AN. INST. BIOL. UNIV. NAL. AUTÓN. MÉXICO 48, SER. ZOOLOGÍA (1): 27-36, 13 figs., 2 tablas

GLYCOGEN CONTENT IN *ISOPARORCHIS HYPSELOBAGRI*

S. P. GUPTA *

MEENAKSHI SRIVASTAVA **

ABSTRACT

Biochemical and histochemical techniques have been employed to determine if the parasite *Isoparorchis hypselobagri* depletes its glycogen reserve in a non nutrient medium and resynthesizes the polysaccharide when placed in a nutrient medium. The glycogen content before starvation was 10.2 ± 0.64 gm/100 gm of F. T. in *Isoparorchis hypselobagri*. After 24, 48 and 72 hours of starvation the glycogen content was 8.8 ± 0.47; 6.9 ± 0.23 and 5.9 ± 0.47 gm/100 gm of F. T. respectively. When parasites were incubated in a nutrient medium after starvation, the amount of glycogen was found to be 8.3 ± 0.14 gm/100 gm of F. T. This shows that parasites are able to synthesize the lost glycogen. The histochemical data showed that the suckers, parenchyma, pharynx and cirrus sac gave a more intense reaction for glycogen. The oesophagus, vitellaria, caeca, receptaculum seminis, seminal vesicle, ova, Mehli's gland and uterine eggs also contained a small amount of glycogen while cuticle and excretory system did not contain any glycogen. The glycogen content of the reproductive structures was not depleted during starvation.

Key words: Parasite, Biochemestry, Trematode Glicogen, Histochemical.

RESUMEN

Se utilizaron técnicas bioquímicas e histoquímicas para determinar si el parásito *Isoparorchis hypselobagri* abate su reserva de glicógeno en un medio no nutriente y si resintetiza el polisacárido cuando se pone en un medio nutriente. El contenido de glicógeno antes de la inanición fue de 10.2 ± 0.64 gm/100 de F. T. en *Isoparorchis hypselobagri*. Después de 24, 48 y 72 horas de inanición el contenido de glicógeno fue de 8.8 ± 0.47; 6.9 ± 0.23 y 5.9 ± 0.47 gm/100 gm de F. T. respectivamente. Cuando se incubaron los parásitos en un medio nutritivo después de la inanición, se encontró que el glicógeno fue de 8.3 ± 0.14 gm/100 gm de F. T. Esto muestra que los parásitos pueden sintetizar el glicógeno perdido. Los datos histoquímicos mostraron que las ventosas, el parénquima, la faringe y la bolsa del cirro dieron una reacción más intensa para glicógeno. El esófago, glándulas vitelinas, ciegos, receptáculo seminal, vesícula seminal, óvulos, glándula de Mehlis y huevos uterinos, también tuvieron una pequeña cantidad de glicógeno, mientras que la cutícula y el sistema excretor no lo presentaron. El contenido de glicógeno de las estructuras reproductivas no desapareció durante la inanición.

Palabras clave: Parásito, Bioquímica, Trematodo, Glicógeno, Histoquímica.

INTRODUCTION

Glycogen is the common reserve substance of animals and it has been shown to occur in large amounts in parasitic worms. Biochemical and histochemical techniques have been employed by a number of investigators to determine the

* Department of Zoology, University of Lucknow, Lucknow, India.

