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GLYCOGEN CONTENT IN GASTROTHYLAX CRUMENIFER AND COTYLOPHORON ORIENTALE AFTER THE ISOLATION OF PARASITES, THEIR STARVATION AND REFEEDING WITH VARIOUS CARBOHYDRATES

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Abstract. The average reserve glycogen content in unstarved (control) parasites, 4.71 ± 0.14 g/100 g F. T. (fresh weight of tissue) in *Gastrothylax crumenifer* and 3.15 ± 0.14 g/100 g F. T. in *Cotylophoron orientale* has been estimated biochemically. The consumption of endogenous glycogen was found to be 18.47 %, 44.37 %, 66.66 % and 78.13 % of F. T. in *G. crumenifer* and 14.92 %, 40.95 %, 66.34% and 71.42 % of F. T. in *C. orientale* during 12, 24, 36 and 48 hours of starvation.Both the parasites were able to resynthesize 71.71 % and 75.42 % of lost glycogen (maximum up to 16 hours) on refeeding in nutrient medium containing glucose. The maximum resynthesis of lost glycogen in both the parasites was observed in nutrient medium containing glucose rather than the other carbohydrates such as fructose, lactose, sucrose and trehalose.

Glycogen, the main stored polysaccharide of animal cells, such as liver and skeletal muscles, has also been shown to occur in parasitic worms. The glycogen of parasites has been estimated biochemically, mostly in cestodes and nematodes. Flury and Leeb (1926) and Weinland and Von Brand (1926) investigated in this respect in *Fasciola hepatica*, Odeaug (1955) *Haematoloechus complex* and *Haematoloechus medioplexus*, Goil (1957) *Paramphistomum explanatum* and *Gastrothylax crumenifer*, Mansour (1959) *Fasciola hepatica*, Goil (1961) *Fasciola gigantica*, Muller (1966) and Halton (1967) *Haplometra cylindracea*, Gupta and Srivastava (1976) *Isoparorchis hypselobagri*, Swamy and Rao (1980) *Gastrothylax crumenifer* and *Carmyerius spatiosus* and Gupta and Gupta (1981) *Opisthorchis pedicellata*, yet not much has been achieved.

Accordingly, the present study was carried out to determine the total glycogen content, in vitro variation and effect of various carbohydrates on the resynthesis of lost glycogen in *Gastrothylax crumenifer* (Creplin) (family Gastrothylacidae) and *Cotylophoron orientale* Harshey (family Paramphistomatidae).

MATERIAL AND METHODS

A large number of trematodes, *G. crumenifer* and *C. orientale* were collected from the rumen of freshly slaughtered sheep obtained from local abattoir. Both the parasites were divided separately into 10 groups, each containing "N" number of parasites after washing them several times with ice cold physiological saline. Parasites of 1st group were treated as control and initial glycogen content was estimated immediately, while parasites of groups 2, 3, 4 and 5 were starved for a period of 12, 24, 36 and 48 hours respectively in non-nutrient medium (Tyrode's solution, pH 6.8) containing antibiotics (1,000 unit/ml penicilline and 200 μg/ml streptomycin) at 37 ± 1 °C. The parasites of groups 6, 7, 8, 9 and 10 were starved first for 24 hours and then refed for an additional period of 0, 4,

8, 12 and 16 hours in Tyrode's solution containing 0.2 % carbohydrate (any one of glucose, fructose, lactose or trehalose at a time). The medium was changed regularly after every two hours in each case.

For measuring the glycogen content, the parasites were washed properly, blotted on Whatman filter paper, weighed quickly and homogenized in 5.0 ml of 10 % (W/V) trichloroacetic acid (TCA). The homogenate was centrifuged at 2000 r. p. m. for 10 minutes. The glycogen content was precipitated with 95 % ethanol, separated from supernatant and the total amount was estimated by the method of Roe et al. (1961).

The experiments were repeated four times before calculating the final mean results.

