Calcium changes in Bradybaena similaris (Férussac, 1821) (Mollusca, Xanthonychidae) under starvation[1]

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ABSTRACT: The effects of different periods of starvation on the calcium in the hemolymph and its deposits in the shell of *Bradybaena similaris* (Férussac, 1821) were studied. The calcium content in the hemolymph of fed snail was 20.76 mg/dl being reduced in 83.77% after thirty days of starvation. The calcium content in the shell of the starved snail was 46.37 ppm $CaCO_3$/g of shell, wet weight, with a reduction of 24.78% after the same period of starvation. The changes on calcium deposits in the shell were lower than that observed in the hemolymph, but both varied significantly. The results were discussed.

Key Words: *Bradybaena similaris,* starvation, calcium, shell, hemolymph.

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INTRODUCTION

The physiology of the molluscs has been studied and many authors showed that the changes occurred in the snails when infected with larval trematodes are similar to that raised in response to the starvation (BECKER, 1980; PINHEIRO, 1996; PINHEIRO *et al*., 2001). But there are few studies on the changes caused in the inorganic metabolism of the snails when submitted to different physiological conditions.

The calcium is a metal that exerts an essential role in the biology of the snails, once that this ion is the main component of the shell in these animals. The calcium has been found to be an important limiting factor to the distribution and survival of the adult snail, in the rate of egg laying and in the survival and development of the eggs and embryos (THOMAS *et al*., 1974; NDUKU & HARRISON, 1976; APPLETON, 1978; DAWIES & ERASMUS, 1984).

The calcium is used in several enzymatic reactions and is also involved in metabolic processes related to the acid-base balance of the hemolymph by maintaining the calcium carbonate ($CaCO_3$) saturation in this site (SMINIA *et al*., 1977, DE WITH & SMINIA, 1980).

The intense degradation of proteins during the starvation in *Bradybaena similaris* (Férussac, 1821) showed by LIRA *et al*. (2000), causes an increase of the nitrogenous products of degradation in the hemolymph, which alter the pH of the internal environment of the snail (SOUZA *et al*., 2000). Thus, a buffering mechanism is needed to ensure the normal values of the pH in the extracellular liquid. The ion calcium, present as $CaCO_3$, may dissociate to form bicarbonate ion, which participates in the main extracellular buffer system (bicarbonate buffer).

The neuropeptide, named calfluxin (CaFl), which is secreted by the snails, is involved in the regulation of the influx of calcium in the mitochondria of the secretory cells of the albumen gland in the freshwater snail *Lymnaea stagnalis* (Linnaeus, 1758) (DICTUS *et al*., 1987), and physiological conditions that alter the CaFl activity lead to a reduced number of mitochondria containing calcium deposits, which cause a reduction of the rate of egg laying by the snail.

The snail *B. similaris* is widely distributed throughout the Brazilian territory, being considered a plague to the horticulture and is also used as intermediate host by some parasites of medical and veterinary importance, as *Angiostrongylus costaricensis* Morera & Céspedes, 1971 (Nematoda, Angiostrongylidae) (THIENGO, 1995) and *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907 (Trematoda, Dicrocoeliidae) (MATTOS Jr. 1987), respectively. In spite of this great importance, there are few studies on the biology of this species of mollusc and on the alterations caused by different physiological conditions.

This study reports the alterations in the calcium concentration in the hemolymph and in the shell of starved *B. similaris*.

MATERIALS AND METHODS

Snails collection and maintenance - Due to the great number of snails needed to the acievement of the experiments, it was very difficult to obtain the amount of snails laboratory bred. So, specimens of *B. similaris* were collected from a vegetation constituted by lettuce (*Lactuca sativa* L.), cabbage (*Brassuca* Lour.) and basil (*Ocimun* L.) in gardens located at Água Santa, Rio de Janeiro, RJ, Brazil and transported to Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil. The snails were observed under stereomicroscope through their transparent shell to verify the presence of *Phostarmostomum gallinum* Witenberg, 1923 (Trematoda, Brachylaemidae) metacercariae in the pericardial cavity. A sample of the snails was randomly chosen and dissected to verify the presence of parasites in its tissues. Snails free of infections with 12 mm of shell diameter, were transferred to glass vivaria, with earth at the bottom and maintained under laboratory conditions (room temperature 25 ± 3 ºC). They were fed with cabbage leaves (*Brassica* sp.) *ad libitum* and the vivaria earth was moistened with tap water on alternate days.

