PHYSIOLOGICAL RESPONSE TO PARASITISM I. CHANGES IN CARBOHYDRATE RESERVES OF THE MOLLUSCAN HOST

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INTRODUCTION

The presence and distribution of carbohydrate in the tissues of various classes of molluscs under different physiological conditions has been reviewed (Martin, 1961; Goddard and Martin, 1966; Goudsmit, 1972). The metabolism of the host snails during trematode infection has largely been ignored in these reviews although they are of undoubted importance to medical malacologists. The available reports show that the carbohydrate reserves of the snail host influence the development and emission of cercariae (Kendall, 1949; Megalhaes and De Almeida, 1956; Sindermann *et al.*, 1957) and that the larval trematodes deplete the carbohydrate reserves of the host (Cheng and Snyder, 1962; James, 1965; Wright, 1966; Cheng, 1963c, 1967).

However, Fried and Blumenthal (1967) reported no significant difference between the reducing sugar levels of uninfected *Nassarius obsoletus* and those infected with *Stephanostomum tenue*. Robson and Williams (1971) found the concentration of glycogen in digestive gland and foot of *Littorina littorea* infected with redia of *Himasthla leptosoma* to be essentially the same as in corresponding uninfected tissues. *Proctoeces maculatus* does not significantly alter the carbohydrate reserves of *Mytilus edulis* according to Dennis *et al.*, (1974). Therefore it appears reasonable to expect carbohydrate replenishment to take place in infected molluscs. Although, it is reported that the host snail absorbs sugar from the environment (Cheng and Burton, 1966) and feeds voraciously when infected (Hurst, 1927; Wesenburg-Lund, 1934) the information is inadequate to satisfactorily explain how the mollusc meets the requirements of such a large number of developing larvae, in addition to its own and, how it replenishes its own carbohydrate lost to the parasite. The present investigation was therefore designed to elucidate on the physiological and metabolic disruptions caused by the developing intramolluscan larval trematodes.

MATERIALS AND METHODS

Lymnaea luteola were collected in the months of September to December from a single population in a selected paddy field. They were washed in tap water and acclimated to laboratory conditions for a fortnight. They were fed *ad lib* with half boiled leaves of *Amaranthus viridis*. They were then thoroughly washed in running tap water and suspended in isolation in 20 ml of dechlorinated tap water in 3″ × 1″ specimen tubes. Periodic observation for cercaria shed enabled us to sort out the snails with mature infections. These were considered as 'infected' and used for analysis. The others not shedding the cercariae, were resuspended in isolation periodically. Those that did not shed cercariae after repeated suspension were temporarily considered as uninfected until their tissues were teased in saline and

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examined under a stereobinocular dissection microscope. Only those that harboured no larval stages of the parasite were considered as 'uninfected' and used for analysis. In the present study the snails in the weight range of 300-400 mg were used. Infected snails bore single infections of xiphidiocercariae of *Prosthogonimus* species (Manohar *et al.*, 1972). Samples of uninfected and infected stocks were used for the determination of water content in the tissues, others for the estimation of glycogen and sugar and the rest were subjected to experimental starvation.

Starvation and re-feeding experiments : The snails were starved by isolation in 20 ml of dechlorinated tap water in specimen tubes for 72 hours, with the water changes, at 12 hour intervals. Beyond 72 hours the mortality rate, particularly for infected snails, was too high and one half of these were sacrificed as 'starved' snails for carbohydrate analyses. The remainder were kept in isolation in 10 ml of 0.025M Analar D-glucose for 20 hours in sufficiently large Petri dishes so that their feet alone were immersed. The sugar medium contained 500 units of streptopenicillin. At the end of the period they were washed thoroughly in running tap water for 5 minutes and used as re-fed snails for analysis.

Collection of tissues : The snails were chilled on ice and the body removed from the shell carefully. The digestive gland, foot and mantle were isolated under a stereobinocular dissection microscope. The foot, which is practically free of the parasites, and the mantle, with very few cercarial stages in its sinus (Manohar and Venkateswara Rao, 1975) were separately minced and washed in cold (5°C) saline (Carriker, 1946). They were weighed quickly on a torsion balance after rolling on to filter paper and stored at −10°C. The digestive gland was teased thoroughly in cold saline with fine glass needles under a dissecting microscope in a cold room (16 ± 1°C) and washed with fine jets of saline from a pipette. This helped washing away parasites, if present from the parasitized gland or confirmed the absence of infection. As suggested by Cheng and Burton (1965) the tissues isolated from individual snails were kept separately in numbered containers until confirmation was sought. Glands with immature parasites were not used. The collection of body fluid and the subsequent preparatory procedures are described elsewhere (Manohar *et al.*, 1972).

