Aminotransferases Activity in the Hemolymph of *Bradybaena similaris* (Gastropoda, Xanthonychidae) under Starvation

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Aminotransferases (GOT and GPT) activities in the hemolymph of Bradybaena similaris *under experimental condition of starvation were studied. At the 10th day of starvation, GOT activity was 416.6% higher than that observed in the fed snails, being reduced and ranging values near to that shown by the control group onwards. GPT activity only varied significantly at the day-30 of starvation. The results were discussed.*

Key words*: Bradybaena similaris* - snail - starvation - aminotransferases - L-aspartate:2oxoglutarate aminotransferase - L-alanine:2oxoglutarate aminotransferase

Aminotransferases, also called transaminases, constitute a group of enzymes that catalyzes the interconversion of amino acids in α-keto acids by transferring amino groups (Moss & Henderson 1998). The aminotransferases have an important role in the linking of the amino acids and carbohydrate metabolism, being an essential group of enzymes in the gluconeogenesis pathway. Beyond this, the aminotransferases are good indicators of tissue lesions.

Aminotransferase activity has been shown by some authors in the hemolymph of molluscs infected with larval trematodes (Manohar et al. 1972), in tissues of trematode-infected (Christie & Michelson 1975) and uninfected snails (Nabih et al. 1990). The activity of these enzymes was also studied in starved snails (El-Emam & Ebeid 1989).

Starvation is a physiological condition that resembles trematode infection. Starvation and trematode infection caused quantitative and qualitative changes in protein and carbohydrate levels in snails (Becker & Schmale 1975, Becker & Hirtbach 1975, Schmale & Becker 1977, Stanilawsky & Becker 1979a,b). Pinheiro and Amato (1994) observed a marked reduction in the glycogen deposits of *Bradybaena similaris* (Férussac, 1821) infected with *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907 larva. This alteration was accompanied by a significant reduction of the glucose concentration in the hemolymph of the parasitized snails. Pinheiro (1996) observed that the starvation caused a similar reduction of the glycogen and galactogen deposits of *B. similaris*. Thirty days of starvation caused the same percentage of reduction as 140 days of *E. coelomaticum* infection. In response to these carbohydrate depletion caused by starvation, the snail uses other substrates to obtain the energy needed for its maintenance. Lira et al. (2000) observed that the total protein content in the hemolymph of *B. similaris* changes after a short period of starvation. Thus, aminotransferase activity in the starved snails must also be changed.

This study reports the changes in L-aspartate:2-oxoglutarate aminotransferase (E.C.2.6.1.1) (GOT) and L-alanine:2-oxoglutarate aminotransferase (E.C.2.6.1.2) (GPT) activities in the hemolymph of starved *B. similaris*.

MATERIALS AND METHODS

Snails collection and maintenance - Specimens of *B. similaris* were collected from gardens located at km 37.5, Avenida Brasil, Rio de Janeiro, RJ, Brazil and transported to Universidade Federal Rural do Rio de Janeiro. The snails were observed under stereomicroscope through their transparent shell to verify the presence of *Phostarmostomum gallinum* 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 10 mm of shell diameter, were transferred to glass vivaria, with earth at the bottom and maintained under laboratory conditions (25 ± 3ºC). They were fed with cabbage leaves (*Brassica* sp.) ad libitum and the vivaria earth was moistened with tap water on alternate days.

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Starvation and hemolymph collection - Groups of 50 snails were formed. The food supplies were suspended and the snails were 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 snails was collected by punction of the pericardial cavity using a syringe (B-D Plastipak®) at 0ºC and stored in microtubes at -10ºC until its utilization.

Biochemical analysis - Aminotransferases, GOT and GPT, activities in the hemolymph of starved *B. similaris* were determined by the method of Reitman and Frankel (1957) according to bioMérieux. The results were expressed as URF/ml.

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

RESULTS

The GOT activity in the control group was 19.367 URF/ml (Table). In starved snails, there was an increase in the GOT activity at 10th day of starvation, being 100.05 URF/ml, which represents an activity 416.6% higher than that observed in the control snails. But, after this period of starvation GOT activity was reduced, ranging values near to that observed in fed snails, being the lowest value observed at 30 days of starvation, when the activity was 8.1% lower than that observed to control group.

The polinomial regression test showed a negative relation between the time of starvation and the GOT activity in the hemolymph of *B. similaris*, but this relation was not very significant ($r^2 = 0.56$) (Fig. 1).

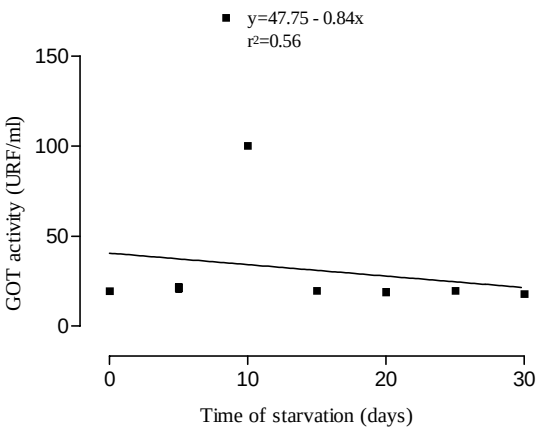

Fig. 1: the negative relation between the L-aspartate:2-oxoglutarate aminotransferase (GOT) activity, expressed im URF/ml, in the hemolymph of *Bradybaena similaris* and the time of starvation, expressed in days.

