Bull. Inst. Zool., Academia Sinica **24**(2): 177-186 (1985)

CARBOHYDRATE METABOLISM IN ADULT *CLONORCHIS SINENSIS* (TREMATODA)

HSIU-HUI SHIH, SHIU-NAN CHEN and SHIN LIOU

Department of Zoology, National Taiwan University
Taipei, Taiwan 107, Republic of China

(Received March 7, 1985)

Hsiu-Hui Shih, Shiu-Nan Chen and Shin Liou (1985) Carbohydrate metabolism in adult *Clonorchis sinensis* (Trematoda). *Bull. Inst. Zool., Academia Sinica* **24**(2): 177-186. This study attempts to detect the activities of the glycolytic enzymes and key enzymes for anaerobic metabolism in the extraction of adult *Clonorchis sinensis*. In addition, intracellular distribution of respiratory enzymes was also investigated. Based on these findings, the metabolic pathway is proposed. The results showed that adult *Clonorchis sinensis* is equipped with all enzymes required for Embden-Meyerhof pathway of glucose degradation. In comparison with phosphoenolpyruvate carboxykinase, the activity of pyruvate kinase is relatively low. This result may suggest that phosphoenolpyruvate is catalyzed to oxaloacetate by decarboxylation of phosphoenolpyruvate carboxykinase in *C. sinensis*. Oxaloacetate is subsequently converted to malate by the catalysis of malate dehydrogenase.

In the study of distribution of enzymes related to pyruvate metabolism of *C. sinensis*, activities of lactate dehydrogenase, pyruvate kinase and malate dehydrogenase are present mainly in cytosolic fraction. Pyruvate decarboxylase and phosphoenol-pyruvate carboxykinase were detected in both cytosolic and mitochondrial fractions. However, no activity of pyruvate dehydrogenase, pyruvate decarboxylase (partial reaction of pyruvate dehydrogenase) or pyruvate oxidase (phosphate-linked) were detected in *C. sinensis*.

Polyacrylamide disc gel electrophoresis analysis of *C. sinensis* showed a distinct predominance of LDH-1, LDH-4 and LDH-5 for lactate dehydrogenase isozyme.

Most studies on carbohydrate metabolism of liver fluke have been concerned with the *Fasciola hepatica* (Barrett, 1976, 1981; Lloyd and Barrett, 1983) and few attempts have been made to investigate biochemical aspects of *Clonorchis sinensis*. Ueda and Sawada (1968) analysed the distribution of low molecular weight carbohydrates in acid hydrolysed tissue of *C. sinensis* and reported that 73% of glucose; 10% of galactose; 8% of mannose; 6% of ribose and 3% of myo-inositol were included. In the study of metabolic products of *C. sinensis*, Read (1961) reported that 50% organic acid resulted from the glucose degradation was lactic acid.

The present study attempts to detect the presence of glycolytic enzymes and anaerobic or aerobic key enzymes in adult *C. sinensis*. The role of these enzymes in the carbohydrate metabolism was also evaluated.

MATERIALS AND METHODS

Sources and Preparation of *Clonorchis sinensis*

Metacercaria of *Clonorchis sinensis* were obtained from naturally infected fish, *Hemicultex keneri*, collected from Sun-Moon Lake, Nantou, Taiwan. To collect metacercaria, musculature of fish were homogenized in

physiological saline using a Nihonseiki (Tokyo, Japan) homogenizer. The metacercaria were then harvested under an Olympus (Japan) dissecting microscope using a pasteur pipette. Approximately 300 metacercaria were orally inoculated into a 2.5 kg rabbit. Adults were collected from the bile duct and liver of freshly slaughtered animal 45 days after infection and proceeded immediately for the experimental uses. Prior to the experiment, the worms were washed twice in Hedon—Fleig solution and once in normal saline, blotted dry using filter paper and weighed.

Preparation of Extract of *C. sinensis*

For glycolytic enzyme assays, each gram of *C. sinensis* were homogenized in 5 ml of buffered solution (0.15 M KCl solution containing 12.5% of 0.02 M $KHCO_3$ solution) using Polytron PTA 20S homogenizer (Switzerland) with a speed of 10,000 rpm. Homogenization was performed for two consecutive minutes in an 4°C water bath. The homogenate was then centrifuged at 12,000 g in a Beckman J-21C refrigerated centrifuge for 15 min and the supernatant was used immediately for detecting the activities of glycolytic enzymes with an exception of glucokinase. For glucokinase assay, the homogenate was centrifuged at 12,000 g for 15 min and the supernatant was then re-centrifuged at 100,000 g in a Hitachi SCP85H refrigerated ultracentrifuge for 30 min. The supernatant was used for the detection of glucokinase activity.

