HEXOSE UTILIZATION AND GLYCOGEN SYNTHESIS BY *SETARIA CERVI* (NEMATODA)*

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Setaria cervi, the filarial parasite of the Indian water-buffalo (*Bubalus bubalis* Linn.), when incubated in a sugar free Kreb's Ringer Bicarbonate medium released lactic and pyruvic acids. On incubation with glucose, mannose, fructose or galactose, the worms consumed these hexoses and formed lactic and traces of pyruvic acids. Glucose and mannose were utilized faster than fructose or galactose. The worms remained motile for 24 hr in a mineral medium containing glucose or mannose.

The radiocarbon of glucose-U-^{14}C or fructose-U-^{14}C was incorporated into a number of constituents of the parasite, the highest incorporation being into the free pool and glycogen. The label picked up from glucose was 8-10 times more than from fructose. Worms exposed to labelled glucose and later incubated in a mineral medium, released radioactivity indicating that the newly synthesized glycogen was broken down into metabolic end products.

INTRODUCTION

Carbohydrate is the major, if not the exclusive source of energy for many parasitic helminths and is often used as an essential constituent of media employed to maintain helminths *in vitro* (von Brand 1973). Most helminth parasites become immotile when they are kept in a sugar free medium. However, during starvation the worms can survive at the expense of their body glycogen which apparently is synthesized from hexoses. Thus glycogen plays an important role in providing energy to the worms.

No information is available regarding the capacity of *Setaria cervi*, a filarial parasite found in the peritoneal cavity of the water-buffalo (*Bubalus bubalis* Linn.) to utilize sugar when kept under *in vitro* conditions for prolonged periods. The present study on the utilization of hexoses and synthesis of glycogen by the adult females of *S. cervi* was carried out with the objective of filling this lacuna.

MATERIALS AND METHODS

Motile adult females of *S. cervi* (average length, 6.0±1.0 cm; average weight, 35±6 mg), collected from the peritoneal cavity of freshly slaughtered buffaloes in

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the local slaughterhouse, were brought to the laboratory in normal saline (0.9 per cent NaCl) and were used for metabolic studies within 2 hr from the time they were removed from the habitat.

Parasites (300–400 mg) were incubated aerobically in 10 ml Kreb's Ringer Bicarbonate buffer (KRB; DeLuca and Cohen, 1964), *p*H 7.4, containing 5.55 μmoles/ml of the sugar (glucose, mannose, fructose or galactose) in the presence of an antibiotic mixture (3000 units Penicillin G and 1 mg dihydrostreptomycin sulphate), at 37°C for 2 hr. At every two hour interval, the worms were removed, washed 2–3 times with plain KRB and then transferred to a fresh set of flasks containing identical media. For overnight incubation, twice the amount of sugar was used. Controls were run simultaneously, which contained all the constituents excepting sugar. On completion of the experiment, the concentrations of the residual sugar (glucose, mannose, fructose or galactose), and lactic and pyruvic acids formed in each set of flasks were determined.

For incorporation of labelled sugars, the motile worms were incubated aerobically in KRB medium, *p*H 7.4, supplemented with glucose-U-^{14}C (3.5 μc/g wet weight of worms) or fructose-U-^{14}C (3.0 μc/g wet weight of worms) in the presence of carrier glucose (18 mg/g wet weight of worms) or fructose (15 mg/g wet weight of worms) at 37°C for 2 hr. At the end of the incubation period the worms were removed and washed several times with KRB buffer containing carrier glucose or fructose for removing the adhering radioactivity. The washed worms were then homogenized with water (1:10 w/v) and used for the separation of glycogen, lipid, nucleic acids and proteins by the following procedures; glycogen was isolated from an aliquot of the homogenate by the method of Good *et al.* (1933). The remaining portion of the homogenate was treated with cold perchloric acid (PCA, final concentration 5 per cent) for the removal of polysaccharide and free pool and the lipids were extracted from the PCA sediment with chloroform: methanol (2:1). The lipid-free residue was kept for 20 hr with 1 N KOH, neutralized and mixed with 5 per cent PCA. The supernatant thus obtained, contained ribonucleic acid (RNA), while deoxyribonucleic acid (DNA) was isolated from the sediment by keeping it with 5 per cent PCA at 90°C for 10 min. The residue containing protein was dissolved in 0.1 N NaOH.