The distribution of larval trematodes in a snails first intermediate host population is overspread, where there are few specimens infected, but harbouring a great number of larva. As

we never collected snails infected with larval helminths, it was possible to assure that the molluscs used in these experiments were free of infection.

Starvation, hemolymph and shell collection – Groups of 50 snails were formed, the food supplies were suspended and to the snails was only given tap water on alternate days. The snails were submitted to 0 (control group), 5, 10, 15, 20, 25 and 30 days of starvation. The hemolymph of, at least, 25 active snails was collected by punction of the pericardial cavity using a syringe (B-D Plastipakâ) at 0ºC and stored in microtubes at – 10ºC until its utilization. After the hemolymph collection, the snails were immerged in a becker with boiling water and the soft tissues were removed with tweezers. So, the shells were washed with tap water and dried at room temperature. The dried shells were weighed and stored until its utilization.

Calcium determination – The calcium content in the hemolymph of *B. similaris* was determined according to GINDLER & KING (1972), being the results expressed in mg of calcium/dl of hemolymph. The calcium content in the shell was determined according to PINHEIRO & AMATO (1995), being expressed as ppm de $CaCO_3$/g of shell, wet weight.

Statistical analysis – The results obtained were expressed by mean and standard deviation and were submitted polinomial regression and Tukey-Kramer multiple comparison test (a=5%).

RESULTS

The calcium content in the hemolymph of the fed snails was 20.76 mg/dl (Table). In starved snails, there was a significant reduction in the calcium content in this site from the tenth day of starvation onward, ranging 3.37 mg/dl, which represented a content 83.77% lower than that observed in the control group. The polinomial regression test showed a significant negative relation between the period of starvation and the calcium content in the hemolymph of *B. similaris* (r^2=0.96) (Fig. 1).

The calcium content in the shell of *B. similaris* was 46.3 ppm $CaCO_3$/g of shell, being higher than that values observed at the 20 and 30 days of starvation, 35.6 and 34.9 ppm $CaCO_3$/g of

Table 1. Calcium content in the hemolymph, expressed as mg/dl, and in the shell, expressed as ppm of $CaCO_3$/g of shell, wet weight, of *Bradybaena similaris* through 30 days of starvation. X ± SD=mean ± standard deviation; percentual variation in relation to the control group; different letters indicate means with significant difference among them (a=5%).

Time of starvation (days)	Calcium content in the hemolymph (mg/dl) X ± SD	Percentual variation (%)	Calcium content in the shell (ppm$CaCO_3$/g of shell) X ± SD	Percentual variation (%)
0 (control group)	20.76 ± 0.382^{a}	0	46.3 ± 0.664^{a}	0
5	20.83 ± 0.477^{a}	+0.33	45.8 ± 0.396^{a}	-1.14
10	18.22 ± 0.599^{b}	-12.23	43.5 ± 0.775^{a,b}	-5.92
15	9.85 ± 1.881^{c,d}	-52.55	46.1 ± 1.467^{a}	-0.42
20	8.10 ± 1.464^{c}	-60.93	35.6 ± 2.864^{b}	-23.16
25	10.08 ± 0.779^{d}	-51.45	44.0 ± 2.449^{a,b}	-4.29
30	3.37 ± 0.001^{e}	-83.77	34.9 ± 7.660^{b}	-24.78

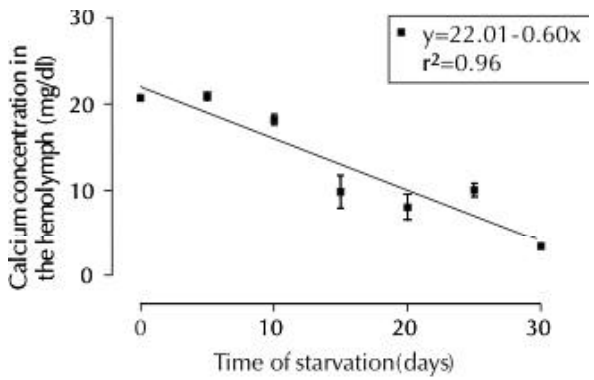

Figure 1. The negative relation between the calcium content in the hemolymph of *Bradybaena similaris*, expressed in mg/dl, and the time of starvation, expressed in days.