Estimation of glycogen and reducing sugar : The tissues were homogenized in 5 ml of 30% cold methanol in a cold room (16 ± 1°C) with a Potter-Elvejhem glass homogenizer. The homogenates were centrifuged at 1500g for 15 minutes. Glycogen in the sediment and reducing sugar in the supernatant were estimated by the method of Kemp and Kitts Van Heijningen (1954). The body fluid was diluted to 5 ml with 80% cold methanol, mixed thoroughly, centrifuged at 1500g for 15 minutes and the supernatant saved for analysis. Evaporation of methanol was carried out in a vacuum drying oven set at 37°C. The color density of hydroxymethylfurfurol complex was read at 520 mμ in a Hilger and Watts (England) Senior Spectrophotometer using glass cuvettes of 10 mm path length. A standard curve was plotted with different concentrations of Analar D-glucose. The values were expressed as mg of glucose per gm dry weight of tissue or per ml of body fluid.

RESULTS

The glycogen and reducing sugar content of the infected and uninfected snails exposed to three different physiological conditions (normal, starved and refed) are presented in Table 1. Per cent changes on infection, starvation and refeeding are given in Table 2.

Changes in digestive gland : Glycogen and sugar levels were significantly higher in the

Table 1

Comparative statement of glycogen and sugar levels in uninfected and infected snails under three different physiological conditions (unstarved, starved and re-fed).

(Values expressed as mg of glucose per gram dry weight* of tissue or per ml of body fluid. Each value is the mean of a minimum of 9 and maximum of 24 individual observations)

Condition of the snail		Glycogen or sugar in			
		Digestive gland	Foot	Mantle	Body fluid
Uninfected and Unstarved	Glycogen	37.83 ± 3.90	67.06 ± 3.90	53.23 ± 5.80	
	Sugar	18.71 ± 3.17	37.36 ± 11.28	47.66 ± 13.50	1.63 ± 0.53
Infected and Unstarved	Glycogen	92.39 ± 3.79	86.35 ± 11.00	128.00 ± 10.07	
	Sugar	31.82 ± 8.60	8.97 ± 3.36	9.86 ± 3.68	2.42 ± 1.06
Uninfected and Starved	Glycogen	17.60 ± 2.13	15.32 ± 2.88	16.95 ± 2.44	
	Sugar	17.18 ± 3.46	11.85 ± 1.61	13.86 ± 1.30	0.43 ± 0.08
Infected and Starved	Glycogen	23.28 ± 4.41	48.01 ± 4.70	35.16 ± 6.60	
	Sugar	20.37 ± 8.07	6.11 ± 0.89	9.65 ± 3.01	1.50 ± 0.38
Uninfected and Re-fed	Glycogen	43.22 ± 4.03	32.02 ± 4.80	42.57 ± 4.52	
	Sugar	41.54 ± 5.04	14.79 ± 2.24	21.02 ± 3.65	0.97 ± 0.44
Infected and Re-fed	Glycogen	29.03 ± 3.63	66.65 ± 9.77	44.00 ± 4.65	
	Sugar	35.16 ± 6.30	35.06 ± 12.84	39.34 ± 7.83	1.51 ± 1.08

*No significant difference was observed in the percentage of water content of infected and uninfected tissues.

infected (Figs. 1, 5) than in the uninfected snails. Although starvation led to severe depletion of carbohydrate in infected snails (Figs. 1, 6, 7), these continued to maintain higher levels (Figs. 1, 8) than corresponding uninfected starved snails. On re-feeding, both glycogen and glucose increased more in the uninfected snails than in the infected snails (Figs. 1, 9, 10). While both glycogen and sugar could exceed prestarvation levels in uninfected re-fed snails (Fig. 1), only sugar could be restored to pre-starvational state by the infected re-fed snails. The carbohydrate quantity is actually lower in infected snails after re-feeding (Fig. 11).