The GPT activity did not vary so markedly as GOT (Table). The mean value observed at 30 days of starvation was significantly different and higher than that observed at 10, 15 and 25 days of starvation, but it was not different from the activity observed in the control snails. The polinomial regression test showed a positive relation between the time of starvation and the GPT activity in the hemolymph of *B. similaris* (Fig. 2), but this relation was also weakly significant ($r^2 = 0.63$).

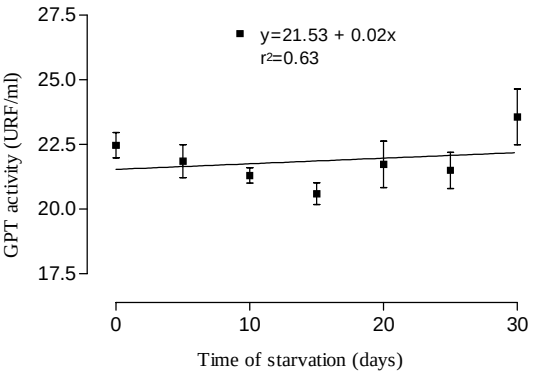

Fig. 2: the negative relation between the L-aspartate:2-oxoglutarate aminotransferase (GPT) activity, expressed im URF/ml, in the hemolymph of *Bradybaena similaris* and the time of starvation, expressed in days.

DISCUSSION

The snails use carbohydrates as the main source of energy for their metabolic processes of maintenance and reproduction, being these substances stored as polysaccharides, as glycogen and galactogen, in tissues of the digestive gland, cephalopedal mass and albumen gland. When the snails are exposed to conditions of stress, such as parasitism by larval trematodes and starvation, other substrates may be used for the same objective (Joosse & Van Elk 1986).

In the snail *B. similaris*, starvation causes a depletion of the glycogen and galactogen reserves (Pinheiro 1996). Lira et al. (2000) observed an increase in the total protein concentration in the hemolymph of *B. similaris* at day 10 of starvation, when this value was 198% higher than that observed in the hemolymph of the fed snails and turning to values near to that of the control group at the end of the period of starvation analyzed (30 days). These authors proposed that the total protein content in the hemolymph of *B. similaris* raised on the tenth day of starvation due to lesions in the tissues of the snail in response to the physiological changes in the snail.

Mohamed and Ishak (1982) observed that in mitochondrial suspensions of starved *Biom-*

TABLE

The L-aspartate:2-oxoglutarate aminotransferase (GOT) and L-alanine:2-oxoglutarate aminotransferase (GPT) activities in the hemolymph of *Bradybaena similaris* under starvation. Twenty five snails were used at each period analyzed

Time of starvation (days)	GOT activity (URF/ml) X ± SD	GPT activity (URF/ml) X ± SD
0 (control group)	19.367 ± 0.9074[a]	22.467 ± 0.4933[a, b]
5	21.300 ± 2.1630[a, c]	21.850 ± 0.6364[a, b]
10	100.05 ± 0.0707[b]	21.300 ± 0.3000[a]
15	19.667 ± 0.3215[a]	20.600 ± 0.4243[a]
20	18.867 ± 1.3800[a]	21.733 ± 0.8963[a, b]
25	19.633 ± 0.9292[a]	21.500 ± 0.7000[a]
30	17.800 ± 0.3215[a, d]	23.567 ± 1.0790[a, b]

X ± SD: mean of five determinations ± standard deviations; *a*, *b*, *c*, *d*: means with significant difference among them (α = 5%)

phalaria alexandrina and *Bulinus truncatus* maintained, the oxygen consumption decreased. It was suggested that starvation exerts a direct influence on the stored energy of the snail to sustain normal metabolic processes. These changes showed that the starvation is responsible for the inhibition of the mitochondrial respiratory rate. The addition of glutamate and α-ketoglutarate was not able to generate GTP which promotes conversion of oxalacetate to phosphoenol pyruvate. In consequence, they proposed that there must be an accumulation of oxalacetate, inhibiting GOT activity and limiting the importance of this enzyme in the gluconeogenesis pathway.

Thus, the results obtained in the present study corroborate the observations of Lira et al. (2000) showing that the increase in the GOT activity observed at the 10th day of starvation occurred due to lesions in the tissues of the snail, mainly in the digestive gland, decreasing after this period due to degradation by enzymes present in the hemolymph of the mollusc. Douglas and Haskin (1976) observed that in *Crassostrea virginica* infected with *Minchinia nelsoni* the GOT increase was associated to the destruction of the tissues of the gills.

The variation of the GPT activity seems to not be related to the alterations resulting from starvation, varying in a less significant way than the GOT activity.

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