Enzyme Assays

Enzyme assays were accomplished using a Gilford G250 spectrophotometer with 1 cm light path quartz cuvette at a constant temperature of 25°C controlled by a thermoprogrammer (Gilford). Although most of tested enzymes were considered to be stable within several hours' storage at a temperature of approximately 4°C, few enzymes are relatively labile under these conditions. Assays for the activities of glyceraldehydephosphate dehydrogenase (GDH), pyruvate kinase (PK), phosphofructokinase (PFK) and enolase should therefore be finished first as suggested by Shonk and Boxer (1964).

Assay techniques for the following enzymes are based on those described by Shonk and Boxer (1964); glucokinase (EC. 2. 7. 1. 2), phosphoglucoisomerase (EC. 5. 3. 1. 9), phosphofructokinase (EC. 2. 7. 1. 11), aldolase (EC. 4. 1. 2. 13), triosephosphate isomerase (EC. 5. 3. 1. 1), glyceraldehydephosphate dehydrogenase (EC. 1. 2. 1. 12), phosphoglycerokinase (EC. 2. 7. 2. 3), phosphoglyceromutase (EC. 5. 4. 2. 1), enolase (EC. 4. 2. 1. 11), pyruvate kinase (EC. 2. 7. 1. 40), lactate dehydrogenase (EC. 1. 1. 1. 27), glucose 6-phosphate dehydrogenase (EC. 1. 1. 1. 49), alcohol dehydrogenase (EC. 1. 1. 1. 1) and glycerophosphate dehydrogenase (EC. 1. 1. 1. 8).

Phosphoenolpyruvate carboxykinase (EC. 4. 1. 1. 32), malic enzyme (EC. 1. 1. 1. 39) and malate dehydrogenase (EC. 1. 1. 1. 37) were assayed spectrophotometrically at 340 nm as described by Bueding and Saz (1968) and Prichard and Schofield (1968b) respectively.

To detect the production of acetyl phosphate, pyruvate dehydrogenase (EC. 1. 2. 4. 1) and pyruvate decarboxylase (partial reaction of pyruvate dehydrogenase) were assayed at 540 nm spectrophotometrically as described by Reed and Willms (1966).

Pyruvate decarboxylase (EC. 4. 1. 1. 1) activity was assayed by following the decrease in absorption at 340 nm. The reaction mixture consisted of a volume of 1.0 ml solution containing 150 μmoles potassium phosphate buffer, pH 6.0, 0.2 μmoles TPP, 0.3 μmoles $MgSO_4$, 50 μmoles pyruvate, 0.3 μmoles NADH, 75 units ADH and 30 μl of the tissue extract.

A slight modification of experimental procedures as described by Willams and Hager (1961) was used for the detection of pyruvate oxidase (EC. 1. 2. 2. 2) activity. One milliliter of reaction mixture containing 100 μmoles potassium phosphate buffer, pH 6.0, 10 μmoles $MgCl_2$, 0.1 μmoles TPP, 50 μmoles pyruvate, 1.0 μmoles sodium lauryl sulfate, 0.5 mg ovalbumin and 0.1 ml tissue extract was

tested. After equilibration at 30°C, 25 μ moles potassium ferricyanide was added to the cuvette, and the ferrocyanide formation was measured at 540 nm as described by Reed and Willms (1966).

Pyruvate oxidase (phosphate-linked) (EC. 1.2.3.3) activity was detected by a modification of the method described by Hager and Lipmann (1955). The assay consisted of 1 ml of mixture containing 200 μmoles potassium phosphate buffer, pH 6.0, 10 μmoles $MgCl_2$, 0.2 μ moles TPP, 50 μ moles pyruvate, 750 μmoles KOH, 0.5-3.0 μmoles NaF and 30 μl tissue extract. Subsequently, acetyl phosphate production was detected by the method described by Reed and Willms (1966).

All the enzyme activities were expressed as specific activity in μmole product formed/min/mg protein (U/mg protein).

Fractionation of Cell Components

Homogenization and fractionation for mitochondrial and cytosolic fractions of *C. sinensis* were carried out as described by McManus and James (1975). The worms were homogenized in an ice-cold buffered solution containing 0.25 M sucrose and 5.0 mM tris-HCl, pH 7.5. The homogenate was centrifuged at 755 g for 15 min to remove nuclei and cell fragments. Subsequently, part of supernatant was retained as crude extract and the remaining solution was then centrifuged at 9,750 g for 15 min. The supernatant was retained as the cytosolic fraction. The pellet was redispersed in excess homogenizing buffer and re-sedimented by centrifugation at 17,300 g for 15 min. Sediments were subsequently resuspended in homogenizing buffer and used as mitochondrial fraction.