For observing the breakdown of glucose-U-^{14}C incorporated into the body of the worms and excretion of the labelled end products during *in vitro* incubation, worms exposed to labelled substrates were incubated in a sugar free medium for 8 hr and the excretory products were analysed for the presence of lactic and pyruvic acids employing thin-layer chromatography using chloroform : methanol : formic acid (80:12:2) as the solvent system. Appropriate volumes of all the fractions were applied on Whatman No.3 filter paper strips (2×2 cm) and counted in a Packard Tricarb liquid Scintillation counter using scintillation fluid containing 0.4 per cent 2, 5-diphenyloxazol and 0.01 per cent dimethyl-1,4-bis [2(5-phenyloxazol)-benzene].

Glucose was determined by glucose oxidase method (Bergmeyer and Benut 1963), mannose and galactose by Nelson's method (1944) of reducing sugars and fructose by Roe's method (1934). Lactic and pyruvic acids were estimated by the method of Barker and Summerson (1941) and Lardy (1964) respectively, while glycogen was assayed according to Montgomery (1957).

RESULTS

Breakdown of endogenous carbohydrates

Table I shows the amount of lactic and pyruvic acids excreted by the parasite during *in vitro* incubation in a sugar free medium. The rate of the formation of lactic acid was more or less constant up to 8 hr of incubation and thereafter it decreased gradually approaching zero level after 24 hr. In contrast, the pyruvic acid production showed an increasing trend, registering 3-fold increase at 6-8 hr in comparison to 4-6 hr of incubation. It may, however, be mentioned here that the total amount of pyruvic acid released was much less than that of lactic acid.

TABLE I

Breakdown of endogenous carbohydrate and formation of lactic and pyruvic acids by S. cervi *during* in vitro *incubation (Mean ± S.E.)*

Period of incubation (in hr)	µmoles/hr/g wet body weight at 37°C	
	Lactic acid formed	Pyruvic acid formed
0–2	15.25±0.5	0.558±0.02
2–4	12.37±0.9	0.649±0.02
4–6	12.65±0.8	0.694±0.01
6–8	11.65±0.7	1.800±0.02
8–10	10.80±0.6	1.374±0.02
10–12	6.53±0.4	1.197±0.03
12–24	2.92±0.3	0.293±0.03
24–26	0	0

Note: Number of determinations were four in each case.

Utilization of hexoses

When the worms were incubated with sugars (glucose, mannose, fructose or galactose), they utilized these hexoses and produced mainly lactic and traces of pyruvic acids. Data for the utilization of various hexoses by four sets of motile worms are summarised in Tables II, III and IV. Among the four hexoses used, glucose and mannose were consumed at a much faster rate than fructose or galactose. Glucose and mannose were utilized more or less at the same rate during the initial hours of incubation but later on the rate of mannose utilization decreased to half of glucose while fructose and galactose were not utilized during overnight incubation. As evident from the data the worms produced more lactic acid when kept in a medium containing glucose or mannose in comparison to one containing fructose or galactose.