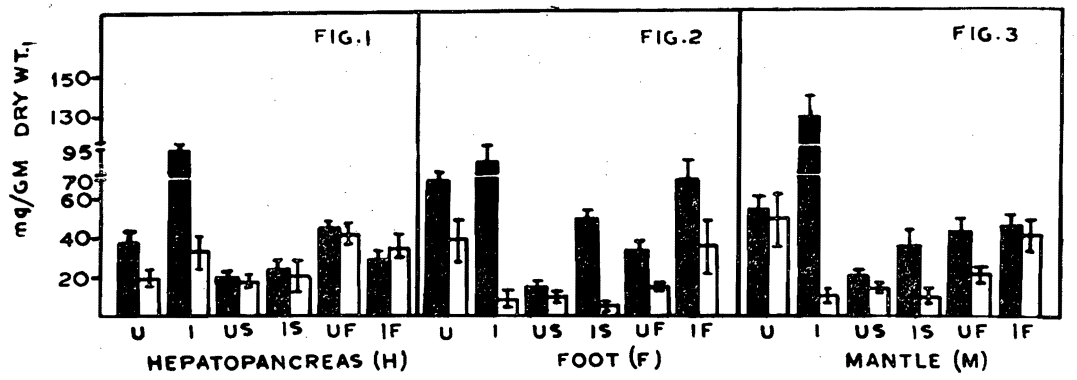
Changes in foot tissue and mantle : In the infected snails the glycogen content was enhanced and sugar was decreased significantly over the uninfected snails (Figs. 2, 3). This picture did not change even when starvation led to carbohydrate depletion in both the groups (Figs. 2, 3, 6, 7, 8). While refeeding resulted in significant increase in carbohydrate content in both infected and uninfected snails over corresponding controls (Figs. 9, 10), the former always had conspicuously higher levels (Fig. 11). The uninfected refed snails could not restore neither glycogen nor sugar to their prestarvation levels while the infected snails could not only regain glycogen to a large extent but could also augment sugar content over the prestarvation level (Figs. 2, 3).

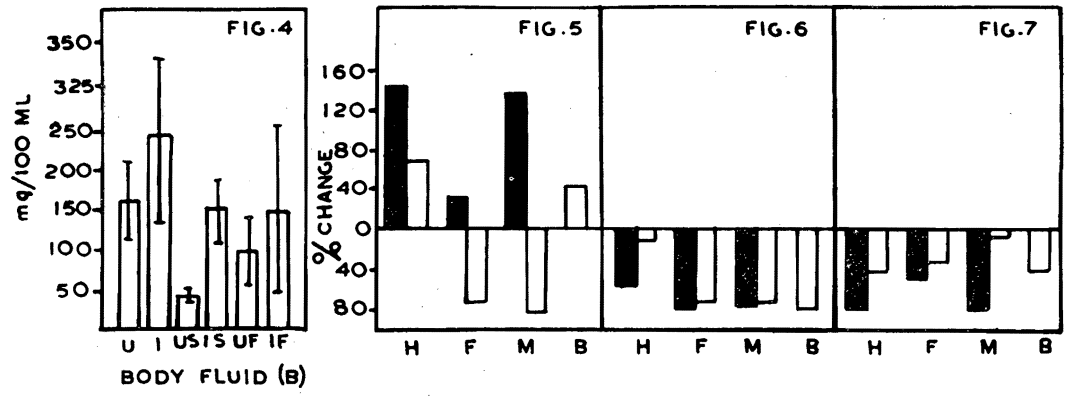
Changes in body fluid : Infected snails invariably had higher quantities of reducing sugar (Figs. 4, 5) than the uninfected snails. They remained hyperglycaemic (Fig. 8) even after starvation when compared to uninfected starved snails, although there was a general decrease in both groups (Figs. 6, 7). On re-feeding, the blood sugar content in uninfected snails increased significantly over

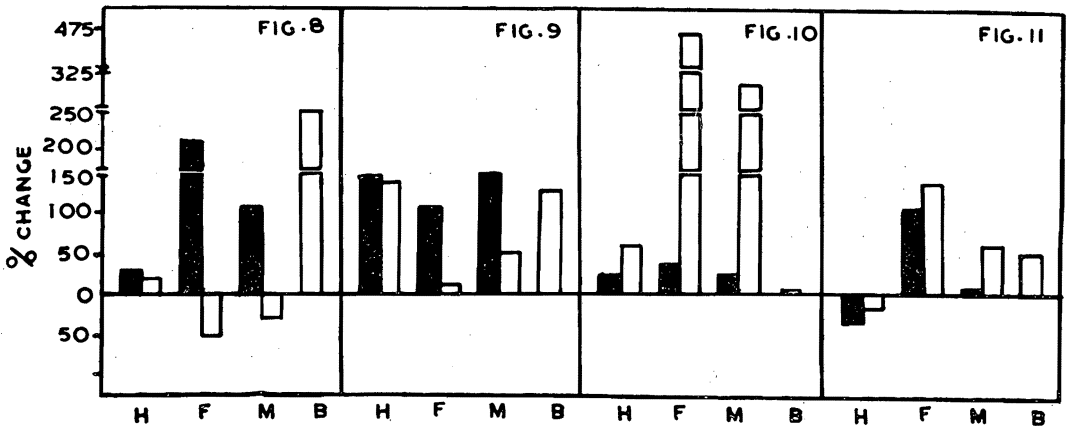
Table 2

Per cent changes in glycogen and sugar levels of snails on infection, starvation and refeeding.

