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Carbohydrate Reserves and Hemolymph Sugars of the African Giant Snail, *Achatina fulica* in Relation to Parasitic Infection and Starvation

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Abstract. In the snail, *Achatina fulica,* parasitized by the rat lungworm *Angiostrongylus cantonensis,* levels of carbohydrate in hemolymph and digestive glands were determined. The normal level of hemolymph glucose of 11.7 mg% dropped to 4.25 mg% in the infected snails, a significant difference after only one week of infection. The level of total reducing sugar in the hemolymph also decreased significantly (12.3 mg% to 3.6 mg%) in this period. Later on, the snails were capable of adapting themselves to the parasitic infection and the concentration of hemolymph sugars returned to the normal range. Starvation caused a decrease in the carbohydrate reserves of the digestive gland only when the starved snails had been previously infected.

Key words: Carbohydrates – *Achatina fulica* – *Angiostrongylus cantonensis* – Starvation.

Introduction

It is generally believed that one contribution to the pathology of parasitic infections is an alteration in the components of body fluids and reserves which results from food competition between the host and parasite. Lee and Cheng (1972) have demonstrated a significant drop in hemolymph protein in *Biomphalaria glabrata* infected with *Schistosoma mansoni.* In this host-parasite model, the blood glucose level of the infected snails is also decreased (Cheng and Lee, 1971). Decrease in carbohydrate content of the digestive gland has been reported for *Littorina littorea* infected with trematodes (Robson and Williams, 1971). It has been confirmed by Christie et al. (1974) that qualitative changes in glycogen and galactogen occur in *B. glabrata* infected with *S. mansoni* before and after cercarial maturation. However, the situation of biochemical alterations in snails infected with nematodes appears to be different. Previously, Brockelman (1978) reported that infection by *Angiostrongylus cantonensis* in the snail, *Achatina fulica* did not cause any significant changes in the concentration of hemolymph protein. The snails lost their ability to maintain the hemolymph protein within the normal range only under additional stress such as repeated bleeding

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and/or starvation. Furthermore, some preliminary observations on *A. fulica* infected with *A. cantonensis* revealed no significant change in glucose levels in the hemolymph (Sitthithavorn, unpublished data).

For this communication, we therefore decided to examine parasitized and stressed snails for the levels of glucose and total reducing sugar in the hemolymph and to compare these levels with those of the same sugars and other carbohydrates in the digestive gland. We hoped that the knowledge obtained would lead to a better understanding of tolerance to stress in snails.

Materials and Methods

1. Experimental Animals

The snails, *Achatina fulica* were originally collected from an uninfected population in Cholburi Province, Thailand in 1974. The snails reproduced well in our laboratory making it possible to establish a colony. Four to six snails were maintained in an eight liter earthenware bowl containing wet sand and garden soil which had been heat sterilized prior to use. Unless stated otherwise, the snails were usually fed ad lib. on carefully washed lettuce supplemented with Hygro fish food, sodium algenate, and calcium carbonate.

The parasite, *Angiostrongylus cantonensis* has been maintained in our laboratory since 1974 by passing from the rat definitive host and then to the snail intermediate host at least three times a year.

2. Handling of the Snails

Procedures for estimation of snail size and weight, bleeding to collect hemolymph, and experimental infection of the snails were similar to those described in a previous study (Brockelman, 1978). For this investigation, snails of 4.5–6.0 cm were used and the infection dose was 2,000–5,000 first stage larvae per snail.

3. Experimental Design

Estimation on Total Reducing Sugar (Fig. 1). One month before the infection, 48 laboratory reared snails were bled from the viseral sac for 0.1 ml of hemolymph. Concentrations of total reducing sugar were determined and used to group the snails into eight classes of six animals each. Half of each class was infected with 5,000 larvae whereas the remaining half was kept as uninfected controls. After the experimental infection, all snails were allowed to feed only on lettuce within the limit of 20 g per four snails per one rearing bowl. Fresh lettuce was supplied at 5 p.m. every day and was usually eaten up by 7 p.m. Bleeding of the snails was consistently performed at 9 a.m. on the following day. Because previous experiments (Brockelman, 1978) showed that snails bled more than five times at four day intervals showed delayed regeneration of shells, we scheduled the bleeding on days 2, 5, 8, 11, 14, 21, 28, and 35.