Disc Gel Electrophoresis

Polyacrylamide disc gel electrophoresis was conducted as described by Davis (1964). 10% of acrylamide concentration gel was used for the experiment. Approximately 80 μg protein of sample was applied onto each gel (8×0.6 cm) and 0.1 M tris-glycine, pH 8.3, was used as tray buffered solution. Electrophoresis was run at a constant current of 3 mA per gel. After termination of electrophoresis, gels were stained for lactate dehydrogenase activity as described by Siciliano and Shaw (1976) with DL-lactate as substrate.

Determination of Protein Concentration

Protein determination was accomplished according to the method of Bradford (1976), and bovine serum albumin (BSA) was used as standard.

RESULTS

Enzyme Levels in *Clonorchis sinensis* Tissue

The activities of glycolytic enzymes in the extract of adult *Clonorchis sinensis* are presented in Table 1. The results show that significant activities were demonstrated in glucokinase, glucose 6-phosphate dehydrogenase, phosphoglucoisomerase, phosphofructokinase, aldolase, triosephosphate isomerase,

TABLE 1
Activity of glycolytic enzymes and phosphoenolpyruvate carboxykinase in *Clonorchis sinensis*

Enzyme	Enzyme activity (U/mg protein)
Glucokinase	0.311±0.081
Phosphoglucoisomerase	11.040±1.796
Phosphofructokinase	0.172±0.027
Aldolase	0.183±0.033
Triosephosphate isomerase	31.686±5.593
Glyceraldehydephosphate dehydrogenase	3.763±1.093
Phosphoglycerokinase	10.934±2.714
Phosphoglyceromutase	1.751±0.613
Enolase	4.314±1.421
Pyruvate kinase	0.276±0.089
Lactate dehydrogenase	2.895±0.613
Alcohol dehydrogenase	0.621±0.221
Glucose 6-phosphate dehydrogenase	0.270±0.055
Glycerol phosphate dehydrogenase	0.361±0.074
Phosphoenolpyruvate carboxykinase (GTP)	1.007±0.537

Each reading is the mean± standard deviation from 6 measurements.
The assay conditions were described in the section of Materials and Methods.

glycerophosphate dehydrogenase, glyceraldehydephosphate dehydrogenase, phosphoglycerokinase, phosphoglyceromutase, enolase, pyruvate kinase, alcohol dehydrogenase and lactate dehydrogenase. The results also showed a significant activity of phosphoenolpyruvate carboxykinase in the extract of *C. sinensis* (Table 1). The ratio of pyruvate kinase to phosphoenolpyruvate carboxykinase activities in adult *C. sinensis* was 0.274.

The intracellular distribution of some key enzymes for pyruvate metabolism in *C. sinensis* was also investigated and the results are presented in Table 2. The activity of malate dehydrogenase, pyruvate kinase or lactate dehydrogenase were mainly detected in cytosolic fraction of *C. sinensis*. In contrast, the malic enzyme and pyruvate oxidase activities were predominately distributed in the mitochondrial fraction (Table 2). The results also showed that phosphoenolpyruvate carboxykinase and pyruvate decarboxylase activities are obtained in both cytosolic and mitochondrial fractions of *C. sinensis* (Table 2). No

TABLE 2
Intracellular distribution of some key enzymes related to pyruvate metabolism in *Clonorchis sinensis*

