ORIGINAL ARTICLE

Biomphalaria glabrata: EXPOSURE TO DIFFERENT AMOUNTS OF CALCIUM CARBONATE INFLUENCING GROWTH, SEXUAL MATURATION AND PEARL'S FORMATION

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ABSTRACT

Calcium plays a fundamental role in the life of snails, by regulating different process such as motility, growth, and cell division. Calcium directly influences the shell growth, fecundity, oviposition, mortality, internal metabolism and homeostasis. The aim of this work was to describe the pearl formation in *Biomphalaria glabrata* exposed to different amounts of calcium carbonate in laboratory conditions. The pearls were observed in the digestive gland and gut in the exposed group given 20 and 60 mg/L after 45 days. The results show that the snails produce pearls as a reservoir of calcium carbonate before reaching sexual maturity.

KEY WORDS: *Biomphalaria glabrata.* Pearls. Calcium carbonate. Schistosomiasis. Planorbidae.

RESUMO

Biomphalaria glabrata: exposição a diferentes concentrações de carbonato de cálcio influenciam o crescimento, a maturidade sexual e a formação de pérolas

O cálcio desempenha um papel fundamental na vida dos caramujos, regulando diferentes processos como motilidade, crescimento e divisão celular. O cálcio influencia diretamente o crescimento da concha, fecundidade, oviposição, mortalidade, metabolismo interno e homeostase. O objetivo deste trabalho foi descrever a formação de pérolas em *Biomphalaria glabrata* expostos a diferentes quantidades de carbonato de cálcio em condições de laboratório. As pérolas foram observadas na glândula digestiva e no intestino no grupo de caramujos expostos a 20 e 60 mg /L de carbonato de cálcio após 45 dias. Os resultados mostram que os caramujos produzem pérolas como um reservatório de cálcio antes de atingirem a maturidade sexual.

DESCRITORES: *Biomphalaria glabrata.* Pérolas. Carbonato de cálcio. Esquistossomose. Planorbidae.

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INTRODUCTION

Calcium carbonate ($CaCO_3$) is generally found in nature in two forms: aragonite and calcite crystal. Snails may produce many different forms of calcium carbonate, examples being pearls, which consist mainly of aragonite, and the outer shell of marine mollusks, which consist mainly of calcite. Calcium carbonate is easily affected by acids as well as ammonium salts and is not dependent on carbon dioxide (CO_2). In addition, it is soluble in water, which disaggregates the CO_2 to form sodium bicarbonate (Moreira et al., 2003).

Calcium plays a fundamental role in the life of snails, by regulating contraction, secretion, endocytosis, membrane transport, and processes such as motility, growth and cell division. For *B. glabrata*, calcium directly influences the shell growth, fecundity, oviposition, mortality, internal metabolism and homeostasis. Snails obtain calcium through their diet. Both the growth rate and oviposition of *B. glabrata* are influenced by the concentration of calcium absorbed (Thomas et al., 1974; Dawies & Erasmus, 1984).

In the hemolymph of *B. glabrata*, the calcium ion is allocated to various parts of the body, such as tissues, mantle and shell; in the latter, in the form of solid calcium. This metal acts in several places, being a key element in regulating electrochemical mechanisms, contributing to homeostasis of snails. The balance of these ions in the shell and hemolymph is regulated by the excretion rate, circulation and amount of calcium available (Magalhães et al, 2011).

The process of biomineralization in snails occurs in the shell matrix, but outside the cells. The shell matrix is formed by various substances such as proteins, glycoproteins, polysaccharides and lipids and those associated with the mineral aragonite precursor are secreted by the mantle epithelium, forming the shell. One of the probable functions of the matrix is to be a cell signal with calcifying epithelium. The affinity of the matrix for calcium has not yet been measured, but its function is associated with removal of calcium ions for their excretion when required (Marxen & Becker 1997, 2000; Marxen et al., 2003; Marie et al., 2007).

Calcium ions are the most important for snails' internal defense mechanisms. In defensive reactions, the phagocytic activity of hemocytes and lectins cooperates, depending on the presence of calcium ions in the hemolymph (Zelck & Becker, 1992) and the process of polymerization of filaments induced by foreign material seems to be calcium-ion dependent (Matricon-Gondran & Letocart, 1999). During the defense process, snails may form pearls as a reaction to different pathogens. In the literature, the formation of pearls in snails has been associated with genetic factors. Richards (1970, 1972) was the first to report pearl formation in *Biomphalaria glabrata*. He presented evidence that pearl formation in snails is determined by a single genetic factor, as a simple recessive event, and that this characteristic probably occurred as the result of a spontaneous mutation in a natural population or in snails kept in laboratory conditions. Two *B. glabrata* populations

were observed with this characteristic: the Santa Lucia island lineage and the colony kept for the NIH-BPR-M genetic experiment by Richards.

Since it is not yet clear exactly what triggers pearl formation in *B. glabrata*, the aim of this work was to describe pearl formation when *B. glabrata* is exposed to different amounts of calcium carbonate.