% Change in	When compared to	% change in						
		Digestive gland		Foot		Mantle		Body fluid
		Glycogen	Sugar	Glycogen	Sugar	Glycogen	Sugar	Sugar
Infected	Uninfected	+ 144.20 P < 0.001	+ 70.01 P < 0.001	+ 32.30 P < 0.001	– 76.00 P < 0.001	+ 136.80 P < 0.001	– 79.31 P < 0.001	+ 48.28 P < 0.001
Uninfected and starved	,,	– 53.47 P < 0.001	– 8.17 N.S.	– 77.15 P < 0.001	– 68.28 P < 0.001	– 68.15 P < 0.001	– 70.91 P < 0.001	– 73.70 P < 0.001
Infected and starved	Infected	– 74.80 P < 0.001	– 35.96 P < 0.001	– 45.76 P < 0.001	– 31.83 P < 0.002	– 72.11 P < 0.001	– 2.12 N.S.	– 38.02 P < 0.02
,,	Uninfected and starved	+ 32.20 P < 0.02	+ 18.50 N.S.	+ 212.70 P < 0.001	– 48.80 P < 0.01	+ 107.20 P < 0.001	– 30.40 P < 0.01	+ 250.80 P < 0.001
Uninfected starved and re-fed	Uninfected and starved	+ 145.50 P < 0.001	+ 141.80 P < 0.001	+ 109.00 P < 0.001	+ 24.80 P < 0.01	+ 150.56 P < 0.001	+ 51.6 P < 0.001	+ 130.40 P < 0.001
Infected starved and re-fed	Infected and starved	+ 24.70 P < 0.01	+ 72.60 P < 0.001	+ 38.80 P < 0.001	+ 473.40 P < 0.001	+ 25.10 P < 0.01	+ 307.60 P < 0.001	+ 0.943 N.S.
,,	Uninfected starved and re-fed	– 32.80 P < 0.001	– 15.30 P < 0.05	+ 108.10 P < 0.05	+ 137.00 P < 0.001	+ 3.60 N.S.	+ 87.10 P < 0.001	+ 53.50 N.S.







Figs. 1-4—Glycogen ■ and sugar □ levels in the tissues and body fluid of uninfected and infected snails. U = Uninfected unstarved; I = Infected unstarved; US = Uninfected starved; IS = Infected starved; UF = Uninfected re-fed; IF = Infected re-fed.

Figs. 5-11—Effect of infection, starvation and re-feeding individually and in combination is shown as per cent changes in glycogen ■ and sugar □ levels in digestive gland (H), foot (F), mantle (M), and body fluid (B). Fig. 5—depicts changes in I when compared to U. Fig. 6—in US when compared to U. Fig. 7—in IS when compared to I. Fig. 8—in IS when compared to US. Fig. 9—in UF when compared to US. Fig. 10—in IF when compared to IS. Fig. 11—in IF when compared to UF.

starved controls but still failed to reach pre-starvation levels (Figs. 4, 9). Though no such increase could be noticed in the re-fed infected snails, they still remained hyperglycaemic compared with re-fed uninfected snails (Fig. 11).

DISCUSSION

With the object of determining whether the physiological disruption caused by infection and the consequent problem of how such an infected snail could meet the demands of the developing trematode larvae, comparison of carbohydrate levels of various tissues of infected and uninfected snails, before and after starvation as well as on refeeding, is made here.

Although it was impossible to eliminate all parasitic material from the infected glands, the higher carbohydrate content could not be attributed simply to the presence of the parasites since there was a similar increase in parasite free organs such as the foot. Robson and Williams (1971) previously found concentrations of glycogen in the digestive gland and foot of *Littorina littorea* infected with *Himasthla leptosoma* to be essentially the same as in corresponding uninfected tissues. Furthermore no statistically significant loss of glycogen could be observed in the digestive gland of *Mytilus edulis* infected with *Proctoeces maculatus* (Dennis *et al.*, 1974). Moreover, similar increased glycogen levels were reported in insect larvae of *Pieris brassicae* when parasitized by *Apanteles glomeratus* (Fuhrer, 1972).