The worms remained motile even after 24 hours when they were kept in a medium fortified with glucose or mannose while the motility was lost within 10–12 hr when

TABLE II

Glucose consumption and formation of lactic and pyruvic acids by S. cervi *during* in vitro *incubation (Mean ± S.E.)*

Period of incubation (in hr)	µmoles/hour/g wet body weight at 37°C			Lactate/ Glucose
	Glucose consumed	Lactic acid formed	Pyruvic acid formed	
0– 2	73.47±5.2	27.48±2.7	0.350±0.03	0.37
2– 4	65.04±3.8	25.54±3.1	0.461±0.05	0.39
4– 6	57.01±5.8	26.16±1.3	0.673±0.03	0.46
6– 8	60.16±5.1	24.58±2.5	0.782±0.04	0.49
8–10	46.01±4.0	23.77±1.6	1.121±0.04	0.52
10–12	38.94±3.8	23.01±1.4	1.296±0.03	0.59
12–24	27.59±4.1	18.51±1.3	0.548±0.04	0.67
24–26	20.96±2.9	13.98±2.8	0.591±0.03	0.67
26–28	14.21±2.7	11.70±1.4	0.674±0.02	0.82

Note: Number of determinations were four in each case.

TABLE III

Mannose consumption and formation of lactic and pyruvic acids by S. cervi *during* in vitro *incubation (Mean ± S.E.)*

Period of incubation (in hr)	µmoles/hour/g wet body weight at 37°C			Lactate/ Mannose
	Mannose consumed	Lactic acid formed	Pyruvic acid formed	
0– 2	58.89±2.0	20.11±2.3	0.368±0.06	0.34
2– 4	53.56±1.8	20.87±1.9	0.546±0.05	0.38
4– 6	48.65±1.7	18.78±2.3	0.621±0.02	0.39
6– 8	44.42±2.1	17.94±1.6	0.712±0.02	0.40
8–10	40.62±1.5	17.22±1.0	0.832±0.02	0.42
10–12	33.17±2.0	16.55±1.3	1.266±0.03	0.49
12–24	13.23±1.5	13.63±1.7	0.506±0.01	1.03
24–26	10.80±1.0	9.54±0.7	0.563±0.02	0.88
26–28	6.32±1.4	6.76±0.5	0.614±0.01	1.07

Note: Number of determinations were four in each case.

incubated with fructose or galactose. Thus the utilization rates of these four sugars were found to be directly dependent on the motility of the worms.

Incorporation of labelled sugars

Table V represents the data of glucose-U-^{14}C and fructose-U-^{14}C incorporation into glycogen and other macromolecules of *S. cervi*. Significant amount of radiocarbon present in labelled glucose was incorporated into the whole worms during

TABLE IV

Consumption of fructose and galactose and formation of lactic acid by S. cervi *during* in vitro *incubation (Mean ± S.E.)*

Sugar	Period of incubation (in hr)	μmoles/hr/g wet body weight at 37°C		Lactate/ Sugar
		Sugar consumed	Lactic acid formed	
Fructose	0–2	21.14±3.7	14.20±1.3	0.67
	2–4	18.37±2.0	12.64±1.4	0.68
	4–6	17.87±1.6	11.98±0.8	0.67
	6–8	14.48±0.8	10.15±1.0	0.70
	8–10	11.72±1.0	9.45±1.3	0.80
	10–12	6.06±1.0	5.49±0.5	0.90
Galactose	0–2	16.57±1.2	12.71±1.4	0.76
	2–4	15.01±1.3	11.00±1.5	0.73
	4–6	13.21±1.2	10.25±1.2	0.77
	6–8	11.05±1.2	8.59±0.5	0.78
	8–10	7.36±2.0	7.20±0.8	0.97
	10–12	4.80±1.2	5.00±0.3	1.04

Note: Number of determinations were four in each case.

TABLE V

Incorporation of labelled sugars in S. cervi *during* in vitro *incubation*

Fraction	Glucose-U-^{14}C		Fructose-U-^{14}C	
	cpm×10^4/g wet worms	% Incorporation	cpm×10^4/g wet worms	% Incorporation
Whole worms	71.62	100.00	7.49	100.00
Glycogen*	17.90	25.14	0.24	3.20
Cold PCA soluble fraction	40.86	57.10	—	—
Lipid	0.29	0.40	—	—
RNA	0.59	0.83	—	—
DNA	0.06	0.09	—	—
Protein	0.13	0.20	—	—

* Isolated either separately from the whole worms or from the cold PCA soluble fraction.
— not determined.

