Z. Parasitenk. 51, 275–283 (1977)

Zeitschrift für
Parasitenkunde
Parasitologic Research

Setaria cervi: Enzymes of Glycolysis and PEP-Succinate Pathway*

Nuzhat Anwar[1], Afzal A. Ansari[2], S. Ghatak[1], and C.R. Krishna Murti[3]

[1] Division of Biochemistry, Central Drug Research Institute, Lucknow—226001, India
[2] Central Research Institute, Kasauli (Simla Hills) 173205, India
[3] Industrial Toxicology Research Centre, Lucknow—226001, India

Summary. *Setaria cervi*, the filarial parasite inhabiting the Indian water buffalo (*Bubalus bubalis* Linn.) contained almost all the enzymes involved in glycogen degradation. Significant activities of glycogen phosphorylase, glucokinase, phosphoglucomutase, phosphoglucose isomerase, phosphofructokinase, FDP-aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphopyruvate hydratase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were detected in cell-free extracts of whole worms.

The presence of PEP-carboxykinase, malate dehydrogenase, fumarase and fumarate reductase revealed the functioning of the PEP-succinate pathway in addition to phosphorylating glycolysis and pentose phosphate pathway in the parasite. Excepting fumarate reductase all other enzymes were localized in the particulate-free cytosol fraction, although small amounts of glycogen phosphorylase, aldolase and lactate dehydrogenase were also detected in the mitochondrial fraction.

Introduction

Evidence for the functioning of the Embden-Meyerhof scheme of glycolysis in parasitic helminths has been adduced mainly by the demonstration of the enzymatic steps or the identification of the intermediates of the pathway. In many cases, only a few enzymes have been explored and the operation of a full glycolytic pathway in which lactic acid accounts for all the glucose utilized occurs in *Dracunculus insignis* (Bueding and Oliver-Gonzalez, 1950), *Dirofilaria uniformis* (von Brand et al., 1963), *Chandlerella hawkingi* (Srivastava et al., 1968; Srivastava and Ghatak, 1971) and *Litomosoides carinii* (Bueding, 1949; Srivastava et al., 1970a). However, no generalization concerning the metabolism of filarial parasites can be made on the basis of studies on one member of a particular group, notwithstanding morphological and taxonomical relationship among them (Bueding and Most, 1953).

* Communication No. 2112 from the Central Drug Research Institute, Lucknow—226001, India. Part of the work was presented at the Annual Meeting of the Society of Biological Chemists, Ludhiana, India (November 1974).

Besides, investigations on the filarial parasites of the jungle crow or the cotton rat carried out in this laboratory have revealed differences and thus pointed out the necessity to study each member as a separate entity.

Setaria cervi, the filarial parasite inhabiting the Indian water-buffalo, *Bubalus bubalis* Linn. (Ansari, 1964) preferentially utilizes glucose and mannose for its survival (Anwar et al., 1975). This communication demonstrates the presence of glycolytic and related pathways of carbohydrate metabolism in adult females of this parasite.

Materials and Methods

Parasitic Material. Adult female *S. cervi* (average length, 6.0 ± 1.0 cm; average weight, 35 ± 6 mg) were obtained from the intestinal folds of freshly slaughtered water buffaloes, brought to laboratory in physiological saline (0.9% NaCl) containing 1% glucose.

Tissue Extracts. The motile worms were cleaned and homogenized in ice cold KCl (150 mM; 1 : 10, w/v) using a high speed tight fitting Potter-Elvehjem homogenizer. The homogenate thus obtained was centrifuged at 800 g (10 min), 11,000 g (30 min) and 105,000 g (60 min) for the separation of cell debris, mitochondrial and microsomal fractions respectively.

Chemicals and Enzymes. Barium salts of FDP, F6P, 2PGA, 6PG and sodium salts of ADP, ATP, GDP, G6P, NADP and PEP, glycogen, oxalacetic acid, crystalline aldolase, rabbit muscle LDH (pyruvate kinase free), pig heart MDH, G6P-DH and α-GDH-TPI were obtained from Sigma Chemical Company, St. Louis, U.S.A. Tris, AMP, cysteine, hydrazine sulfate, L-malate and sodium pyruvate were procured from E. Merck, Darmstadt, West Germany; while NAD, $NADH_2$ and GlP were supplied by the Biochemical Unit, Patel Chest Institute, Delhi, India.

All other chemicals used were of analytical grade. Oxalacetic acid and cysteine were neutralized before use with KOH and the barium salts were converted to sodium salts by standard procedures.

