tissue is known in a few species of gastropods, usually species important in medical parasitology. This study aimed to locate and characterize morphologically the hematopoietic tissue of two gastropod species: Bradybaena similaris (Ferussac, 1821) (exotic), and Megalobulimus abbreviatus (Bequaert, 1948) (native).

MATERIAL AND METHODS

Collection and maintenance of gastropods analyzed in this study -

Individuals of B. similaris were collected in the urban area of Canoas, state of Rio Grande do Sul, Brazil. Samplings were carried out in two weekends of October 2011, and a final weekend of June 2012 and in another in July 2012, for the maintenance of the population in the laboratory. Individuals of M. abbreviatus were collected in the municipality of Barra do Ribeiro, state of Rio Grande do Sul, Brazil, and their spawn were handed over by ‘Museu de Ciências Naturais da Fundação Zoobotânica’ (MCN - FZB) in Porto Alegre, state of Rio Grande do Sul, Brazil. The snails were kept in the laboratory under not controlled environmental conditions (luminosity and humidity).
They were placed in plastic boxes with litter substrate and a plastic container with water to maintain moisture and to supply their water needs, and they were fed with cabbage leaves. The eggs, when present, were separated into smaller boxes with litter substrate and a container with water, until hatching.

**Histology of hematopoietic tissue** - The specimens selected were separated in a box without litter for at least 24 hours before the procedure to make sure that the intestinal tract was clean. The snails were killed under anesthetic action, by been placed on water and menthol crystals under refrigeration. Small individuals were kept for ± 24 hours, while large individuals were kept for ± 48 hours. Then, the specimens were removed from the shells and the incision and fixation of the renopericardic region and part of the mantle were made. For fixing, the fastener Duboscq-Brasil solution modified from HUMASON (1972) (600 ml ethanol 80° GL, 250 ml of 37% filtered formalin and 150 ml of glacial acetic acid) was used. The tissues (renopericardic region and part of the mantle) were processed following standard protocol for histology, and embedded in paraffin. Serial sections were made with an 8μm thickness, and remained drying, fixed on slides, in a dry slide dryer (60° C) for at least 24 hours. Sections were stained with Harris hematoxylin and eosin (HUMASON, 1972), to highlight the cells in mitotic division. The identification of the hematopoietic tissue was based on studies by KINOTI (1971), LIE *et al.* (1975), JOENG *et al.* (1983), & SOUZA & ANDRADE (2006). Such studies were used to characterize the hematopoietic tissue of the species studied here. Microphotographs of the hematopoietic tissue from *B. similaris* and *M. abbreviatus* were taken with a microscope Leica DMR Hc.

**Measurements of the hematopoietic tissue** - The hematopoietic tissue was measured according to the method of NODA (1992), where the central parts of each hematopoietic tissue were chosen for measuring the thickness.

**RESULTS**

**Location and characterization of the hematopoietic tissue** - The hematopoietic tissue or organ was characterized in the same way for individuals of both species. Through morphological analysis of the histological sections, it was possible to identify the site of origin of hemocytes which are free in the hemolymph, and within the connective tissues. Hemocytes originated via a special tissue, a cluster of cells located...
in the renopericardic region, next to the atrium and the ventricle, in the pericardial sac (Fig.1, sections A and D). This tissue corresponds to a narrow stratified epithelial tissue, with primary hemocytes, and is characterized by the presence of oval to spherical nucleus (Fig.1, sections B-C and E-F), located on the upper side of the pericardial sac, near the saccular portion of the kidney. It was possible to observe that in some points of hematopoietic tissue of *M. abbreviatus* and *B. similaris* there was more than one band of cells (primary hemocytes). In individuals of *M. abbreviatus* some points with agglomeration of hemocytes (formations resembling a small mountain peak) were observed, but it was not enough to characterize hyperplasia, because they present few cells in mitosis (Fig.1, section C). In general, the hematopoietic tissue was fairly uniform, without major alterations. It was possible to observe hyperplasia in the hematopoietic tissue of individuals of *B. similaris* (Fig. F), because possessed many cells in mitosis as work of JOENG *et al.* (1983). In both species, we observed the presence of round or cuboid cells grouped in bands. The hemocytes were also observed in the interstitial tissue, between the heart fibers, but the largest amount of them was located within the ventricular cavity.