MATERIAL AND METHODS

Description of the breeding conditions

Specimens of *B. glabrata* (Belo Horizonte – BH lineage), maintained at the Experimental Schistosomiasis Laboratory/Fiocruz, Rio de Janeiro, Brazil, with shell diameters between 8 and 10 mm, were placed in 30-liter polyethylene aquaria. The average water temperature was 21 to 28 °C and the relative humidity varied from 70 to 78% throughout the experiment. Once a week the aquaria were cleaned with dechlorinated water (MS, 2008) and the snails were fed *ad libitum* with lettuce leaves (*Lactuca sativa* L).

Snails measuring

The *B. glabrata* specimens (200) at the age of 60 days were divided into five experimental groups of 40 snails each, one of which was the control. The experimental groups were exposed to four different amounts of calcium carbonate (20, 40, 60 and 80 mg/L) during 45 days. Over this period, 30 snails were randomly selected from each group, they were measured (shell diameter) and observed for the start of sexual maturity by observation of egg masses.

The presence of pearls in the tissue of snails exposed to different amounts of calcium carbonate

After the 45 days from the start of the experiment, ten snails from each group were sacrificed, dissected and observed for presence of pearls. The sacrifice was conducted by extraction of the hemolymph by cardiac puncture. After that, the snails were dissected and the digestive gland was separated and examined for the presence of pearls. Photographs were taken of the pearls found at the time of dissection.

Statistical analysis

The results for the size of the snails were evaluated by ANOVA, with the Tukey-Kramer test for comparison of the means ($\alpha = 5\%$) and Student's-*t* test for unpaired data ($\alpha = 5\%$).

RESULTS

Table 1 showed the shell size of the snails exposed to different amounts of calcium carbonate and the survival rate. The results showed that the highest survival rate was in the group exposed to 60 mg/L $CaCO_3$. The average growth was 1.9 mm in all exposed groups and there was a significant difference in relation to different times of exposure: 7-14 days in the control group ($q = 7.195$, $p < 0.001$) and 37-45 days in the groups receiving 20mg / L ($q = 8.968$, $p < 0.001$), 40 mg / L ($q = 5.648$, $p < 0.01$) and 80 mg / L ($q = 5.798$, $p < 0.01$). Figure 1 shows continuous growth of exposed snails after 75 days of life, not observed in snails from the control group.

Table 1. Mean and standard deviation of the shell size (mm) and survival rate of snails exposed to different amounts of $CaCO_3$ during 45 days

Control	0	07	14	21	30	37	45	Survival rate
	5.97±1.14a,b	5.76±1.11a	7.08±1.33a*	6.65±0.81a	6.83± 0.92a	6.50±0.70a	7.28±1.25a	17.5
20mg CaCo3	5.47± 0.75a	5.82±0.93a	6.16± 0.92b	7.30±0.73a,b	7.84± 0.47b	6.92±0.49a,b	8.54±0.68b*	27.5
40mg CaCo3	5.71± 0.99a	5.85±0.99a	6.13± 1.22b	7.32±1.21a,b	8.13± 1.14c	6.42±0.86a	8.18±0.87a,b*	27.5
60mg CaCo3	5.97±0.73a,b	6.34±0.74a,b	6.57±0.88a,b,c	7.85±0.75b,c	8.50±0.92b,c,d	6.42±0.86a	8.57± 0.75b	40
80mg CaCo3	6.35± 0.92b	6.77±0.92b	6.94±1.013a,c	8.46±0.85c	9.03± 0.99d	7.54±0.82b,c	9.18± 0.98b*	27.5

Different letters: significant difference between groups. Asterisk (*) significant difference versus time.

In relation to start of sexual maturity, the group exposed to 80 mg/L of $CaCO_3$ began laying egg masses at 14 days after exposure. The control group began laying egg masses 30 days after the beginning of the experiment (Figure 1). The specimens exposed to higher concentrations of calcium carbonate (60 and 80mg / L) had higher shell growth (Figure 2) and began to lay eggs earlier.

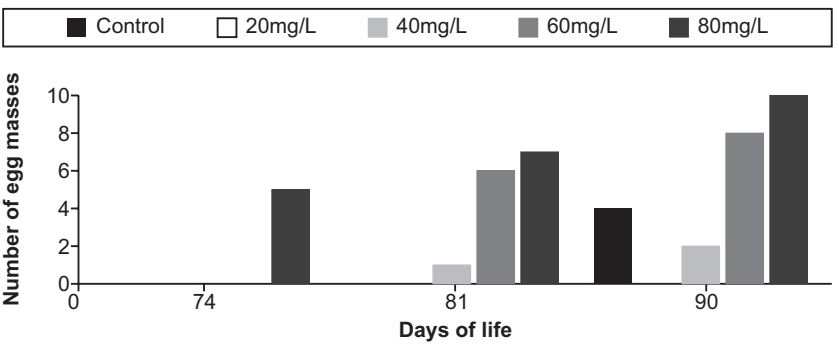

Figure 1. Number of egg masses observed in *B. glabrata* exposed to different amounts of calcium versus age (days)

Pearls were found in the digestive gland and the gut of the snails in the groups exposed to 20 and 60 mg/L after 45 days (Figure 3).