** Upgraded Department of Pathology and Bacteriology, K. G. Medical College, Lucknow, India.

distribution and amount of glycogen present in the tissues to trematodes. Ortner-Schönbach (1913) reported glycogen in the muscle cells and parenchyma of *Haplometra cylindracea* from the lungs of *Rana temporaria* and from similar tissues in *Gorgodera cygnoides* and *Polystomum integerrimum* from the urinary bladder of *Rana esculenta*. Flury and Leeb (1926) and Weinland and von Brand (1926) found 13% and 21% respectively, of the dry weight of *Fasciola hepatica*, to be composed of glycogen. Wilmoth and Goldfischer (1945), employing Best's carmine technique, Lugol's solution, and the Feulgen-Bauer reaction, identified glycogen granules in muscle tissue, eggs and parenchyma of *Haematoloechus* sp. from *Rana pipiens*. Axmann (1947) found glycogen in the form of granules, fibres and lumps, in the oral and ventral suckers of *Haematoloechus, Gorgodera* and *Gorgoderina*. Bueding and Koletsky (1950), working with *Schistosoma mansoni*, reported a sexual difference with respect to glycogen content; they found 13.6-29% of the dry weight of males to be glycogen but only 2.7% of the females. Bair (1954) found that the glycogen content of *Haematoloechus medioplexus* varied from 0.50% to 1.37% of the wet weight of the parasites. Odlaug (1955) made quantitative determination of glycogen and found that the lung fluke, *Haematoloechus complexus* and *H. medioplexus*, have significantly smaller amounts of glycogen than do the encysted *Clinostomum* metacercariae and the frog tape-worm, *Crepidobothrium saphena*. Bladder flukes, *Gorgodera amplicava* and *Gorgoderina attenuata* showed a glycogen content closer to that of the lung forms rather than to the intestinal forms. Starvation of the host appeared to have little effect on glycogen content of encysted forms within the host. According to Goil (1957) glycogen amounts to 7.20% of F. W. or 30. 32% of D. W. in *Paramphistomum explanatum* and 6.5% of F. W. in *Gastrothylax crumenifer*. Dawes and Muller (1957) used histochemical means to show the depletion of body glycogen in *Haplometra cylindracea* maintained in glucose free saline. Mansour (1959) observed that *Fasciola hepatica* utilized 50% of its glycogen content in 24 hours. The glycogen concentration in freshly isolated *Fasciola hepatica* was about 15-20% of D. W. of tissue. Rao (1959) found glycogen in the vitellaria of *Fasciola hepatica*. Bruskin (1959) in those of *Opisthorchis felineus*. Goil (1961) observed in *Fasciola gigantica* a glycogen content before starvation of 5.4% of F. W. or 25.35% of D. W. which fell, after 10 hours of starvation, to 3.5% of F. W. or 16.29% of D. W. of tissue. Von Brand and Mercado (1961) made histochemical studies on *Fasciola hepatica* and found that during starvation glycogen disappears from the parenchymal cells and the muscular organs while during starvation periods of 12 or 24 hours duration no glycogen decrease could be observed in the vitellarian cells and uterine ova. Burton (1962) tested histochemically *Haematoloechus medioplexus* from *Rana pipiens* for glycogen by using the Bauer-Feulgen method. He showed major concentrations of glycogen in oral sucker, pharynx, anterior subcuticular parenchyma and spermatozoa. Worms maintained, in vitro, in glucose-saline showed no depletion of tissue glycogen after 50 hours, while worms maintained in only saline showed partial loss of glycogen from the oral sucker, pharynx and subcuticular parenchyma. Fried and Kramer (1968) made histochemical studies to determine if *Echinostoma revolutum* depletes its glycogen reserves in a non nutrient medium and resynthesizes the polysaccharide, when placed in a nutrient medium or on the chick chorioallantois. They demonstrated that glycogen was depleted primarily from the parenchyma and musculature during a 72

hours period of starvation. The vitellaria, cuticle and subcuticle did not contain glycogen. Flukes starved for 48 hours and then incubated on the chick chorioallantois for 48 hours resynthesized lost glycogen, but not to the same extent as those maintained in Tyrode's-glucose after stravation. Probert *et. al.* (1972) observed that in *Fasciola gigantica* glycogen was mainly concentrated in yolk glands, parenchyma, mature eggs and the non-muscular regions of both oral and ventral suckers.

The present studies have been made to determine whether the parasite *Isoparorchis hypselobagri* depletes its glycogen content when placed in a non-nutrient medium and resyntheses polysaccharide when they are refed in a nutrient medium after starving.

MATERIALS AND METHODS

A large number of trematodes, *Isoparorchis hypselobagri*, were collected from the air bladder of a freshwater fish, *Wallagonia attu*, obtained locally from the fish market. These parasites were washed several times with distilled water prior to further processing. For both biochemical and histochemical studies the parasites were divided into 5 groups, each consisting of 10 parasites. Group 1 parasites were untreated controls, while parasites of groups 2, 3 and 4 were starved for 24, 48 and 72 hours respectively in a non-nutrient medium, i. e. Tyrode's solution (Paul, 1960) without glucose and with antibiotics (1,000 unit/ml Penicillin and 200, µg/ml Streptomycin). During the inanition period parasites were maintaned individually at room temperature (25-30°C) and at pH 7.5. The parasites of group 5 were starved for 72 hours and then refed for an additional period of 48 hours in Tyrode's solution containing 0.1% glucose and antibiotics.

