Eurytrema coelomaticum (Digenea, Dicrocoeliidae): the Effect of Infection on Carbohydrate Contents of its Intermediate Snail Host, Bradybaena similaris (Gastropoda, Xanthonychidae)

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The interface Eurytrema coelomaticum/Bradybaena similaris was studied by quantifying the amount of glucose on the hemolymph and the content of glycogen in the cells of the digestive gland and the cephalopedal mass of infected and uninfected snails. Samples were analyzed on days 0, 30, 90 and 150 post-infection. The infected snails had less glucose in the hemolymph, with a reduction of 67.05% at 30 days, and 62.09% at 90 days post-infection. The reduction in glycogen content was 86.41% in the digestive gland and 79.1% in the cephalopedal mass at 30 days, and 92.71% and 90.89% in these organs respectively at 90 days post-infection. It is proposed that the sporocysts absorb glucose directly from the hemolymph.

Key words: Eurytrema coelomaticum - Bradybaena similaris - host/parasite interface - glucose - glycogen

Among the effects of larval trematodes on their snail intermediate hosts, the depletion of the energy sources and storage is the most striking one. Investigations on the interrelationship between the larval trematode and its snail host have showed several alterations in the carbohydrate metabolism, specially with reference to glucose (Snyder & Cheng 1961, Cheng & Snyder 1962, Moore & Halton 1973, Ishak et al. 1975, Mohamed & Ishak 1982, Joosse & van Elk 1986). However, most of these studies have been done on freshwater snails.

The terrestrial pulmonate snail Bradybaena similaris (Férussac, 1821) is widely distributed throughout Brazil, being a pest to horticulture and an intermediate host of some parasites. The dicrocoeliid trematode Eurytrema coelomaticum (Giard et Billet, 1892) Looss, 1907, parasitic in the pancreatic ducts of ruminants, can cause losses in milk and meat production (Lapage 1958).

Ishak et al. (1975) observed a high glycogen depletion in Biomphalaria alexandrina and Bulinus truncatus infected with Schistosoma mansoni and S. haematobium, respectively. This reduction was related to the inhibition of the enzymes of the respiratory chain inhibiting the aerobic metabolism of the snails with an acceleration of the anaerobic metabolism of

glucose; because the latter has a low energetic yield a larger amount of glucose is used (Mohamed & Ishak 1982). According to Schwartz and Carter (1982) the direct utilization of glucose by the digenetic larvae can cause a reduction of this carbohydrate in the hemolymph and in the stored glycogen in the tissues of the snail host.

The effects of the infection by E. coelomaticum on B. similaris were studied by measuring the glucose level in the hemolymph, and glycogen contents in the cephalopedal mass and digestive gland of B. similaris during different periods of the intramolluscan larval development.

MATERIALS AND METHODS

Maintenance and infection of the snails - Specimens of B. similaris were collected from gardens located at km 45, Highway BR 465, Itaguaí, RJ, Brazil, and maintained under laboratory conditions (25 ± 3°C; 80 ± 7% relative humidity). The molluscs were examined through their transparent shells for the occurrence of a previous infection, such as the presence of Postharmostomum gallinum metacercariae in the pericardial cavity. Molluscs free of these parasites were randomly chosen and dissected to verify the presence of other parasites.

Molluscs, free of infection, were cultured in glass vivaria with earth at the bottom, moistened with tap water. They were fed ad libitum with cabbage leaves (Brassica sp.) and ration elaborated according to Frantz and Mossmann

(1989). Eggs of *E. coelomaticum* were obtained from adult worms collected from the pancreas of naturally infected bovines and slaughtered at the "Matadouro Industrial de Santa Cruz", Rio de Janeiro, RJ. The eggs were selected according to criteria of Tang (1950) and smeared on pieces of cabbage leaves placed in Petri dishes lined with filter paper moistened with tap water. Five or ten snails, with 10 mm of shell diameter, and 10 eggs/snail were place on these leaves and left overnight. The next day they were transferred to the vivaria.

Hemolymph sampling and glucose quantification - The hemolymph of five groups of 25 snails, for each treatment (control, 30 and 90 days post-infection) was collected using 1.0 ml disposable syringes (B-D Plastipak@) introduced into the pericardial cavitity of the live snail, through a small aperture made in the shell. The hemolymph was kept at -18°C until subjected to analysis. The hemolymph was treated by the method of Nelson (1944) to remove the proteins and to quantify the glucose, the results being expressed in mg %.

Glycogen determination - Snails of nine groups of 25 individuals each, were dissected. The digestive gland (DG) and cephalopedal mass (CM) were separated and kept at -18°C until utilization. The glycogen was extracted and isolated by the method used by Becker (1978) and quantified according to that of Sumner (1924), being expressed in mg glucose/g tissue, wet weight.

Statistical analysis - The results were submitted to the variance analysis (α=5%) and the polinomial regression test, except those relative to hemolymph glucose that were submitted only to the variance analysis (α=5%).