Assay of Enzymes. Spectrophotometric measurements were performed in a final volume of 3 ml using a Beckman DU spectrophotometer with silica cuvettes (1 cm light path). All assay mixtures were incubated for 3 min at room temperature (28 ± 2° C) before starting the reaction by the addition of the substrates. Changes of absorbance at 340 nm were noted prior to the addition of the substrate as well as for the period of actual enzymic reaction following the addition of the substrate.

Colorimetric assays were carried out at 37° C in reaction mixture of 1 ml after the addition of substrate. In controls, substrate was added after stopping the reaction. For all enzyme assays the concentration of substrates, coenzymes, ions and added enzymes (protein) were chosen to give maximal reaction rates.

Abbreviations Used

AMP, Adenosine-5′-monophosphate
ATP, Adenosine-5′-triphosphate
F6P, Fructose-6-phosphate
G6P, Glucose-6-phosphate
G6P-DH, Glucose-6-phosphate dehydrogenase
LDH, Lactate dehydrogenase
NAD, Diphosphopyridine nucleotide
NADP, NAD phosphate
6PG, 6-phosphogluconate
S.E., Standard error
PK, pyruvate kinase
ADP, Adenosine-5′-diphosphate
FDP, Fructose-1 : 6-diphosphate
G1P, Glucose-1-phosphate
GDP, Guanosine diphosphate
α-GDH-TPI, α-glycerophosphate dehydrogenase-triosephosphateisomerase
MDH, Malate dehydrogenase
$NADH_2$, Reduced NAD
PEP, phosphoenol pyruvate
2PGA, 2-phospho-D-glycerate
TCA, Trichloroacetic acid
PEPCK, PEP-carboxykinase

Spectrophotometric Assays

Glucokinase (ATP : D-glucose-6-phosphotransferase, EC 2.7.1.2) was assayed by the method of Parry and Walker (1966). The assay mixture contained: tris–HCl (pH 7.4), 50 mM; $MgCl_2$, 10 mM; ATP, 10 mM; glucose, 100 mM; KCN, 10 mM; NADP, 0.24 mM; G6P-DH, 2 units and enzyme protein, 300–500 μg.

G6P-dehydrogenase (G6P : NADP oxidoreductase, EC 1.1.1.49) was assayed according to Shonk and Boxer (1964). The assay mixture contained: tris–HCl (pH 7.4), 200 mM; $MgCl_2$, 10 mM; G6P, 10 mM; NADP, 0.24 mM and enzyme protein, 250–500 μg.

6PG-dehydrogenase (6-phospho-D-gluconate : NADP oxidoreductase, EC 1.1.1.44) was assayed according to Shonk and Boxer (1964). Assay mixture contained: tris–HCl (pH 7.4), 200 mM; $MgCl_2$, 10 mM; 6PG, 5 mM; NADP, 0.24 mM and enzyme protein, 300–600 μg.

Phosphofructokinase (ATP: F6P-phosphotransferase, EC 2.7.1.11) was assayed according to Racker (1947). Assay mixture contained: tris–HCl (pH 8.6), 200 mM; $MgCl_2$, 10 mM; F6P, 10 mM; ATP, 10 mM; $NADH_2$, 0.24 mM; aldolase, 1 unit; TPI, 17 units; GDH, 2 units and enzyme protein, 250–350 μg.

Glyceraldehyde Phosphate Dehydrogenase (D-glyceraldehyde 3-phosphate : NAD oxidoreductase (phosphorylating), EC 1.2.1.12) was assayed according to Srivastava et al. (1970a). Reaction mixture consisting of tris–HCl (pH 7.4), 200 mM; $MgCl_2$, 5 mM; cysteine, 10 mM; sodium arsenate, 20 mM; FDP, 10 mM and aldolase, 1 unit was incubated for 15 min at 37° C to produce adequate amounts of glyceraldehyde 3-phosphate. Subsequently NAD, 0.3 mM and enzyme protein, 100–200 μg were added and change in absorbancy was followed for 3 min.

Phosphopyruvate Hydratase (D-2-phosphoglycerate hydro-lyase, EC 4.2.1.11) was assayed according to Warburg and Christian (1942). Assay mixture contained: tris–HCl (pH 7.4), 200 mM; $MgCl_2$, 10 mM; 2PGA, 5 mM and enzyme protein, 150–300 μg.