Correlation Between Hemolymph Glucose and Glycogen under Parasitic Stress (Table 1). Twenty snails were infected with larvae of *A. cantonensis*, and the other twenty snails were used as controls. On day 0 and every seven days after infection until day 28, four infected and four control snails were bled to death and the hemolymph glucose was determined. Digestive glands were dissected carefully, weighed, and assayed for glycogen content. In this experiment, snails were allowed to feed on an unlimited amount of fresh lettuce.

Effects of Parasitic Stress and Starvation (Tables 2 and 3). For this experiment, 56 snails were used. Twenty-eight of them were each infected with 5,000 larvae of *A. cantonensis*. After the

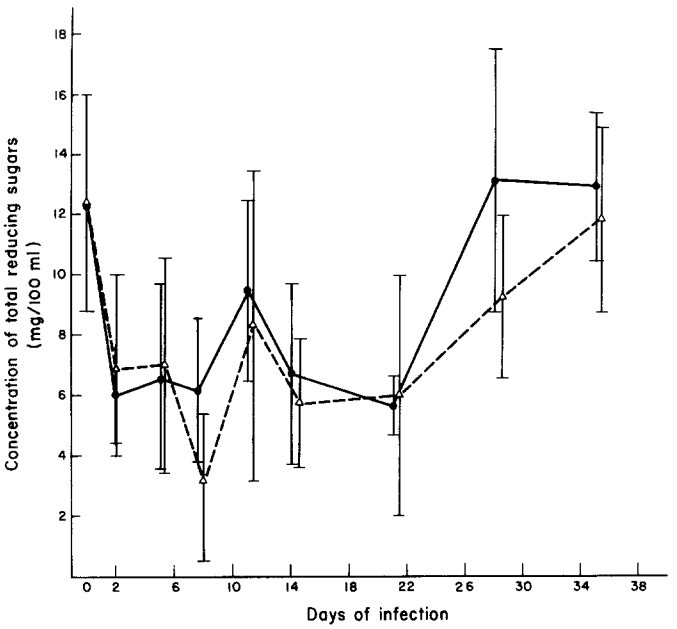

Fig. 1. Concentrations of total reducing sugar in the hemolymph of non-infected (●——●) *Achatina fulica* compared with those of infected (△--△) snails. Vertical bars represent standard deviations of the means

Table 1. Comparison of the glucose concentrations (mg in 100 ml) in hemolymph samples of *Achatina fulica* non-infected and infected with 2,000 larvae of *Angiostrongylus cantonensis*. Measurements were made at weekly intervals after infection. All groups consisted of eight snails

Time (weeks)	Control		Infected		*P*-value
	Mean	SD	Mean	SD	
0	11.7	4.07	–	–	–
1	19.05	5.94	4.25	0.96	<0.10
2	10.47	6.42	4.53	1.56	>0.10
3	2.33	0.67	2.50	1.04	>0.10
4	7.60	5.29	13.29	6.32	>0.10

infection, 14 of them were fed on 5 g of lettuce per snail per day throughout the two month experimental period. The other 14 snails were kept without food at very low moisture to induce aestivation. The other 28 non-infected snails were divided into two groups of 14, and only one group was fed (Table 3). The aestivated snails were kept in empty rearing bowls until they were sacrificed and dissected for the digestive gland.