2 hr of incubation and over 50 per cent of the radioactivity was recovered in PCA soluble fraction (presumably containing polysaccharide, unidentified glycolytic intermediates, unpolymerized purine and pyrimidine nucleotides and amino acids) while only traces were detected in lipid, RNA, DNA and proteins. About 25 per cent of the total recovered radioactivity was in the form of glycogen (isolated either separately or from the cold PCA soluble fraction). Under the same conditions employing fructose-U-^{14}C as the exogenous carbohydrate, the incorporation in the whole body of the parasite was much lower.

Table VI represents the data of another set of experiments where the worms were incubated, first for 2 hours with glucose-U-^{14}C and then starved in sugar free medium for 8 hours. The labelled worms when incubated in a medium devoid of sugars released radioactivity into the medium. Radioactivity in the excretory products and in the starved worms remaining after the experiment accounted for nearly 97 per cent of the total radioactivity present in the labelled unstarved worms. Thin layer chromatography of the excretory products indicated the presence of labelled lactic and pyruvic acids.

TABLE VI

Incorporation of glucose-U-^{14}C and excretion of labelled end products by S. cervi *during* in vitro *incubation*

Fractions	cpm×10^4/g wet body weight	% Recovery
Total radioactivity incorporated in whole worms	71.62	100.0
Radioactivity left in starved worms	6.24	8.7
Glycogen in starved worms	2.96	—
Excretory products	62.84	87.7

DISCUSSION

Filarial parasites are known to thrive well in a medium fortified with sugars. However, the survival rates of these parasites are usually dependent on the nature of the sugars present in the medium (von Brand 1973). *Litomosoides carinii* (Bueding 1949) utilizes all four hexoses, i.e., glucose, mannose, fructose and galactose but *S. cervi,* like *Chandlerella hawkingi* (Srivastava *et al.* 1968; Srivastava and Ghatak 1974) preferentially utilizes glucose and mannose and remains motile up to 24 hr while it becomes immotile after 10-12 hr when incubated with fructose or galactose. In contrast, *Dracunculus insignis* (Bueding and Oliver-Gonzalez 1950) and *Dirofilaria uniformis* (von Brand *et al.* 1963) metabolize considerable amount of glucose only. The consumption rate of glucose and mannose in *S. cervi* was more or less the same whereas fructose and galactose were not significantly utilized. Therefore, it appears that glucose and mannose not only can serve as energy sources but can also be used as the constituents of the medium for *S. cervi* under *in vitro* conditions as well as in the elucidation of the mechanism of action of antifilarial agents particularly

effecting the motility of the worms, while fructose and galactose cannot serve this purpose.

L. carinii (Srivastava 1969) and *C. hawkingi* (Srivastava *et al.* 1968) did not release endogenous lactic and pyruvic acids when incubated in a sugar-free medium but *S. cervi* released these acids and resembles *Ascaridia galli* (Srivastava *et al.* 1970) in this respect.

Parasites like *D. insignis* (Bueding and Oliver-Gonzalez 1950), *D. uniformis* (von Brand *et al.* 1963), *C. hawkingi* (Srivastava *et al.* 1968; Srivastava and Ghatak 1971) and *Schistosoma mansoni* (Bueding 1950; Bueding and Saz 1968), which exclusively convert glucose into lactic acid resemble vertebrate tissues in possessing a classical glycolytic pathway; while *Ascaris lumbricoides* (Bueding and Yale 1951; Bueding and Saz 1968), *Hymenolepis diminuta* (Fairbairn *et al.* 1961; Bueding and Saz 1968), *Haemonchus contortus* larvae (Ward *et al.* 1968*a*,*b*), *Trichinella spiralis* (Agosin and Aravena 1959) and *Fasciola hepatica* (Mansour 1959; Prichard and Schofield 1968) converting 0–9 per cent glucose into lactic acid, have a modified glycolytic pathway diverging at phosphoenolpyruvate (PEP) level towards the formation of oxaloacetate. The bovine filarial parasite which converts about 20–26 per cent of the glucose into lactic acid has recently been shown to possess both glycolytic and PEP-oxaloacetate pathways (Anwar *et al.*—*In press*) thereby showing to occupy a medium position.