The works of Friedl (1961) and Vernberg and Hunter (1963) suggest that some trematodes actively metabolise non-carbohydrate substrates. Therefore, in the present host-parasite system the observed increase in glycogen levels on infection might be attributed to a "sparing effect" on the sugars. However, the higher percentage loss of carbohydrate (Fig. 7) in the infected digestive gland caused by starvation, strongly suggests demands for sugar by the parasite (Cheng and Snyder, 1962, 1963; Cheng, 1963a; James, 1965; Cheng and Burton, 1966; James and Bowers, 1967; Patnaik, 1968; Cheng and Lee, 1971; Hoskin and Cheng, 1973) or rapid glycolysis due to acute anaerobic conditions prevailing in the infected glands (Cheng, 1963b; James, 1965; James and Bowers, 1967). The failure on the part of the parasitized glands to augment their carbohydrate store (Figs. 10, 11) even after re-feeding reflects some sort of impairment in glycogen synthesizing and/or storage capacity of this gland. It could be mass destruction of sites of glycogen synthesis and storage (Cheng and Burton, 1966) or disturbance in the metabolism (Cheng, 1964; James and Bowers, 1967) or physiological inhibition (Cheng and Burton, 1965) consequent to trematode infection. It is difficult to conceive, without involving the role of other tissues, how the digestive gland of an infected snail with this physiological impairment can have a store of glycogen and sugar, richer than that of the uninfected animals.

The relatively low sugar levels of infected foot tissue (comparable to that of starved uninfected foot) in spite of its high glycogen content, is suggestive of rapid depletion of sugar from this organ. This observation coupled with others such as maintenance of higher glycogen content by the infected foot in spite of the imposed starvation stress (Figs. 2, 8) and restoration to prestarvation levels on re-feeding, make it apparent that the foot is involved in meeting the demands of the parasite.

The changes observed in the mantle of infected snails are *via media* to the foot and hepatopancreas. Like the foot it also starves for free carbohydrate. Although the higher glycogen content in the infected mantle may mean its increased efficiency in glycogen

synthesizing and/or storage capacity, rapid depletion on starvation (Figs. 3, 7) and negligible increase in glycogen on refeeding (Figs. 3, 10, 11) suggest that this efficiency is not of the same high order as observed in the foot. This organ however, seems to be more efficient than the digestive gland, in that there is only an insignificant loss in sugar on starvation (Fig. 7), four fold increase in sugar (Fig. 10) and increase in glycogen content on re-feeding (Table 3 and Fig. 10).

Undoubtedly the infected snails are hyperglycaemic in spite of the large degree of variation. This condition has already been suggested in earlier reports (James 1965; Cheng and Burton, 1966; James and Bowers, 1967). Moreover, the observation that infected snails even after starvation were still hyperglycaemic not only to uninfected starved (Fig. 8) but also to uninfected re-fed snails (Fig. 4) vindicates earlier work. Although the hyperglycaemia may be explained as due to release of host hyperglycaemic factor (Goddard *et al.*, 1964) or glycogen splitting enzymes of parasite origin (Cheng and Snyder, 1962; Cheng, 1963b; James, 1965), the role of the foot and mantle which suffer from acute hypoglycaemia during infection (Figs. 2, 3, 5) in spite of their rich glycogen reserves, cannot be ignored.

It is apparent from this study that the infected snails accumulated more carbohydrates in spite of the parasite drain. This can be accomplished only when they make up the loss either by voraciously feeding (Hurst, 1927; Wesenberg-Lund, 1934), or by absorbing glucose from the environment (Cheng and Burton, 1966).

The first possibility appears improbable in view of the observations of Meuleman (1973) who noticed a lesser feeding rate in the infected snails. The results obtained in the current investigation rule out both possibilities, since the infected snails remained hyperglycaemic even after starvation. They indicate the presence of an inherent mechanism that are pressed into service during infection. The contrasting behaviour of the digestive gland and foot in parasitized and non-parasitized snails under different physiological conditions suggests the existence of such a mechanism in the foot rather than in the digestive gland. Thus, the foot may play a crucial role in maintaining hyperglycaemic levels in the body fluid from which the parasites pick up their requirements. This may enable the infected digestive gland to reduce its own involvement in meeting the parasite demands and to build up a buffer stock of glycogen.

SUMMARY

A comparison of tissue carbohydrate levels is made between larval trematode infected and uninfected snails under three physiological conditions, i.e., unstarved, starved and ré-fed. Under all these conditions, infected snails were found to be hyperglycaemic compared to uninfected snails. Contrasting behaviour of the digestive gland and the foot under these conditions, suggests that some inherent mechanism in the foot is activated which enables it to take up an additional role of seplenishing carbohydrate lost to the pararites.

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