Pyruvate Kinase (ATP : pyruvate phosphotransferase, EC 2.7.1.40). Assay was based on Bücher and Pfleiderer (1955), modified by the use of tris-buffer instead of triethanolamine buffer. Assay mixture contained: tris–HCl (pH 7.4), 200 mM; KCl, 100 mM; $MgCl_2$, 10 mM; ADP, 5 mM; PEP, 5 mM; LDH, 4 units; $NADH_2$, 0.24 mM and enzyme protein, 100–200 μg.

Lactate Dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) was assayed according to Kornberg (1955). Assay mixture contained; tris–HCl (pH 7.4), 200 mM; KCl, 100 mM; sodium pyruvate, 5 mM; $NADH_2$, 0.24 mM and enzyme protein, 5–15 μg.

PEP-carboxykinase (GTP : oxalacetate carboxylase (phosphorylating), EC 4.1.1.32) was assayed according to Ward et al. (1969). Assay mixture contained: tris–HCl (pH 7.4), 200 mM; $MnCl_2$ 10 mM; $NaHCO_3$, 20 mM; GDP, 1 mM; MDH, 8 units; PEP, 5 mM; $NADH_2$ 0.24 mM and enzyme protein, 200–400 μg.

Malate Dehydrogenase (L-malate : NAD oxidoreductase, EC 1.1.1.37) was assayed according to Shonk and Boxer (1964). Assay mixture contained: tris–HCl (pH 8.0), 200 mM; $MgCl_2$, 10 mM; oxalacetate, 10 mM; $NADH_2$, 0.24 mM and enzyme protein 0.6–1.5 μg.

Fumarase (L-malate hydro-lyase, EC 4.2.1.2) was assayed according to the method of Racker (1950). The reaction mixture consisting of potassium phosphate buffer (pH 7.4), 150 mM; L-malate, 30 mM; and enzyme protein, 40–80 μg. Increases in absorbancy was measured at 240 nm for 3 min.

Fumarate Reductase (Succinate : (acceptor) oxidoreductase, EC 1.3.99.1). The assay based on that described by Prichard (1973) was performed with mitochondrial preparations. The reaction mixture contained phosphate buffer (pH 7.0), 100 mM; $MgCl_2$ 10 mM; $NADH_2$, 0.3 mM and enzyme protein, 2–3 mg. The reaction was started by the addition of 10 μmoles of sodium fumarate.

Malic Enzyme (L-malate : NADP oxidoreductase (decarboxylating), EC 1.1.1.40) was assayed according to Ochoa (1955). Assay mixture contained: tris–HCl (pH 7.4), 200 mM; $MnCl_2$ 10 mM; L-malate, 10 mM; NADP, 0.24 mM and enzyme protein, 0.5–1.5 mg.

Colorimetric Assays

Glycogen Phosphorylase (α-1,4-glucan : orthophosphate glucosyl transferase, EC 2.4.1.1) was assayed essentially according to Rall et al. (1957). The assay mixture contained 1.14 mg glycogen, 3.76 mg G1P, 0.84 mg NaF, 0.292 mg AMP and enzyme protein, 400–800 μg. The reaction was terminated after 30 min with chilled TCA (10%, w/v), sodium acetate (0.1 M, 0.4 ml) was added to raise the pH for preventing spontaneous hydrolysis of GIP and Pi was estimated according to Taussky and Shorr (1953).

Phosphoglucomutase (D-G1P phosphotransferase, EC 2.7.5.1) was assayed according to Sutherland (1949). The assay mixture contained: tris–HCl (pH 7.4), 50 mM; $MgCl_2$, 10 mM; GlP, 10 mM; cysteine, 10 mM and enzyme protein, 150–300 μg. After 30 min the reaction was terminated with 5N-H_2SO_4 and labile phosphorus was estimated.

Phosphoglucose Isomerase (D-G6P ketol-isomerase, EC 5.3.1.9) was assayed according to Bodansky (1961). The assay mixture contained: tris–HCl (pH 8.6), 50 mM; $MgCl_2$, 10 mM; G6P, 10 mM and enzyme protein, 5–10 μg. The reaction was stopped with an equal volume of TCA (10%) after 15 min and F6P was estimated.

Aldolase (FDP-D-glyceraldehyde 3-phosphate lyase, EC 4.1.2.13) was assayed according to Khan et al. (1970). The assay mixture contained: tris–HCl (pH 8.6), 50 mM; hydrazine sulfate (pH 8.6), 28 mM; FDP, 2.5 mM and enzyme protein, 20–40 μg. The reaction was terminated after 30 min with equal volume of TCA (10%) and triose phosphates formed were estimated colorimetrically.