BIOCHEMICAL STUDIES

The parasites were washed with several changes of distilled water, blotted on a filter paper and quickly weighed. After heating for 30 minutes with 30% KOH glycogen was precipitated with alcohol according to the procedure of Good *et. al.* (1933). The precipitate was then dissolved in a known volume of distilled water and glycogen was estimated by the method of Montgomery (1956).

HISTOCHEMICAL STUDIES

The parasites fixed in 70% alcohol and Carnoy's fluid at 4°C for 24-48 hours and embedded in paraffin, were sectioned and stained for glycogen by Best's carmine method given by Pearse (1960). The glycogen granules were stained red.

RESULTS

Biochemical Studies: The mean values of glycogen estimated in the parasites unstarved, starved for 24, 48 and 72 hours, and in the parasites starved for 72 hours and refed for 48 hours, are shown in Table I and Fig. 1.

The results of twenty analyses carried out with several lots of parasites showed that the glycogen content of *Isoparorchis hypselobagri* was 10. $\pm$ 0.64 gm/100 gm of fresh tissue. After 24 hours of starvation the amount of glycogen estimated was reduced to 8.8 $\pm$ 0.47 gm/100 gm of fresh tissue. At the end of 48 hours and 72 hours of starvation the amount of glycogen estimated was further re-

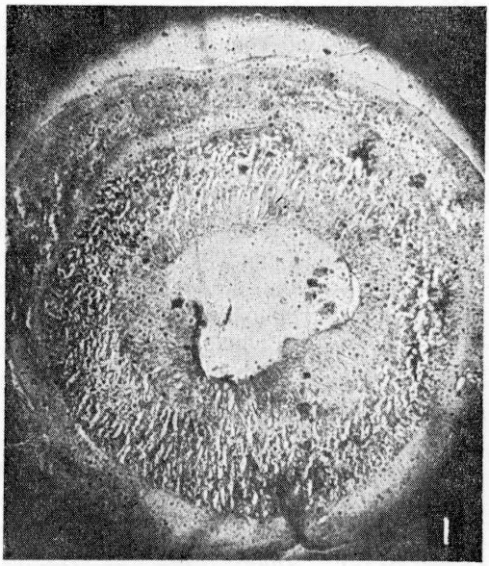

Fig. 1. Cross section of Oral sucker showing intensely stained glycogen masses.

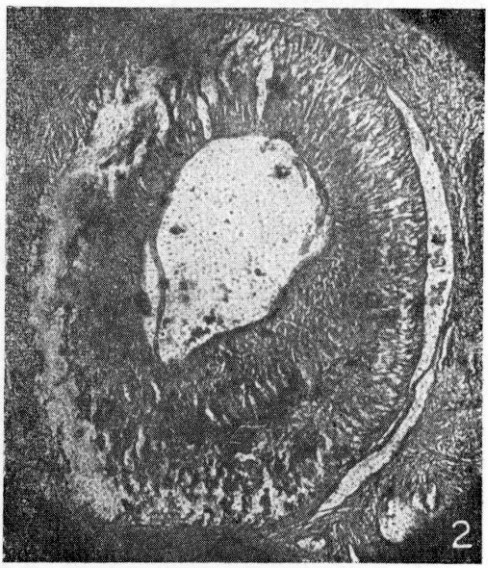

Fig. 2. Cross section of pharynx showing intensely stained glycogen masses.

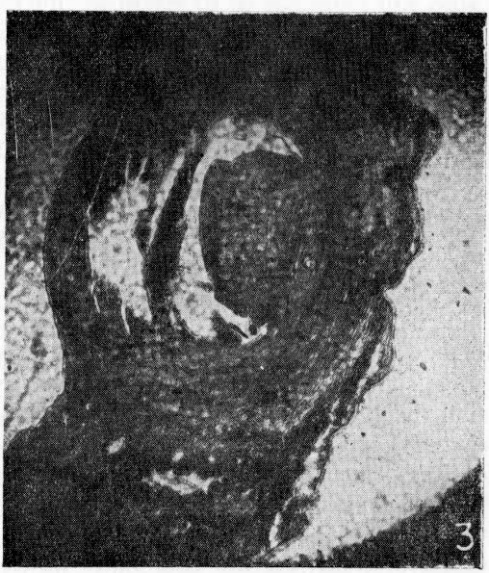

Fig. 3 Cross. section of ventral sucker showing intensely stained glycogen masses.