RESULTS

The results obtained are shown in Fig. 1. The total glycogen content was found to range from 4.52 to 4.86 with an average of 4.71 $\pm$ 0.14 g/100 g of F. T. and from 2.93 to 3.25 with an average of 3.15 $\pm$ 0.14 g/100 g of F. T. in *G. crumenifer* and *C. orientale*,

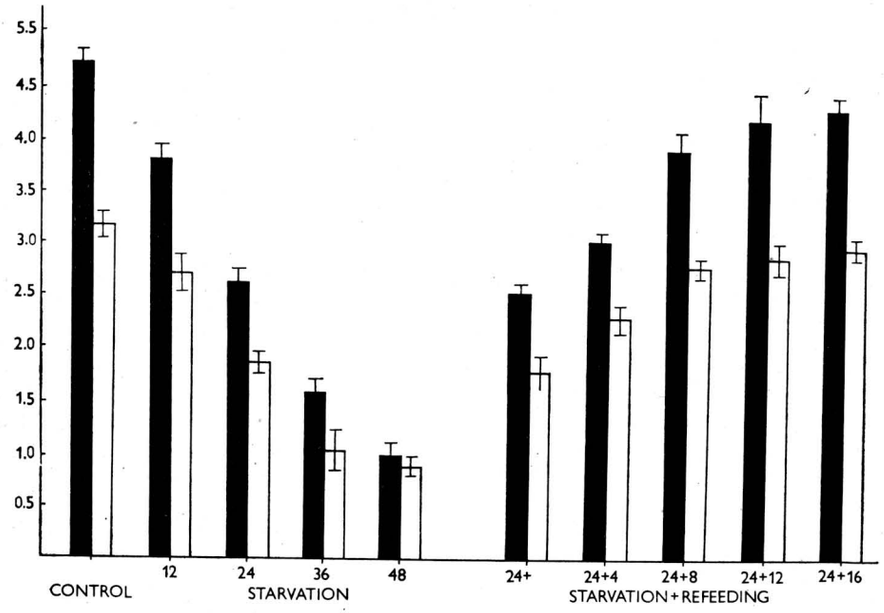

Fig. 1. In vitro variation of glycogen content in *Gastrothylax crumenifer* and *Cotylophoron orientale:* abscissa — period in hours, ordinate — glycogen content in g/100 g F. T. Black columns — *G. crumenifer*, white columns (right) — *C. orientale*. (Read 0 in control and 24 + 0 in the first columns of starvation + refeeding).

respectively. The endogenous glycogen was found to decrease with the increase in the period of starvation from 0 to 48 hours. The parasites became inactive and ultimately disintegrated due to the loss of endogenous glycogen and nonavailability of food.

The consumption of endogenous glycogen was found to be 18.47 %, 44.37 %, 66.66 % and 78.13 % of F. T. in *G. crumenifer* and 14.92 %, 40.95 %, 66.34 % and 71.42 % of F. T. in *C. orientale* during 12, 24, 36 and 48 hours of starvation, respectively.

The refeeding experiments showed that the parasites were able to resynthesize the lost glycogen. The resynthesis of glycogen was found to decrease with the prolongation of the period of starvation. The effect of exogenous carbohydrates on resynthesis of

glycogen in *G. crumenifer* and *C. orientale* are given in Figs. 2 and 3. The maximum glycogen resynthesis was found in nutrient medium containing glucose as compared to other carbohydrates like fructose, lactose, sucrose and trehalose.