Succinate Dehydrogenase (Succinate:(acceptor) oxidoreductase, EC 1.3.99.1). It was assayed according to Slater and Bonner (1952), the reaction mixture consisting of phosphate buffer (pH 7.2), 150 mM; KCN, 30 mM; $K_3Fe(CN)_6$, 6 mM; sodium succcinate, 20 mM and enzyme protein, 2–4 mg. Equal volume of 10% TCA was added after incubation for 30 min and the supernatant was read at 400 nm against water blank.

Glucose-6-Phosphatase (D-G6P phosphohydrolase, EC 3.1.3.9) was assayed according to Shull et al. (1955). Assay mixture contained: tris–HCl (pH 7.4) or citrate buffer (pH 6.5), 50 mM; $MgCl_2$, 10 mM; G6P, 10 mM and enzyme protein, 1.0–1.5 mg. The reaction was stopped with an equal volume of TCA (10%) after 60 min and Pi estimated.

Fructose Diphosphatase (D-FDP-1 phosphohydrolase, EC 3.1.3.11) was assayed according to Freedland and Harper (1959). Reaction mixture contained: tris–HCl (pH 8.6), 50 mM; $MgCl_2$, 10 mM; cysteine, 12 mM; FDP, 10 mM and enzyme protein, 500–800 μg. The reaction was terminated after 60 min with TCA (10%) and Pi was estimated.

Definition of Enzyme Unit and Specific Activity

One unit of enzyme activity is the amount of enzyme required to catalyze the transformation of one nmole substrate or the formation of one nmole product per min under specified experimental conditions. Specific activity is units/mg of enzyme protein.

The extinction for oxidation or reduction of pyridine nucleotides was measured at 340 nm while increase in optical density due to the formation of PEP was recorded at 240 nm. An extinction coefficient of 6.22×10^6 cm^2/mole (Horecker and Kornberg, 1948) was used to calculate reduced NAD or NADP.

For fumarase the molar extinction coefficient used was $2.4 \times 10^3 \times m^{-1}$ M^{-1} at 240 nm (Mahler et al., 1958).

Analytical Procedures. Protein content of the enzyme preparations was estimated spectrophotometrically according to Layne (1957) or colorimetrically using the method of Lowry et al. (1951). F6P was determined by the Roe (1934) method as adapted by Bodansky (1961) while Pi was estimated by the method of Sumner (1944).

Results

Cell-free extracts of *S. cervi* were found to contain various enzymes of glycolysis and alternate pathways of carbohydrate metabolism. Tables 1 and 2 show the comparative activities of the enzymes present in the cell-free, mitochondria-free and microsome-free extracts of the parasite. The worms contained glycogen phosphorylase, glucokinase, phosphoglucomutase, phosphoglucose isomerase, phosphofructokinase, FDP-aldolase, glyceraldehyde phosphate dehydrogenase, phosphopyruvate hydratase, pyruvate kinase, lactate dehydrogenase, PEP-carboxykinase, malate dehydrogenase, fumarase, fumarate reductase (succinic dehydrogenase) and malic enzyme. Significant activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and the phosphatases acting on glucose-6-phosphate and fructose-1,6-diphosphate were also present. Glycogen phosphorylase and glucose-6-phosphatase were detected only in the supernatant fluid obtained from low speed centrifugation. Fumarate reductase (succinic dehydrogenase) was present

Table 1. Distribution of enzymes of *Setaria cervi* in supernatants obtained after differential centrifugation[a]