Table 2. Comparison of the glycogen concentrations (mg/g) of fresh digestive gland of *Achatina fulica* non-infected and infected with 2,000 larvae of *Angiostrongylus cantonensis*. Measurements were made at weekly intervals after infection. All groups consisted of eight snails

Time (weeks)	Control		Infected		*P*-value
	Mean	SD	Mean	SD	
0	2.10	0.50	–	–	–
1	3.89	2.84	5.29	3.84	>0.10
2	1.59	1.72	2.00	0.88	>0.10
3	7.43	1.56	12.07	7.15	>0.10
4	9.38	0.18	8.53	4.02	>0.10

Table 3. Mean concentrations of polysaccharide from the digestive glands of *Achatina fulica* which have been subjected to parasitic infection and/or starvation. Vertical lines connect insignificantly different means according to the Student-Neuman-Keuls test procedure

Treatment of snails	Polysaccharide Mean concentrations (mg/g dry weight)	$\alpha=0.05$	$\alpha=0.01$
non-infected, fed	85.33		
infected, fed	81.76		
non-infected, starved	76.71		
infected, starved	56.61		

Chemical Analysis

Determination of Glucose. Hemolymph (0.2 ml) bled from individual snails was added to 1.8 ml triple distilled water then deproteinized by Somogyi's method (1930). After separation of the protein by centrifugation at $1,200 \times g$ for 20 min, the supernatant was used to determine the glucose content using glucostat reagent (Worthington Biochem. Corp).

Determination of Total Reducing Sugar. One half ml of hemolymph was added to 1.5 ml of triple distilled water and acidified with concentrated acetic acid in order to flocculate the hemolymph protein. After centrifugation at $1,200 \times g$ for 15 min, the supernatant was assayed for total reducing sugar (Nelson, 1944).

Polysaccharide Determination. Digestive glands were dissected from the snails, weighed, and used directly for extraction. In one of the experiments (total polysaccharides) the glands were frozen immediately and freeze-dried at $-4°$ C before their carbohydrate reserves as polysaccharide concentration were determined.

Glycogen was extracted from the tissue and determined using the method described by Montgomery (1957). Generally, 0.25 g of digestive gland was dropped into 2 ml of 30% KOH in a centrifuge tube and boiled in a water bath for 20 min. After complete cooling, the glycogen was precipitated with 95% ethyl alcohol. The precipitate was redissolved in 2 ml of distilled water and assayed for glycogen. Shellfish glycogen was used as the standard.

Before the determination of total polysaccharide, 50 mg of freeze dried digestive gland tissue was hydrolyzed in one ml of hot 2N HCl. The concentration of hexoses was determined by the primary cysteine sulfuric acid method (Dische and Danilchenko, 1967). Galactose was used as the standard.

Results

When the snails, *A. fulica,* were experimentally infected with larvae of *A. cantonensis,* the parasitic infection did not cause any great alteration in hemolymph glucose. As summarized in Table 1, the glucose concentrations in hemolymph samples from the infected group were significantly lower than those of the control group only after one week of infection. This result coincided well with decreases in the concentration of total reducing sugar in another group of infected snails which had been bled repeatedly every three days post-infection (Fig. 1). The first bleeding caused a marked drop in the concentration of sugar from 12.3 ± 5.6 mg% to 6.0 ± 2.2 mg% in the uninfected group and to 6.5 ± 3.4 mg% in the infected group. Levels of total reducing sugar in the infected group were lowest on day 8 post-infection and had the mean value of 3.6 ± 2.5 mg%. This value was only one quarter of the mean for the control group. However, soon afterwards the sugar concentrations rose sharply over the following three days in both uninfected and infected snails (means 9.5 mg% and 8.4 mg%, respectively, on day 11). When the bleeding interval was increased from three to seven days, the uninfected snails regained their normal hemolymph sugar content more quickly than the infected snails. The uninfected control group reached the normal level of total reducing sugar (12.0 mg%) by day 28, whereas the infected group took seven days longer. This experiment provided data on total reducing sugar levels in an individual snail over a long period of time. It was apparent from the non-infected individuals that the sugar levels in *A. fulica* fluctuated naturally and that variations among the snails were large. Among the controls, the maximum individual value was 17.91 mg% which was about four and a half time higher than the minimum value of 3.95 mg% from another individual bled on the same day. The levels of total reducing sugar varied even more in the infected group as shown by the standard deviations plotted in Fig. 1.