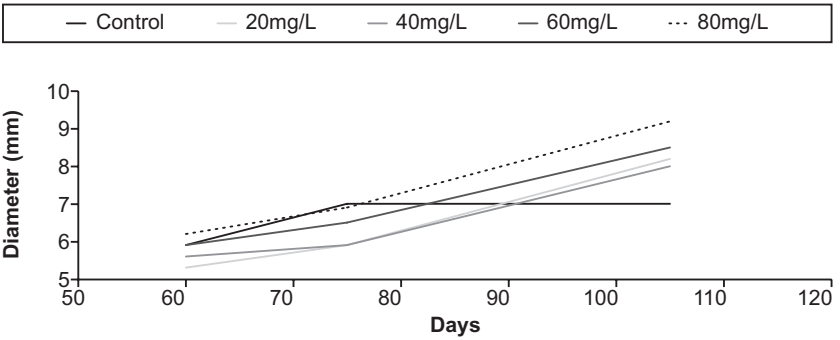

Figure 2. Shell growth (mm) of snails exposed to different concentrations of $CaCO_3$ as a function of time.

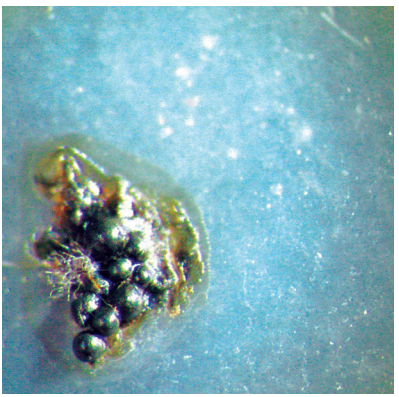

Figure 3. Pearls in gut of *Biomphalaria glabrata* exposed to 60 mg/L calcium carbonate

DISCUSSION AND CONCLUSIONS

Calcium plays an important role in the lives of snails. It is the main component of these animals' shells. The availability of calcium in the environment is a limiting factor for the survival and geographical dispersion of *B. glabrata*, directly influencing egg laying (Moreira et al., 2003).

The availability of calcium influences in shell growth of *B. glabrata* and this influences the beginning of sexual maturity. Costa et al. (2004) reported that shell growth starts slowly until about 60 days, when it accelerates until the snails reach sexual maturity before slowing down to a steady rate until death. Some authors have reported that sexual maturity of this species begins around 30 days (Teles & Carvalho, 2008), whereas others have stated that this period is between

40 to 113 days (Penido et al., 1951; Rey, 1956; Pimentel, 1957; Perlawogora, 1958; Costa et al., 2004; Teles & Carvalho, 2008). There is controversy in the literature about the reasons for differences in the start of sexual maturity. Pimentel (1957) reported that sexual maturity is related to the growth of the shell and not the snail's age. This fact was confirmed by Perlawogora (1958). Kawazoe (1977) related the growth of the shell and laying of egg masses, implying that the shell growth directly affects the fertility of these snails. We used snails of the same age, associating the beginning of sexual maturity with age and size of the shell. The results show there was no statistical difference in shell size between the snails in the control group and the group exposed to 80 mg / L of calcium carbonate. The exposed group reached sexual maturity 15 days before the control group. In this case, it appears that the calcium concentration positively influenced the start of sexual activity. These results agree with those of Thomas et al. (1974) and Dawies & Erasmus (1984), reporting that the growth rate and oviposition of *B. glabrata* are influenced by the concentration of calcium absorbed.

The main contribution of this paper is to first report the finding of pearls in snails bred in laboratory conditions and exposed to different amounts of calcium carbonate. The pearl formation in *B. glabrata* was described for the first time by Richards (1970, 1972). In these two papers, the author reported the presence of pearls six months after sexual maturity and described this phenomenon as a recessive genetic characteristic. However, in this experiment, we observed pearls at the beginning of sexual maturity, before the 90th day of age, as well as shell growth of only 1.9 mm. Furthermore, the pearls were easy to identify due to their size.

The pearls and shell are formed of $CaCO_3$ in the form of aragonite; this nacrezation occurs in invaginations in the epithelium of the mantle (Marxen & Becker 1997, 2000; Marxen et al, 2003). The main function of the pearls may be to act as a reservoir of calcium for these snails. The finding of pearls in the digestive gland, where there are specific cells for calcium storage, and the gut, the site where calcium is absorbed, suggests that calcium was being used to maintain the homeostasis because the environment was unfavorable.

We can conclude that the availability of calcium carbonate directly influences the shell growth and consequently the start of sexual maturity. When the snails were exposed to high amounts of calcium carbonate, they produced pearls as a calcium reservoir.

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