Enzymes		Specific activity (units/mg protein) ± SE			
		800 × *g*	12,000 × *g*	105,000 × *g*	Mitochondria
Glycogen phosphorylase	(3)	21.3 ± 1.9	NA	NA	NA
Glucokinase	(5)	22.0 ± 2.8	29.0 ± 3.4	45.0 ± 2.9	NA
Phosphoglucomutase	(5)	256.4 ± 10.0	288.0 ± 8.1	480.7 ± 8.5	NA
Phosphoglucose isomerase	(6)	810.0 ± 15.3	927.0 ± 12.5	1,490.0 ± 14.2	NA
Phosphofructokinase	(4)	46.0 ± 3.6	69.0 ± 7.5	93.0 ± 4.3	NA
FDP-aldolase	(5)	299.4 ± 8.8	361.4 ± 10.8	384.4 ± 10.0	28.2 ± 1.5
Glyceraldehyde-3-phosphate dehydrogenase	(3)	287.6 ± 9.6	319.7 ± 11.5	428.7 ± 21.9	NA
Phosphopyruvate hydratase	(3)	218.2 ± 10.2	290.8 ± 9.5	307.7 ± 7.4	NA
Pyruvate kinase	(5)	47.0 ± 2.5	82.0 ± 3.2	109.0 ± 4.8	NA
Lactate dehydrogenase	(5)	1,607.0 ± 14.5	2,310.0 ± 15.2	2,750.0 ± 16.0	23.1 ± 2.3
Glucose-6-phosphate dehydrogenase	(5)	53.0 ± 4.4	72.0 ± 3.7	107.0 ± 5.0	NA
6-Phosphogluconate dehydrogenase	(3)	32.0 ± 1.0	53.0 ± 5.0	66.0 ± 4.0	NA
Glucose-6-phosphatase	(3)	17.0 ± 1.0	ND	ND	NA
Fructose-1,6-diphosphatase	(3)	19.0 ± 1.5	24.0 ± 1.6	29.0 ± 2.5	NA

[a] Experimental details of enzyme assays were as described in Materials and Methods. ND = not detectable, NA = not assayed. Enzyme unit = Defined as the amount of enzyme which is required to catalyze the transformation of one nmole of the substrate or the formation of one nmole of the product per min under specified experimental conditions

Table 2. Enzymes of phosphoenolpyruvate-succinate pathway of *S. cervi*[a]

Enzymes		Specific activity (units/mg protein) ± SE			
		800 × *g*	12,000 × *g*	105,000 × *g*	Mitochondria
PEP-carboxykinase	(5)	182.5 ± 3.2	198.0 ± 2.5	262.8 ± 4.0	NA
Malate dehydrogenase	(5)	18,260.0 ± 17.2	19,560.0 ± 16.5	22,780.0 ± 12.5	ND
Malic enzyme	(3)	5.3 ± 0.2	6.8 ± 0.5	7.9 ± 0.3	NA
Fumarase	(3)	462.0 ± 11.2	616.0 ± 10.4	840.0 ± 12.5	NA
Succinic dehydrogenase	(3)	ND	ND	ND	34.9 ± 2.7
Fumarate reductase	(3)	ND	ND	ND	35.3 ± 1.8

[a] Experimental details of enzyme assays were as described in Materials and Methods. Enzyme unit as described in Table 1. ND = not detectable, NA = not assayed

only in the mitochondrial preparation while nearly 10% of the total activity of aldolase and lactate dehydrogenase could be demonstrated in mitochondria. The specific activities of the latter two enzymes were lower in the mitochondria than in the cytosol fraction. Higher specific activities of other enzymes in the particulate-free (105,000 × *g*) supernatant indicated that they were mainly confined to the cytosol fraction.

Among the soluble enzymes malate dehydrogenase was the most active although significantly high activities of lactate dehydrogenase, fumarase, phosphoglucose isomerase, phosphoglucomutase, glyceraldehyde phosphate dehydrogenase, FDP-

aldolase, phosphopyruvate hydratase, PEP-carboxykinase and pyruvate kinase were also observed. Phosphofructokinase, glucokinase, malic enzyme and fructose diphosphatase were less active. The supernatant fluid, however, did not oxidize reduced pyridine nucleotides ($NADH_2$ or $NADPH_2$) without added substrates, indicating the absence of diaphorase (EC 1.6.4.3) activity in the systems. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the enzymes of pentose phosphate pathway, were also present in significant amounts.

Among the PEP-metabolizing enzymes PEP-carboxykinase was 2–3 times more active than pyruvate kinase while the activity of malate dehydrogenase was nearly 8–10 times higher than that of lactate dehydrogenase.

Indirect evidence for the functioning of phosphoglycerokinase (EC 2.7.2.3) and phosphoglyceromutase (EC 2.7.5.3) was obtained, based on the fact that pyruvic and lactic acids were formed from FDP when the extracts were incubated with $MgCl_2$, ADP, cysteine and NAD as the presence of aldolase, glyceraldehyde phosphate dehydrogenase, phosphopyruvate hydratase, pyruvate kinase and LDH required to complete the sequence for the coupled reactions were already established by direct assay procedures.