RESULTS

The glucose content in the hemolymph of *B. similaris* was markedly reduced at the first period of intramolluscan development of *E. coelomaticum* sporocyst. At 30 days post-infection the reduction was calculated in 67.05%. Between 30 and 90 days post-infection it was observed an slight increase of glucose in the hemolymph, and the reduction was 62.09%. Statistical analysis showed that the amount of glucose varied significantly (α - 5%) in the three periods tested (Table I).

The glycogen content in *B. similaris* in both sites studied was highly reduced in response to the parasitism by larval *E. coelomaticum*, being the reduction rate higher than 95% at the end of the larval development (Table II). The analysis of variance test showed no variation in the glycogen contents in CM and in DG, at 90 and 150 days post-infection, but there was significative difference among the values obtained at the others periods of the infection studied (Table III), and

TABLE I

Glucose concentration in the hemolymph of the *Bradybaena similaris*, and its percent reduction through the *Eurytrema coelomaticum* infection

Period of infection (days)	N	Glucose concentration (mg %) X ± SD	Percent reduction (%)
uninfected	5	44.02 ± 1.30[a]	0
30	5	14.56 ± 0.99[b]	67.05
90	5	16.68 ± 0.46[c]	62.09

X ± SD - mean values ± standard deviation. N - number of repetitions.
Leters [a], [b] and [c] indicate means with significant difference among them (α - 5%).

TABLE II

Percent reduction of glycogen content in the cephalopedal mass and digestive gland of *Bradybaena similaris* through the *Eurytrema coelomaticum* infection

| Period of infection (days)	Reduction in the glycogen content Cephalopedal mass (%)	Digestive gland (%)
uninfected	0	0
30	79.10	86.41
90	90.89	92.71
150	96.70	99.14

TABLE III

Glycogen content in the cephalopedal mass and in the digestive gland of *Bradybaena similaris* through the *Eurytrema coelomaticum* infection

Period of infection (days)	N	Glycogen content (mg glucose/g tissue, wet weight) Cephalopedal mass X ± SD	Digestive gland X ± SD
uninfected	9	22.33 ± 2.631[a]	42.09 ± 3.680[a]
30	9	4.79 ± 0.429[b]	5.85 ± 0.302[b]
90	9	2.11 ± 0.195[c]	3.16 ± 0.483[c]
150	9	0.76 ± 0.041[c]	0.38 ± 0.107[c]

X ± SD - mean ± standard deviation; N - number of repetition
Letters [a], [b] and [c] indicate means with significant difference among them (α - 5%).

the polinomial regression analysis revealed a strong negative relation between the time of infection by *E. coelomaticum* and the glycogen content in both sites, MC and DG, in *B. similaris* (Fig.).

Estimated glycogen (mg glucose/g tissue)

Duration of infection (days)
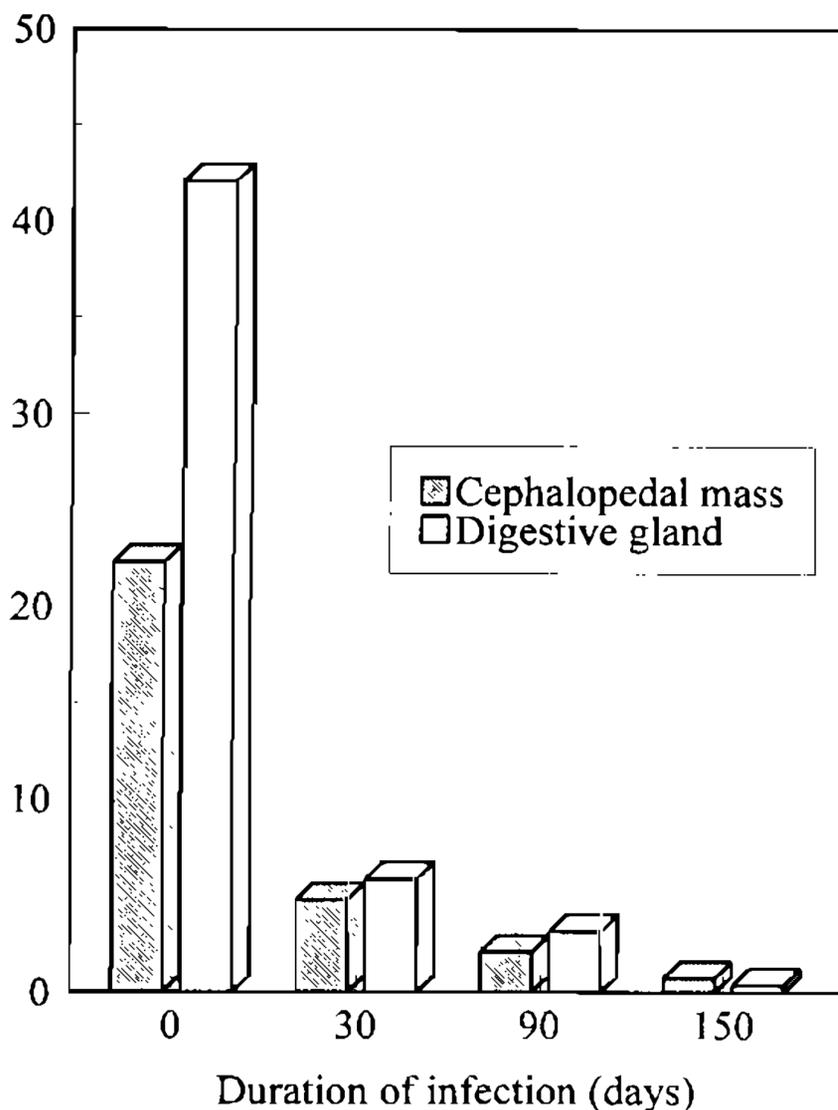
Cephalopedal mass
$y = 22.329998 - 0.8694573x + 0.01065501x^2 - 0.000038784x^3$
$r^2 = 1.0$
Digestive gland
$y = 42.091112 - 1.8285266x + 0.02327272x^2 - 0.000086242x^3$
$r^2 = 1.0$