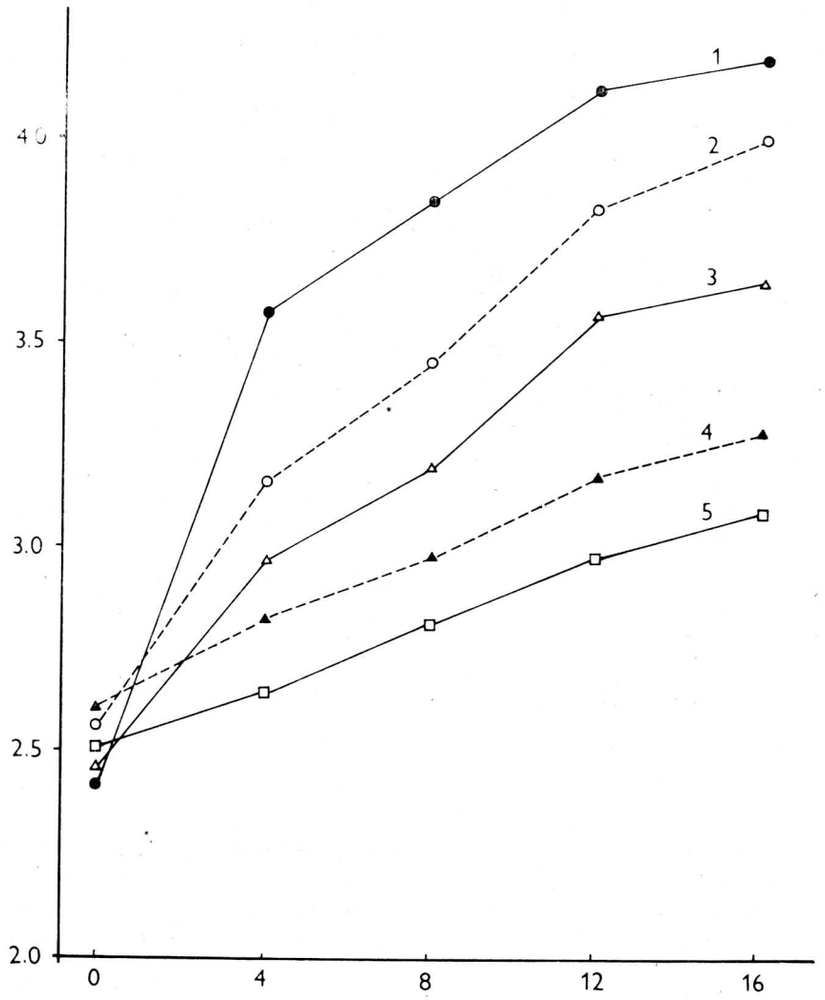

Fig. 2. Glycogen content in *Gastrothylax crumenifer* starved for 24 hours (denoted by 0) and refeeded with various carbohydrates: abscissa — refeeding time (hours), ordinate — glycogen content in g/100 g F. T. 1 — glucose, 2 — fructose, 3 — lactose, 4 — sucrose, 5 — trehalose.

DISCUSSION

Trematodes like other helminth parasites have substantial amount of reserve carbohydrates in the form of glycogen. Our results with total glycogen content in both trematode species are in accordance with the findings of some authors but differ from

the results of others. These differences might be due to different genera and species of trematode parasites, host specificity or experimental conditions.

Similarly, the same factors may be responsible for the differences observed in the consumption of endogenous glycogen showing in some cases higher and in other cases lower values.