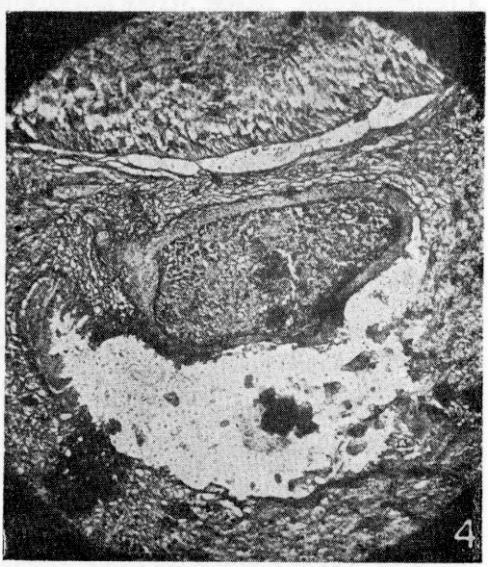

Fig. 4 Cross section of Oesophagus showing moderately stained glycogen masses.

TABLE 1

GLYCOGEN CONTENT OF *ISOPARORCHIS HYPSELOBAGRI* IN UNSTARVED, STARVED AND STARVED/REFED PARASITES IN GM/100 GM OF FRESH TISSUE

Unstarved parasites	*Starved parasites* starvation period in hours			*Starved/refed parasites* starvation and feeding period in hours
	24	48	72	72 + 48
10.2	8.8	6.9	5.9	8.3
S.D. ± 0.64	± 0.47	± 0.23	± 0.47	± 0.14

duced to 6.9 ± 0.23 and 5.9 ± 0.47 gm/100 gm of fresh tissue respectively. After 72 hours of starvation followed by the parasites refeding in Tyrode's solution containing 0.1% of glucose the amount of glycogen increased to 8.3 ± 0.14 gm/100 gm of fresh tissue. This showed that the parasites were able to resynthesise the lost glycogen.

Regarding the rate of consumption of glycogen, *Isoparorchis hipselobagri* consumed 13.72%, 32.35% and 42.15% of glycogen reserves after starving for 24, 48 and 72 hours respectively.

Histochemical Studies: The histochemical localization and relative intensity (amount) of glycogen content of *Isoparorchis hypselobagri* of unstarved, starved for 24, 48 and 72 hours and the parasites refed for 48 hours after 72 hours of starvation are shown in table 2.

TABLE 2

GENERAL DISTRIBUTION AND RELATIVE INTENSITY (AMOUNT) OF GLYCOGEN IN *ISOPARORCHIS HYPSELOBAGRI*

Structure	Unstarved	Starved parasites			Starved/refed parasites starvation and feeding period in hours.
		24	48	72	72 + 48
Cuticle	0	0	0	0	0
Oral sucker	+++	++	+	+	+++
Ventral sucker	+++	++	+	+	+++
Pharynx	+++	++	+	0	++
Oesophagus	++	++	+	0	++
Caeca	++	++	+	0	++
Parenchyma	+++	+++	++	+	+++
Vesicula seminalis	+	+	+	+	+
Testes	+	+	+	+	+
Vitellaria	++	++	++	++	++
Mehli's gland	++	++	++	++	++
Excretory system	0	0	0	0	0
Eggs	+	+	+	+	+
Uterus	++	++	++	++	++

Key; +++ = intensely stained; ++ = moderately stained; + = slightly stained; 0 = no stain.

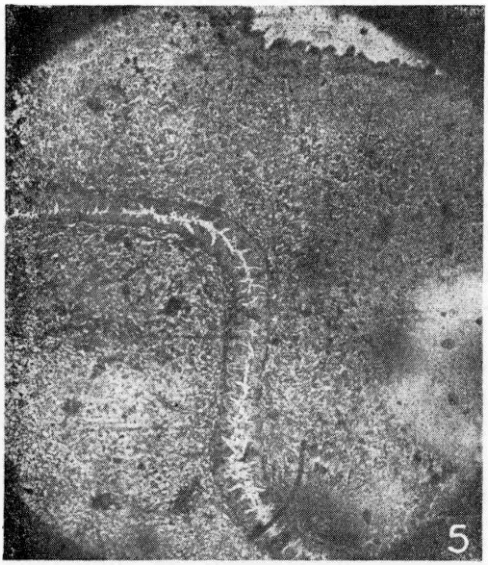

Fig. 5. Cross section of intestinal caeca showing moderately stained glycogen masses.