Exploratory experiments using labelled sugars indicated that when *S. cervi* was incubated with glucose-U-^{14}C, the highest incorporation was into the free pool and glycogen while only traces of radioactivity could be detected in other constituents of the parasite. In case of fructose-U-^{14}C, however, the incorporation rate was nearly 8–10 times less than that of glucose and only a small fraction of it was incorporated into glycogen. Therefore, it appears that *S. cervi,* during the early period of *in vitro* incubation, synthesized glycogen from the exogenous carbohydrates and most of the glycogen was derived from glucose. A low ratio of lactate/glucose was obtained with unlabelled glucose during the early period of *in vitro* incubation. This can be explained on the basis of incorporation studies that whatever sugar was utilized was not converted entirely to lactic acid, but was also channelled towards the synthesis of glycogen. von Brand (1973) mentioned that glycogen synthesis from exogenous carbohydrates takes place in several helminths. Rapid glycogen synthesis was observed in *L. carinii* (Bueding 1949), using labelled glucose. *Ancylostoma caninum* synthesised glycogen from glucose medium fortified with serum (Fernando and Wong 1964). Entner and Gonzalez (1959) using labelled glucose observed that half of the sugar consumed by *A. lumbricoides* was incorporated into glycogen.

When *S. cervi* was kept in a sugar-free medium after prior incubation with radioactive glucose, a depletion of radioactivity incorporated into the glycogen was observed. When the radioactivity remaining in the starved worms was added to that obtained from the excretory products, the sum of these two was more or less same as that accounted by the unstarved whole worms. It may, therefore, be inferred that the newly synthesized glycogen in *S. cervi* was being broken down into lactate, pyruvate and other unidentified metabolic end products through the process of glycolysis.

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References

Agosin, N., and Aravena, L. (1959). Anaerobic glycolysis in homogenates of *Trichinella spiralis* larvae. *Exp. Parasitol.*, **8**, 10–30.

Anwar, N., Ansari, A. A., and Ghatak, S. (1976). Enzymes of glycolysis and carbon dioxide fixation (*In press*).

Barker, B. S., and Summerson, W. H. (1941). The colorimetric determination of lactic acid in biological materials. *J. biol. Chem.*, **138**, 535–554.

Bergmeyer, H. U., and Benut, E. (1963). Methods of Enzymatic Analysis (Ed. Hands-Ulbrich Bergmeyer). Verlag Chemie, GMBH, Weinheim/Bergster, p. 123.

Bueding, E. (1949). Studies on the metabolism of filarial worm *Litomosoides carinii*. *J. exp. Med.*, **89**, 107–130.

——— (1950). Carbohydrate metabolism of *Schistosoma mansoni*. *J. gen. Physiol.*, **33**, 475–495.

Bueding, E., and Oliver-Gonzalez, J. (1950). Aerobic and Anaerobic production of lactic acid by the filarial worm *Dracunculus insignis*. *Br. J. Pharmacol. Chemotherap.*, **5**, 62–64.

Bueding, E., and Saz, H. J. (1968). Pyruvate kinase and phosphoenolpyruvate carboxykinase activities of *Ascaris* muscle, *Hymenolepis diminuta* and *Schistosoma mansoni*. *Comp. Biochem. Physiol.*, **24**, 511–518.