No correlation was found between levels of blood glucose and levels of glycogen in the digestive glands. As summarized in Table 2, there was no significant decrease in hepatopancreas glycogen in the infected group when compared with the non-infected control ($P > 0.10$), although snails of both groups were killed and examined for glycogen every week throughout the course of infection.

Starvation alone did not have any effect on the depletion of carbohydrate reserves in the digestive gland of *A. fulica.* Table 3 shows that carbohydrate contents of starved but non-infected snails did not decrease significantly. Only the snails which had been starved after being infected showed a significant decrease in carbohydrate reserves. The carbohydrate values summarized in Table 3 are the values obtained from the same snails bled repeatedly for total reducing sugars (Fig. 1). Repeated loss of hemolymph in small quantities (0.5 ml) did not influence the use of the carbohydrate reserves of the snails.

Discussion

To a certain extent, this study confirmed previous reports that the levels of glucose in gastropods varied greatly (Cheng and Lee, 1971; Becker, 1972).

As indicated by Altman and Dittmer (1971) the hemolymph glucose concentration in *Achatina fulica* was 7.30 ± 2.10 mg/%. Our mean values varied between 2.33 and 19.05 mg/% despite precautions not to handle the snails unnecessarily. We were aware that vigorous handling might cause a rise in hemolymph glucose levels (Holtz and von Brand, 1940).

Barry and Munday (1959) pointed out that blood sugar levels reflected the carbohydrate intake in *Patella vulgata*. They demonstrated a rise in blood sugar from 2.5 mg% to 30 mg% after injection of 2 mg of glucose into the blood stream. Four hours later the blood sugar level returned to its preinjection level. In our experiment bleeding was performed consistently, about 14 hours after the snails had been fed. Results obtained still varied greatly, suggesting differences in the normal level for individual snails. Therefore it was essential to bleed the snails one month before experimentation, so that they could be classified according to blood sugar levels. In this way the mean concentrations for the control group and infection group could be the same at the beginning of any experiment and this eliminated a possible technical error when the effects of parasitism were investigated.

Parasitic infection of the snails with the nematode, *A. cantonensis* did not change levels of hemolymph carbohydrate throughout the course of infection. The decrease in levels of glucose and total reducing sugar was observed only one week after infection. This period is a crucial time for the host, since this is the time when the first stage larvae have just become lodged in the mantle and started to develop extensively (Brockelman et al. 1976). Their length increases from 240 μm to 360 μm within only four days – the highest growth rate observed for intramolluscan stages of *A. cantonensis*. This growth is probably accompanied by a high rate of carbohydrate intake by the nematode and it may be the time of peak competition with the host.

Infection by *A. cantonensis* did not affect carbohydrate reserves of the digestive gland even though the snails were fed minimum dietary requirements. Our results show that the host-parasite relationship for a nematode infection in a large snail is quite different from that reported for small snails infected with trematode parasites (Cheng and Synder, 1962; James, 1965; Porter, 1970). The causes for the difference should result from the lack of asexual multiplication of the nematode in this molluscan host, and the capacity of the digestive gland of the land snail to store glycogen in large quantities. The carbohydrate reserves must be sufficient for the hibernation period which has been observed to be five dry "winter" months (Sitthithavorn, unpublished observations). Therefore, the two-month starvation period in this experiment did not cause any significant drop in the carbohydrate content of the digestive gland.

Although significant changes in carbohydrate concentrations of the digestive glands were observed when the snails were subjected to simultaneous infection and starvation, this phenomenon was unnatural. In nature, the snails feed less and less when the dry season starts. They begin to lose water, become less active, and burrow in the soil where they retreat in their shells and seal them with an epiphragma. Therefore, ingestion of 5,000 larvae of *A. cantonensis* could not naturally be followed in 48 h by aestivation. Our experiments have demonstrated that the snails can well handle parasitic infection under natural conditions. Hence, the suggestion by Mead (1972) of using *A. cantonensis* for

the biological control of *A. fulica* would probably be unsuccessful. In addition, increase in incidence of angiostrongyliasis would mean increasing a hazard to the public health since this nematode is a primary cause of eosinophilic meningoencephalitis in the Asia-Pacific region (Cross, 1979).

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