Relation between the glycogen content in cephalopedal mass and digestive gland of *Bradybaena similaris* (mg glucose/g tissue) during intramolluscan development of larval *Eurytrema coelomaticum*.

DISCUSSION

The reduction in the glucose concentration in the hemolymph of the snails parasitized by larval trematodes has been reported (Cheng & Lee 1971, Friedle 1971, Lee & Cheng 1972, Stanislawsky & Becker 1979). Our results appear to be in accordance to what has been published by other authors, e.g. Stanislawsky and Becker (1979), who working with the *S. mansoni/B. glabrata* association, found that, at the end of the larval development, the concentration of glucose

in the hemolymph was reduced by 50%. Although we had no values of hemolymph glucose for the end of the larval development of *E. coelomaticum* in *B. similaris*, the results obtained at the 30 and 90 days of infection lead us to suppose that the concentration of this carbohydrate in the hemolymph at the end of the larval intramolluscan development would also be approximately 50%.

In a review, Becker (1980) indicated that larval trematodes are bathed by the hemolymph of their snail host, obtaining from it the nutrients and releasing into it their excreting products, thus altering the normal composition of the hemolymph and causing physiological alterations in the host. In the interface *E. coelomaticum/B. similaris* there was no previous data on glucose concentration in hemolymph of *B. similaris* infected with *E. coelomaticum* being difficult to compare and to discuss our results. However it is evident that this trematode uses the glucose of its snail host for its development.

The glycogen contents in CM and DG of *B. similaris* measured during the intramolluscan development of *E. coelomaticum*, were quickly and markedly reduced during the first period of infection studied, the depletion being lower in CM than in DG.

Many authors have shown glycogen depletion in other host/parasite relationships (Moore & Halton 1973, Christie et al. 1974, Ishak et al. 1975, Joosse & van Elk 1986). In that of *E. coelomaticum/B. similaris*, Paschoal and Amato (in press) pointed out that during the larval intramolluscan development of the parasite, the glycogen storage of the digestive gland tissue was highly reduced and, at the same time, deposits of this polysaccharide increased in the body of the cercaria contained in the daughter sporocysts. Similar results were observed by Snyder and Cheng (1961), Cheng and Snyder (1962, 1963), working with the interface *Glypthelmins pennsylvaniensis/Helisoma trivolvis*. They concluded that the larval trematode could secrete a substance, like an enzyme, that could penetrate the digestive gland cells and hydrolise the glycogen. The resultant glucose molecules could cross the membranes of the digestive gland cells being absorbed by the larvae through its tegument and ounce inside the glycogen would be resynthesized. This hypothesis was widely accepted to explain the glycogen depletion in snails caused by larval trematodes. But if this is true, it should not be expected that, in the interface *E. coelomaticum/B. similaris*, a concomitant reduction of the glycogen in both sites, CM and DG, would be observed, since that there are no larval stages in the CM, and the CM is not a site for development of *E. coelomaticum* sporocysts. The hypothesis of Cheng and Snyder (1962) can not

therefore be used to explain the glycogen depletion caused by this parasite in B. similaris.

Becker (1980) showed that S. mansoni absorbs glucose directly from the hemolymph of B. glabrata and, according to Thompsom and Lee (1986), the snails tend to maintain constant the glucose levels in the hemolymph. Thus, we can suppose that the sporocysts of E. coelomaticum absorb glucose from the hemolymph of the B. similaris and, by doing so, there is a decrease in the contents of this carbohydrate in the hemolymph. To maintain the normal glucose level in the hemolymph, the snails use the stored carbohydrates, like that of the DG, site location of the daughter sporocysts, and also from the CM, causing the deep reduction in the contents of the carbohydrates stored like the glycogen.

ACKNOWLEDGEMENTS

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