shell, wet weight, respectively. At the end of the period of starvation studied, the calcium concentration was 24.78% reduced in relation to the control group (Table). The polinomial regression test showed a negative relation between the calcium content in the shell of *B. similaris* and the period of starvation analyzed (r^2=0.83) (Fig. 2).

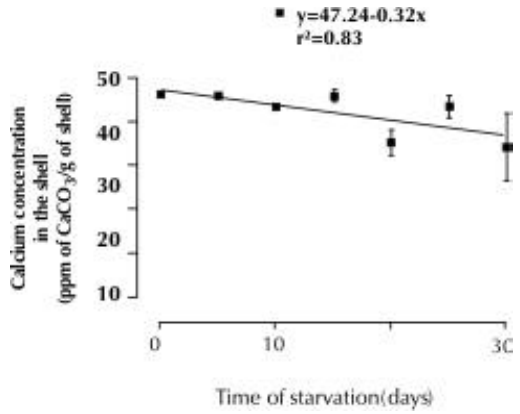

Figure 2. The negative relation between the calcium content in the shell of *Bradybaena similaris*, expressed in ppm of $CaCO_3$/g of shell, wet weight, and the time of starvation, expressed in days

DISCUSSION

The food deprivation established, led to a significant alteration in the calcium deposits in *B. similaris* throughout the 30 days of starvation studied, causing a severe reduction of the concentration of this ion in the both sites analyzed: the hemolymph and the shell. According to DE WITH & SMINIA (1980) and THOMPSON & LEE (1986), the composition of the hemolymph is precisely regulated, maintaining the homeostasis of the internal medium. The alterations recorded by PINHEIRO (1996), SOUZA *et al*. (2000) and LIRA *et al.* (2000) in *B. similaris* under starvation are accompanied by several alterations in the hemolymph composition. The elevated degradation of the carbohydrates, indicating the occurrence of an anaerobic metabolism of these substrates and producing high amounts of organic acids (WIESER, 1981), and the accumulation of nitrogenous products of degradation, due to the utilization of other substrate as source of energy, are factors that cause alterations in the pH of the hemolymph of the snail, the main site where this metabolic products are released. The pH is a parameter of the internal medium that must be precisely regulated, once that it highly influences the enzymatic activity.

FLORKIN & SCHEER (1972) observed that the shell was involved in this process of buffering the hemolymph, once that the calcium located at this site is found as $CaCO_3$, that can be used to form the bicarbonate buffer, the main buffer of the extracellular medium.

In the present study the significative variation of the calcium concentration in the hemolymph occured at the tenth day of starvation, but the same alteration in the calcium content in the shell was significative only at the twentieth day of starvation. This observation indicates that the alterations in the calcium content in the hemolymph of *B. similaris* occurred due to its mobilization in the formation of bicarbonate buffer, causing the withdrawl of this ion of the shell, where the alterations in the calcium content in this site raise as a consequence of that occurred in the hemolymph. The calcium alterations in the shell were highly variable throughout the period of starvation studied. The variations observed may be related to the dormancy periods in response to the food deprivation, reducing the metabolic rate of the snail.

Other situations of physiological stress can cause alterations in the calcium deposits in the shell. PASCHOAL & AMATO (1996) observed that there is a positive relation between the shell diameter and the calcium content in this site in *B. similaris*, but this relation is significatively altered when the snail are infected with larval stages of *E. coelomaticum*. In spite of the reduction of calcium contents in shell of infected snails, the shell diameter was increased. Thus, we conclude that the growth of the shell involve other factors beyond the calcium, as the organic matter constituted by proteic matrix. So, the calcium reduction in the shell observed by us may be expressing an increase of the shell that was not followed by a calcium incorporation, consequently the relation calcium concentration/shell weight is negatively altered.

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