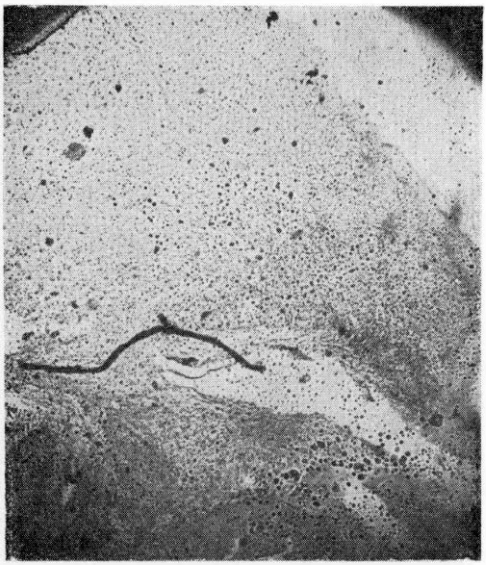

Fig. 6. Cross section of Parenchyma showing intensely stained glycogen masses.

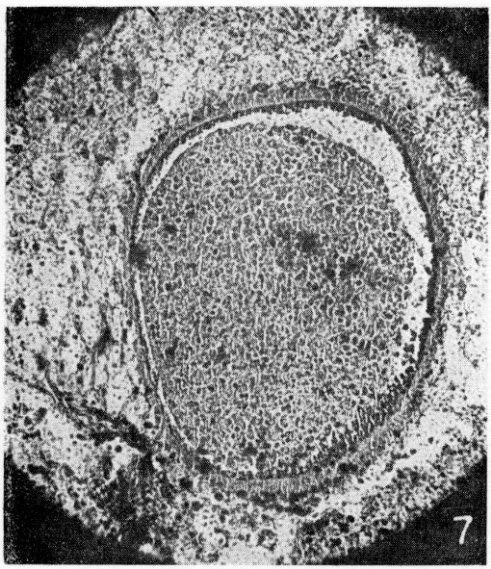

Fig. 7. Cross section of testes showing slightly stained glycogen masses.

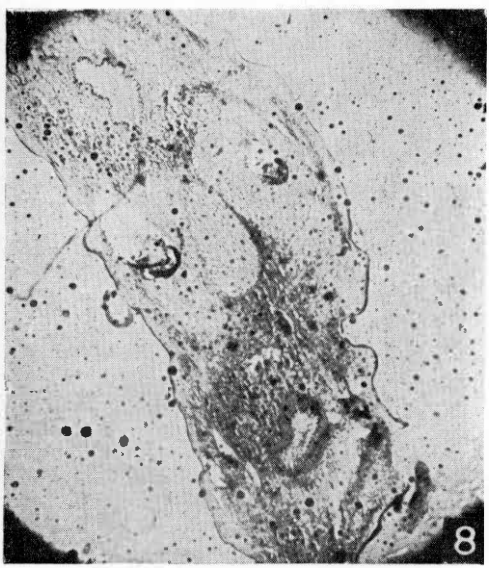

Fig. 8. Cross section of vesicula seminalis showing slightly stained glycogen masses.

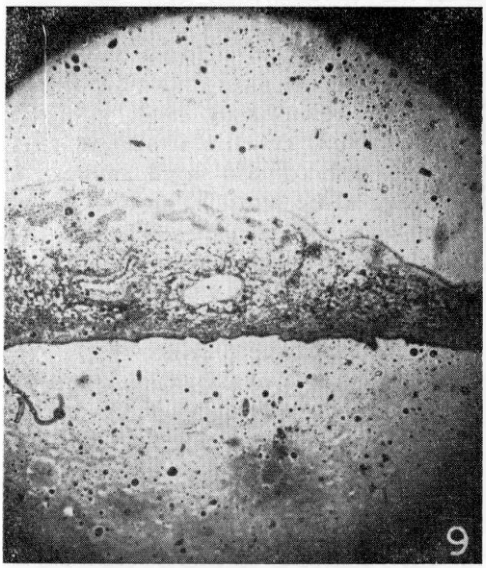

Fig. 9. Cross section of vitellaria showing slightly stained glycogen masses.