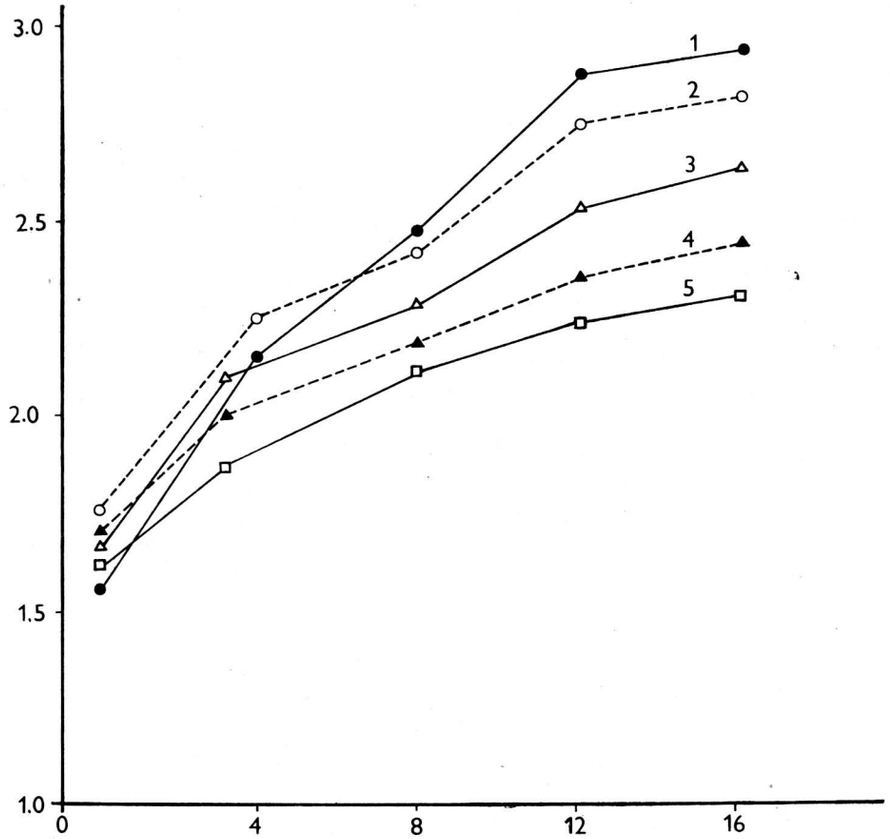

Fig. 3. Glycogen content in *Cotylophoron orientale* starved for 24 hours (denoted by 0) and refeeded with various carbohydrates: abscissa — refeeding time, ordinate — glycogen content in g/100 g F. T. 1—5 — the same as in Fig. 2.

Refeeding of both the parasites in nutrient medium (containing different carbohydrates) shows that both parasites were able to absorb carbohydrates through the cuticle. These experiments also reveal that the parasites starved for 24 hours were quite healthy to absorb exogenous carbohydrate through the cuticle and capable to resynthesize the lost glycogen. The capacity of absorption and resynthesis of glycogen decreased with the increase in starvation period. The absorption and resynthesis of glycogen was also found to decrease gradually when the nutrient medium changed from monosaccharides to disaccharides.

The results of the effect of different exogenous carbohydrates indicate that maximum

resynthesis of lost glycogen occurs in glucose, whereas the other carbohydrates, such as fructose, lactose, sucrose and trehalose, are less effective. This shows that glucose is absorbed through the cuticle more easily than any other carbohydrate. Among disaccharides the absorption, as well as resynthesis, of lost glycogen was comparatively greater in lactose than in others, in both the trematodes.

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СОДЕРЖАНИЕ ГЛИКОГЕНА У *GASTROTHYLAX CRUMENIFER* И *COTYLOPHORON ORIENTALE* ПОСЛЕ ВЫДЕЛЕНИЯ ПАРАЗИТОВ, ИХ ГОЛОДАНИЯ И ВОЗОБНОВЛЕННОГО КОРМЛЕНИЯ РАЗНЫМИ УГЛЕВОДАМИ

Р. Бахадур и П. Ц. Гупта

Резюме. При помощи биохимических методов установлено среднее содержание запасного гликогена у неголодающих (контрольных) паразитов, 4,71±0,14 г/100 г веса сырой ткани у *Gastrothylax crumenifer* и 3,15±0,14 г у *Cotylophoron orientale*. Обнаружено, что потребление эндогенного гликогена 18,47 %, 44,37 %, 66,66 % и 78,13 % веса сырой ткани у *G. crumenifer* и 14,92 %, 40,95 %, 66, 34 % и 71,42 % веса сырой ткани у *C. orientale* во время 12, 24, 36 и 48 часов голодания. Оба вида паразитов ресинтезировали 71,71 % и 75,42 % потерянного гликогена (максимум до 16 часов) возобновленным кормлением в питательной среде, содержащей глюкозу. Максимальный ресинтез потерянного гликогена осуществился в питательной среде, содержащей глюкозу, по сравнению с другими углеводами, как фруктозой, лактозой, сахарозой и трегалозой.

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