Discussion

The bovine filarial parasite appeared to be equipped with almost all the enzymes of glycolytic and oxidative pathways. Except for aldolase and LDH, which were also present in small amounts in the mitochondria, all the enzymes of the Embden–Meyerhof scheme were localized exclusively in the soluble fraction, showing a resemblance with the mammalian system. The activity range of some of the key enzymes in *S. cervi* was higher than the corresponding values reported for other filariids (*L. carinii*, Srivastava et al., 1970a; *C. hawkingi*, Srivastava and Ghatak, 1971), trematodes (*Fasciola hepatica,* Prichard and Schofield, 1968a) and nematodes (*Haemonchus contortus* larvae, Ward and Schofield, 1967; and *Ascaridia galli,* Srivastava et al., 1970b). Hexokinase, specific for the phosphorylation of glucose was detected in *S. cervi* similar to the one functioning in *Schistosoma mansoni* (Bueding and Mackinnon, 1955), *Echinococcus granulosus* (Agosin and Aravena, 1959a) and *Trichinella spiralis* larvae (Agosin and Aravena, 1959b). However, the enzyme reported in *L. carinii* (Srivastava et al., 1970a), *C. hawkingi* (Srivastava and Ghatak, 1971), *A. galli* (Srivastava et al., 1970b), *H. contortus* and rat liver (Ward and Schofield, 1967) was of the non-specific type. G6P dehydrogenase and 6PG dehydrogenase, the enzymes of pentose phosphate pathway, which could not be detected earlier in measurable amounts in crow (Srivastava et al., 1968) and cotton rat (Srivastava et al., 1970a) filarial parasites, were present in the bovine filarial parasite in significant quantities.

Recent investigations on the glycolytic and carbondioxide fixing enzymes have shown that parasites producing succinic and volatile fatty acids in preference to lactic acid as the end products of carbohydrate utilization have a modified scheme of glycolysis diverging at the PEP level. Such parasites usually possess high activities of PEP-carboxykinase and MDH and low levels of pyruvate kinase and LDH, and the glycolytic pathway proceeds only up to the formation of PEP which is then carboxylated to oxalacetate by an active PEP-carboxykinase (von Brand, 1973). The worms coming under this group are *Ascaris lumbricoides* (Bueding and Saz, 1968), *H. contortus* larvae (Ward et al., 1968a, b), *Hymenolepis diminuta* (Bueding and Saz,

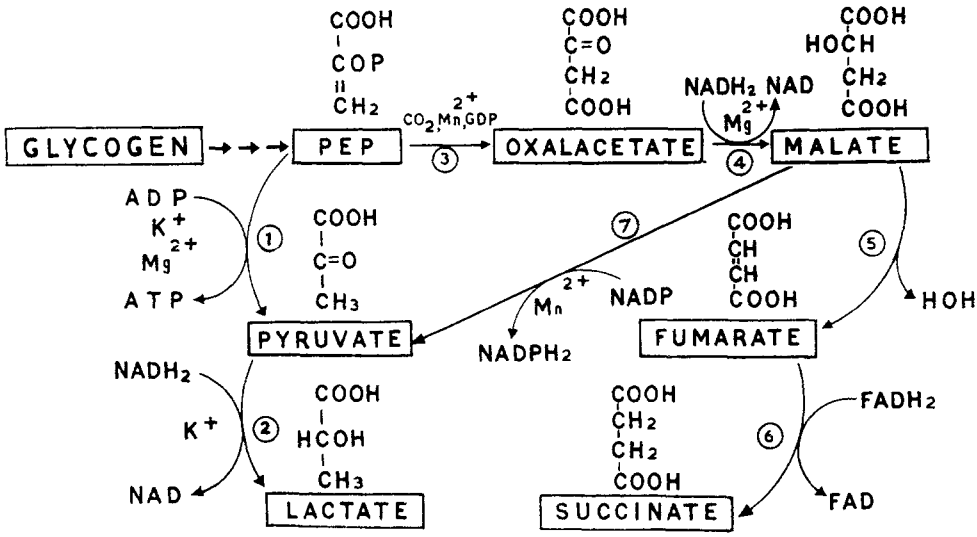

Fig. 1. PEP-Succinate pathway in *Setaria cervi*

1 Pyruvate kinase
2 Lactate dehydrogenase
3 PEP-carboxykinase
4 Malic dehydrogenase
5 Fumarate hydratase
6 Succinate dehydrogenase
7 Malic enzyme