**Thickness of the hematopoietic tissue** - The thickness of the tissue varied from 70-138 µm in *M. abbreviatus* and from 70-162 µm in *B. similaris*.

![Figure 1. Histological sections of the renopericardic region and hematopoietic tissue. (A-C) Histological sections of *Megalobulimus abbreviatus*; (D-F) Histological sections of *Balanoglossus similaris*.](image)
sections of *Bradybaena similaris*; (A, D) pericardial region; Scale bar=100μm, (B, C, E and F) view of the hematopoietic tissue; (C, F) detail the hematopoietic tissue with cells undergoing mitosis; (F) detail of the hematopoietic tissue with hyperplasia; Scale bar=50μm. (a) atrium; (cm) mitotic cells; (sc) pericardial sac; (th) hematopoietic tissue; (r) kidney, (v) ventricle.

**DISCUSSION**

The hematopoietic tissue from both species of this study was morphologically similar, and was located in the same area of the renopericardic region within the pericardial sac. This result is corroborated by KINOTI (1971), LIE et al. (1975), RONDELAUD & BARTHE (1981), JEONG et al. (1983) and SOUZA & ANDRADE (2006), who also identified the hematopoietic tissue within the mantle cavity near the renopericardic region in other species of pulmonate gastropods. In the studies cited above, the authors commented on the format of the tissue, which could be oval or kidney-shaped. In our study, it was not possible to assign a format for this tissue; it seemed to be arranged in bands, with shapeless cell clusters. The presence of hyperplasia was observed only in the hematopoietic tissues of *B. similaris* despite the similar hematopoietic tissue thickness between *M. abbreviatus* and *B. similaris*. The hematopoietic tissue has already been analyzed in studies that aimed to observe the differences between tissues of healthy specimens and specimens infected with digeneans. KINOTI (1971) measured the uninfected hematopoietic tissue of two species of *Bulimus*: *B. truncatus* (Auduoin, 1827) and *B. africanus* (Krauss, 1848) and the mean thickness were two times higher than those found for the species in this study. RONDELAUD & BARTHE (1981) measured the hematopoietic tissue of *Lymnaea trunculata* Muller, 1774 and the mean thickness also was slightly higher than the one found for *M. abbreviatus*, and similar to the maximum value found for the hematopoietic tissue of *B. similaris*. In the researched carried out by Noda (1992), the mean thickness of hematopoietic tissue was smaller and did not vary greatly when compared to the hematopoietic tissue found in individuals of this study. JOKY & MATRICON-GONDRA (1985), did not mention measures, but through microphotographs they showed that the hematopoietic tissue of individuals of *Biomphalaria glabrata* (Say, 1818) tripled in size under the infection by *Echinostoma*...
caproni Richard, 1964, and one of the conclusions of such study was that if there was an infection, there would be more cells in mitosis, and the tissue would present hyperplasia.

The hematopoiesis is continuous, but environmental changes can cause fluctuations in the hematopoietic tissue and the amount of hemocytes present in the hemolymph (VAN DER KNAAP et al. 1993). However, contrary to the previous study, Souza & Andrade (2006) stated that the general appearance of the normal hematopoietic tissue does not change with the presence of parasitic lesions.

According to PAN (1958), MATRICON-GONDRAIN (1990), MONTEIL & MATRICON-GONDRAIN (1991) and SMINIA (1972, 1974), the hemocytes would have a multicentric origin (in the endothelial lining of the vascular spaces and loose connective tissue). However, for LIE et al. (1975), hemocytes are derived from a hematopoietic tissue (organ). According to SOUZA & ANDRADE (2006), to consider the hematopoietic tissue as a central tissue (originating all hemocytes), we would have to take into consideration that the hemocytes formed in hematopoietic tissue would not be able to replicate in peripheral sites (in this study we have observed some free hemocytes in the hemolymph undergoing mitosis). Otherwise, the tissue would have to be invaded by many hemocytes when a strong response against foreign materials or parasites was occurring. The authors also mentioned that if the migration of hemocytes occurs from a central point to multiple reaction sites of a gastropod’s body, this would be reflected on the hemocyte levels in the hemolymph circulation. Thus, the results obtained in this study support the hypothesis that the hematopoietic tissue is a central tissue for the hemocyte production, but there may be other hemocyte producers.

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