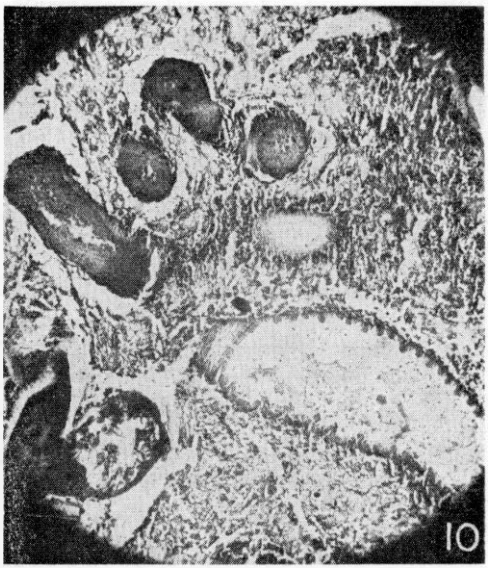

Fig. 10. Cross section of Mehli's gland showing moderately stained glycogen masses.

Fig. 11. Cross section of eggs showing slightly stained glycogen masses.

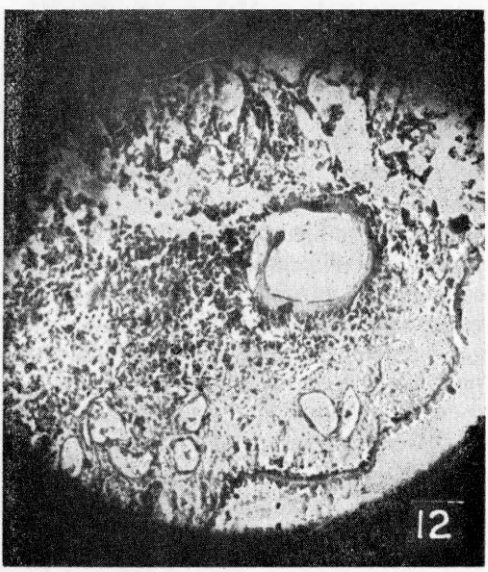

Fig. 12. Cross section of uterus showing moderately stained glycogen masses.

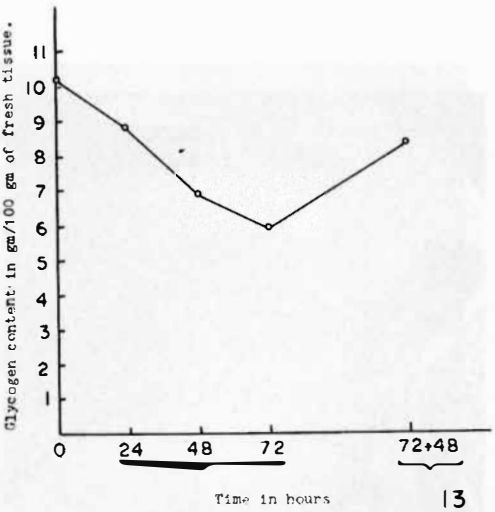

Fig. 13. Showing glycogen content in unstarved, starved and starved/refed parasites.

The data of table 2 show large amounts of glycogen occuring in the musculature, oral sucker, ventral sucker and pharynx. The muscle bands of the pharynx give a uniform reaction of moderate intensity while relatively large glycogen masses of irregular shape are interspersed among the bands. Glycogen occuring in the parenchyma may appear diffuse or granular and is mostly intracellular. In all the regions of the parasite, the parenchymal cells are filled with glycogen granules of various sizes. Therefore, the parenchyma should be considered as the most important storage place. The oesophagus, caeca, vitellaria, Mehli's gland, vesicula seminalis, testes and eggs also contain small amounts of glycogen while cuticle and excretory system were glycogen free. The ova contained little glycogen while most of the polysaccharide of the female reproductive system was found in the mature vitellarian cells and the uterine eggs.

After 24 hours of starvation there was only a slight glycogen depletion in suckers and pharynx. After 48 hours of starvation a further depletion of glycogen was observed, but after 72 hours of starvation, a drastic glycogen depletion in the pharynx, suckers, oesophagus, parenchyma and caeca was observed while the reproductive structures did not utilise their glycogen reserve.

The parasites, which were first starved for 72 hours and then refed for 48 hours in 0.1% glucose-Tyrodes showed an almost complete resynthesis of lost glycogen.