Bueding, E., and Yale, H. W. (1951). Production of α-methylbutyric acid by bacteria-free *Ascaris lumbricoides*. *J. biol. Chem.*, **193**, 411–423.

Deluca, H. F., and Cohen, P. P. (1964). Manometric Techniques, (4th edn.) p. 132 (*ed. by* W. W. Umbreit, R. H. Burris and J. F. Stauffer). Burgess Publishing Company, U.S.A

Entner, N., and Gonzalez, C. (1959). Fate of glucose in *Ascaris lumbricoides*. *Exp. Parasitol.*, **8**, 471–479.

Fairbairn, D., Wertheim, G., Harpur, R. P., and Schiller, E. L (1961). Biochemistry of normal and irradiated strains of *Hymenolepis diminuta*. *Exp. Parasitol.*, **11**, 248–263

Fernando, M. A., and Wong, H. A. (1964). Metabolism of hookworms. II. glucose metabolism and glycogen synthesis in adult female *Ancylostoma caninum*. *Exp. Parasitol.*, **15**, 284–292.

Good, C. A., Kramer, H., and Somogyi, M. (1933). The determination of glycogen. *J. biol. Chem.*, **100**, 485–491.

Lardy, H. A. (1964). Manometric Techniques (IVth edn.) p. 210 (*ed. by* W. W. Umbreit, R. H. Burris and J. F. Stauffer). Burgess Publishing Company, U.S.A.

Mansour, T. E. (1959). Studies on the carbohydrate metabolism of the liver fluke, *Fasciola hepatica*. *Biochim. Biophys. Acta.*, **34**, 456–464.

Montgomery, R. (1957). Determination of glycogen. *Archs. Biochem. Biophys.*, **67**, 378–386.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. *J. biol. Chem.*, **153**, 375–380.

Prichard, R. K., and Schofield, P. J. (1968). Phosphoenolpyruvate carboxykinase in the adult liver fluke, *Fasciola hepatica*. *Comp. Biochem. Physiol.*, **24**, 773–785.

Roe, J. H. (1934). A colorimetric method for the determination of fructose in blood and urine. *J. Biol. Chem.*, **107**, 15–22.

Srivastava, V. M. L. (1969). Biochemical studies on certain pathogenic helminths. Ph.D. Thesis, University of Agra, Agra (*Unpublished*).

Srivastava, V. M. L., and Ghatak, S. (1971). Glycolytic and carbon dioxide metabolizing enzymes in *Chandlerella hawkingi*. *Indian J. Biochem. Biophys.*, **8**, 108–111.

Srivastava, V. M. L., and Ghatak, S. (1974). Utilization of mannose, galactose and fructose by *Chandlerella hawkingi*. *Indian J. exp. Biol.*, **12**, 472-473.

Srivastava, V. M. L., Ghatak, S., and Krishna Murti, C. R. (1968). *Chandlerella hawkingi;* Glucose utilization and glycolytic enzymes. *Exp. Parasitol.*, **23**, 339–346.

———(1970). *Ascaridia galli:* Lactic acid production, glycogen content, glycolytic enzymes and properties of purified aldolase, enolase and glucose-6-phosphate dehydrogenase. *Parasitology*, **60**, 157–180.

von Brand, T. (1973). Biochemistry of Parasites (2nd edn.) Academic Press, New York.

von Brand, T., Bowmann, I. B. R., Weinstein, P. P., and Sawyer, T. K. (1963). Observations on the metabolism of *Dirofilaria uniformis*. *Exp. Parasitol.*, **13**, 128–133.

Ward, C. W., Schofield, P. J., and Johnstone, I. L. (1968*a*). Pyruvatekinase in *Haemonchus contortus* larvae. *Comp. Biochem. Physiol.*, **24**, 643–647.

——— (1968*b*). Carbon dioxide fixation in *Haemonchus contortus* larvae. *Comp. Biochem. Physiol.*, **26**, 537–544.