1968), *F. hepatica* (Prichard and Schofield, 1968b) and *T. spiralis* larvae (Agosin and Aravena, 1959b), which convert negligible amounts to 9% of sugars into lactic acid. On the other hand, the typical lactic acid producers like *S. mansoni* (Bueding and Saz, 1968) and *C. hawkingi* (Srivastava et al., 1968; Srivastava and Ghatak, 1971) converting 80–90% of glucose into lactic acid resemble vertebrate tissues in possessing high levels of pyruvate kinase and LDH and low levels of PEP-carboxykinase and MDH activities. The intermediate case may also occur as in *Dictyocaulus viviparus*, a worm apparently depending on an essentially aerobic life (von Brand, 1973), where the activities of pyruvate kinase and PEP-carboxykinase are more or less of the same order.

S. cervi aerobically converts nearly 28% of glucose into lactic acid (Anwar et al., 1975) and possesses a PK/PEPCK ratio of around 0.4. Hence it appears that both the major pathways of glucose degradation, glycolysis and the PEP-succinate pathway are operating in the bovine filarial parasite (Fig. 1). *S. cervi*, thus, differs metabolically from other filarial parasites viz. *L. carinii* and *C. hawkingi* and resembles more closely the intestinal parasites with regard to the activities of PEP-metabolizing enzymes. The difference in metabolic activity can be explained on the basis of the location of the parasite in the host; *S. cervi* resides on the intestinal folds which have relatively high oxygen tension, while other filarial parasites (*C. hawkingi* and *L. carinii*) thrive in heart and pleural cavity of crow and cotton rat respectively under a rich supply of oxygen.

Acknowledgements. Two of the authors (N.A. and A.A.A.) are grateful to the Council of Scientific and Industrial Research for the award of Fellowships. Thanks are also due to Messrs S. Banerjee, M. M. Khan and P. C. Khandoori.

References

Agosin, M., Aravena, L.: Studies on the metabolism of *Echinococcus granulosus* III. Glycolysis, with special reference to hexokinases and related glycolytic enzymes. Biochim. Biophys. Acta **34**, 90–102 (1959a)

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

Ansari, J.A.: Studies on *Setaria cervi* (Nematoda: Filarioidea) Part II. Its peritoneal transplant and periodicity of the microfilariae in white rats. Z. Parasitenk. **24**, 105–111 (1964)

Anwar, N., Ansari, A.A., Ghatak, S.N.: Hexose utilization and glycogen synthesis by *Setaria cervi* (Nematoda). Proc. Indian Nat. Sci. Acad. **41**, 550–558 (1975)

Bodansky, O.: Phosphohexose isomerase. In: Standard methods of clinical chemistry (D. Seligson, ed.), Vol. III, pp. 182–193. New York: Academic Press 1961

Bücher, T., Pfleiderer, G.: Pyruvate kinase from muscle. In: Methods in enzymology (S.P. Colowick and N.O. Kaplan, eds.), Vol. I, pp. 435–440. New York: Academic Press, 1955

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

Bueding, E., Mackinnon, J.A.: Hexokinases of *Schistosoma mansoni*. J. Biol. Chem. **215**, 495–506 (1955)

Bueding, E., Most, H.: Helminths: metabolism, nutrition and chemotherapy. Ann. Rev. Microbiol. **7**, 295–321 (1953)

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

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

Freedland, R.A., Harper, A.E.: Metabolic adaptation in higher animals V. The study of metabolic pathways by means of metabolic adaptation. J. Biol. Chem. **234**, 1350–1354 (1959)

Horecker, B.L., Kornberg, A.: The extinction coefficients of the reduced band of pyridine nucleotides. J. Biol. Chem. **175**, 385–390 (1948)

Khan, M.A., Ghatak, S., Krishna Murti, C.R.: Chromatographic heterogeneity of FDP-aldolase and lactate dehydrogenase of rabbit tissue; use of DEAE-cellulose in separation of isomeric forms of aldolase. Indian J. Biochem. **7**, 33–39 (1970)

Kornberg, A.: Lactic dehydrogenase in muscle. In: Methods in enzymology (S.P. Colowick and N.O. Kaplan, eds.). Vol. I, pp. 441–443. New York: Academic Press 1955

Layne, E.: Protein estimation by U.V. absorption. In: Methods in enzymology (S.P. Colowick and N.O. Kaplan, eds.). Vol. III, pp. 451–454. New York: Academic Press 1957

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265–275 (1951)