DISCUSSION

Like most other helminth parasites, trematodes have a pronounced carbohydrate metabolism and the most common carbohydrate reserve is glycogen. They contain substantial quantities of glycogen, make extensive use of endogenous glycogen and have a high rate of transport of exogenous glucose into the tissues. Flury and Leeb (1926) recorded the glycogen content of *Fasciola hepatica* as 3.1% of F. W. or 15% of D. W. of tissue and Weinland and von Brand (1926) found 3.7% of F. W. or 21% of D. W. in the same parasite. Goil (1957) determined the glycogen content of *Paramphistomum explanatum* and *Gastrothylax crumenifer* for 7.26% of F. W. or 30.32% of D. W. and 6.5% of F. W. respectively. Goil (1961) found 5.4% of F. W. or 25.35% of D. W. glycogen in *Fasciola gigantica*. The glycogen content in *Isoparorchis hypselobagri* was found 10.2% of F. W. of the parasite. It is difficult to assign any definite reason for this high percentage of glycogen in *Isoparorchis hypselobagri* as compared to other parasites. It may be due to large size of parasites.

The rate of glycogen consumption in *Isoparorchis hypselobagri* was found to be 13.72%; 32.35% and 42.15% after 24, 48 and 72 hours of starvation respec-

tively. Weinland and von Brand (1926) observed that *Fasciola hepatica* utilized 20% of its glycogen content in 5 hours while Mansour (1959) observed 50% of glycogen utilization of the same parasite in 24 hours. Goil (1957) observed that *Paramphistomum explanatum* utilized 36.4% of its glycogen content in 10 hours. Goil (1961) recorded that *Fasciola gigantica* utilized 35.2% of its glycogen in 10 hours. Compared to these, the present parasite *Isoparorchis hypselobagri* consumed less glycogen.

In *Isoparorchis hypselobagri* the highest concentration of glycogen was found in the parenchyma and musculature and no glycogen was detected in cuticle and excretory system of the untreated control. These findings are in accord with other studies on trematodes; Axmann (1947); Mansour (1959); Burton (1962) and Pantelouris (1964). The excretory system of *Isoparorchis hypselobagri* contains no glycogen, this is in agreement with the findings of Axmann (1947). The presence of glycogen in vitellaria in *Isoparorchis hypselobagri* agrees with the findings of Ortner-Schönbach (1913), Axmann (1947), Stephenson (1947), Rao (1959), Govaert (1960) and von Brand and Mercado (1961) who found glycogen in the vitellaria of *Fasciola hepatica*; those of Bruskin (1959) who reported the presence of glycogen in the vitellaria of *Opisthorchis felineus* and those of Guilford (1961) who found glycogen in the cytoplasm of fully developed vitelline cells of *Halipegus eccentricus*. Our findings differ from those of Burton (1962) and Fried and Kramer (1968) who reported the absence of glycogen in the vitellaria of *Haematoloechus medioplexus* and *Echinostoma revolutum* respectively. The presence of glycogen in the vesicula seminalis corresponds to the findings of Axmann (1947) and Burton (1962) who observed glycogen in fully formed spermatozoa of *Haematoloechus medioplexus*. Glycogen depletion in starved *Isoparorchis hypselobagri* is comparable with the findings of von Brand and Mercado (1961) for *Fasciola hepatica;* Burton (1962) for *Haematoloechus medioplexus* and Fried and Kramer (1968) for *Echinostoma revolutum*. Von Brand and Mercado (1961) and Burton (1962) reported a radical depletion in 24 hours and Fried and Kramer (1968) in 48 hours. In the present study the depletion of glycogen reserve was observed after 24 hours which is in agreement with the findings of von Brand and Mercado (1961) and Burton (1962) but differs with those of Fried and Kramer (1968).

Von Brand and Mercado (1961) observed in *Fasciola hepatica* that parenchymal glycogen was utilized faster than muscle glycogen and the uterine eggs did not utilise their glycogen reserves. This type of glycogen utilization is also found in the present parasite. The resynthesis of glycogen when refed after starvation also occur in *Isoparorchis hypselobagri* as in *Fasciola hepatica* (von Brand and Mercado, 1961); *Haematoloechus medioplexus* (Burton, 1962) and *Echinostoma revolutum* (Fried and Kramer, 1968).

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. G. G. Sanwal, Professor of Biochemistry, Lucknow University, Lucknow, for kindly going through the manuscript and giving useful suggestions.

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