Mahler, H.R., Wittenberger, M.H., Brand, L.: Biochemical studies of the developing avian embryo. II. Enzymes of the citric acid cycle. J. Biol. Chem. **233**, 770–782 (1958)

Ochoa, S.: Malic enzyme. In: Methods in enzymology (S.P. Colowick and N.O. Kaplan, eds.), Vol. I, pp. 739–753. New York: Academic Press 1955

Parry, M.J., Walker, D.G.: Purification and properties of adenosine 5′-triphosphate-D-glucose 6-phosphotransferase from rat liver. Biochem. J. **99**, 266–274 (1966)

Prichard, R.K.: The fumarate reductase reaction of *Haemonchus contortus* and the mode of action of some anthelmintics. Int. J. Parasitol. **3**, 409–417 (1973)

Prichard, R.K., Schofield, P.J.: The glycolytic pathway in adult liver fluke, *Fasciola hepatica*. Comp. Biochem. Physiol. **24**, 697–710 (1968a)

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

Racker, E.: Spectrophotometric measurement of hexokinase and phosphohexokinase activity. J. Biol. Chem. **167**, 843–854 (1947)

Racker, E.: Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim. Biophys. Acta **4**, 211–214 (1950)

Rall, T.W., Sutherland, E.W., Barthet, J.: The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. Biol. Chem. **224**, 463–475 (1957)

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

Shonk, C.E., Boxer, G.E.: Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. Can. Res. **24**, 709–721 (1964)
Shull, K.H., Ashmorb, J., Mayer, J.: Hexokinase, glucose-6-phosphatase and phosphorylase levels in hereditarily obese–hyperglycemic mice. Arch. Biochem. Biophys. **62**, 210–216 (1956)
Slater, E.C., Bonner, W.D.: The effect of fluoride on the succinic oxidase system. Biochem. J. **52**, 185–196 (1952)
Srivastava, V.M.L., Ghatak, S., Krishna Murti, C.R.: *Chandlerella hawkingi*: Glucose utilization and glycolytic enzymes. Exp. Parasitol. **23**, 339–346 (1968)
Srivastava, V.M.L., Chatterjee, R.K., Sen, A.B., Ghatak, S., Krishna Murti, C.R.: Glycolysis in *Litomosoides carinii*. Exp. Parasitol. **28**, 176–185 (1970a)
Srivastava, V.M.L., Ghatak, S., Krishna Murti, C.R.: *Ascaridia galli*: Lactic acid production, glycogen content, glycolytic enzymes and properties of purified aldolase, enolase and glucose-6-phosphate dehydrogenase. Parasitology, **60**, 157–180 (1970b)
Srivastava, V.M.L., Ghatak, S.: Glycolytic and carbondioxide metabolizing enzymes in *Chandlerella hawkingi*. Indian J. Biochem. Biophys. **8**, 108–111 (1971)
Sumner, J.B.: A method for the determination of phosphorus. Science **100**, 413–414 (1944)
Sutherland, E.W.: Activation of phosphoglucomutase by metal binding agents. J. Biol. Chem. **180**, 1279–1284 (1949)
Taussky, H.H., Shorr, E.: A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. **202**, 675–685 (1953)
von Brand, T.: Biochemistry of parasites, 2nd edition. New York: Academic Press 1973
von Brand, T., Bowmann, I.B.R., Weinstein, P.P., Sawyer, T.K.: Observations on the metabolism of *Dirofilaria uniformis*. Exp. Parasitol. **13**, 128–133 (1963)
Warburg, O., Christian, W.: Isolation and crystallization of enolase. Biochem. Z. **310**, 384–421 (1942)
Ward, C.W., Schofield, P.J.: Glycolysis in *Haemonchus contortus* larvae and rat liver. Comp. Biochem. Physiol. **22**, 33–52 (1967)
Ward, C.W., Schofield, P.J., Johnstone, I.L.: Pyruvate kinase in *Haemonchus contortus* larvae. Comp. Biochem. Physiol. **24**, 643–647 (1968a)
Ward, C.W., Schofield, P.J., Johnstone, I.L.: Carbondioxide fixation in *Haemonchus contortus* larvae. Comp. Biochem. Physiol. **26**, 537–544 (1968b)
Ward, C.W., Castro, C.A., Fairbairn, D.: Carbondioxide fixation and phosphoenolpyruvate metabolism in *Trichinella spiralis* larvae. J. Parasitol. **55**, 67–71 (1969)

Received September 13, 1976