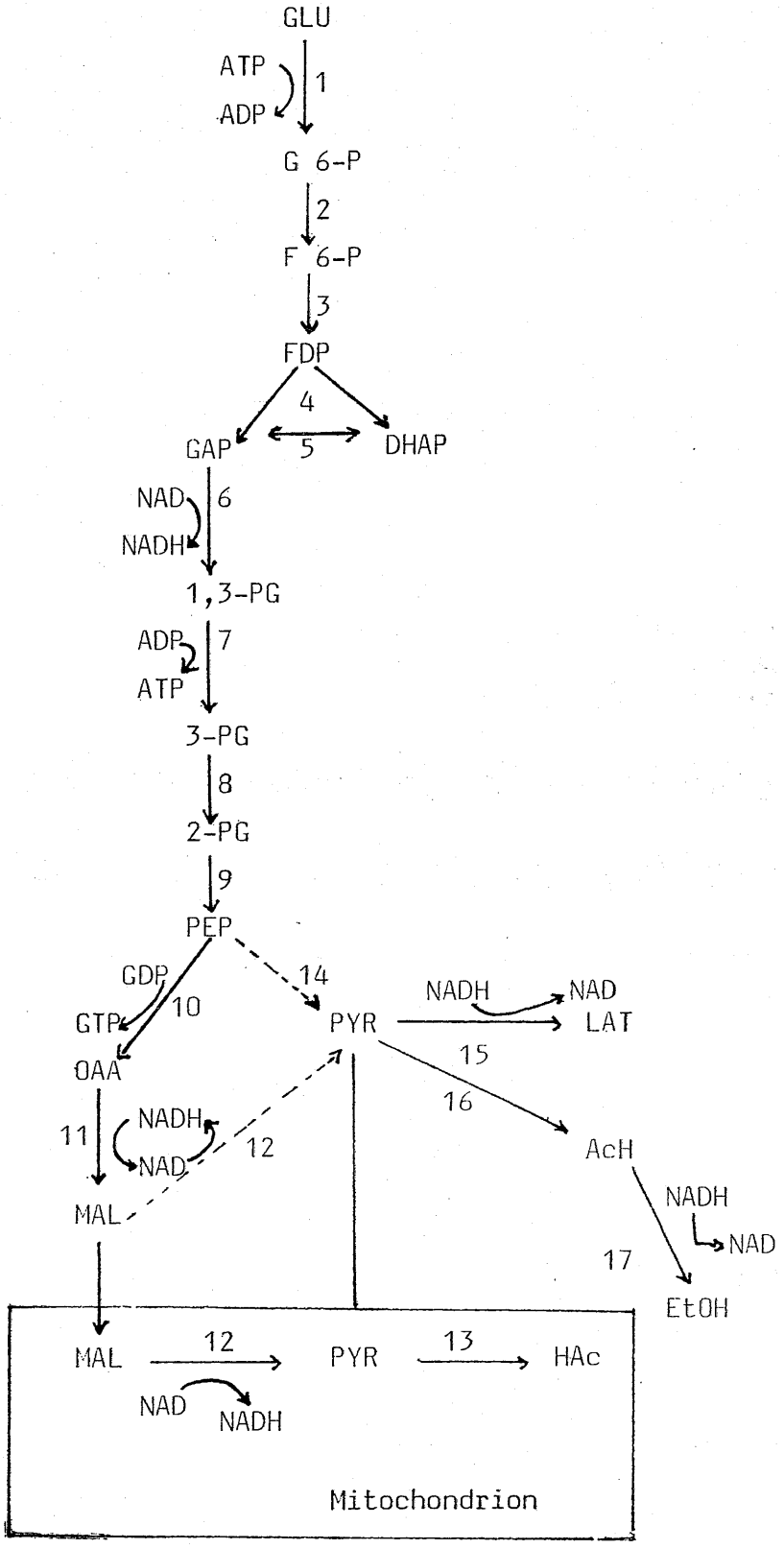
Enzyme	Specific activity (U/mg protein)		
	Crude extract	Cytosolic fraction	Mitochondrial fraction
Pyruvate kinase	0.239±0.030	0.217±0.024	0*
Phosphoenolpyruvate carboxykinase	0.617±0.049	0.772±0.031	0.364±0.043
Lactate dehydrogenase	0.367±0.058	0.374±0.062	0
Malic enzyme	0.776±0.116	0.225±0.061	1.253±0.331
Pyruvate dehydrogenase	0	0	0
Pyruvate decarboxylase (partial reaction of pyruvate dehydrogenase)	0	0	0
Pyruvate decarboxylase	1.847±0.231	1.738±0.122	2.077±0.536
Pyruvate oxidase	2.622±0.371	0.696±0.106	14.502±2.564
Pyruvate oxidase (phosphate-linked)	0	0	0

Activities are expressed as mean ± standard deviation of 4 determinations.
* No activity was observed.
The assay conditions were described in the section of Materials and Methods.

Fig. 1. Proposed pathway of carbohydrate metabolism in *Clonorchis sinensis*. The broken lines denote minor pathways. 1. glucokinase, 2. phosphoglucoisomerase, 3. phosphofructokinase, 4. aldolase, 5. triosephosphate isomerase, 6. glyceraldehyde phosphate dehydrogenase, 7. phosphoglycerokinase, 8. phosphoglyceromutase, 9. enolase, 10. phosphoenolpyruvate carboxykinase (GTP), 11. malate dehydrogenase, 12. malic enzyme, 13. pyruvate oxidase, 14. pyruvate kinase, 15. lactate dehydrogenase, 16. pyruvate decarboxylase, 17. alcohol dehydrogenase.

Abbreviations:

AcH	acetaldehyde	HAc	acetate
DHAP	dihydroxyacetone phosphate	LAT	lactate
EtOH	ethanol	MAL	malate
FDP	fructose 1, 6-diphosphate	OAA	oxaloacetate
F6P	fructose 6-phosphate	PEP	phosphoenolpyruvate
GLU	glucose	PG	phosphoglycerate
GAP	glyceraldehyde 3-phosphate	PYR	pyruvate
G6P	glucose 6-phosphate		


GLU
ATP
ADP
1
G 6-P
2
F 6-P
3
FDP
4
5
GAP
DHAP
NAD
NADH
6
1,3-PG
ADP
ATP
7
3-PG
8
2-PG
9
PEP
GDP
GTP
10
14
NADH
NAD
PYR
LAT
15
16
OAA
11
NADH
NAD
12
AcH
NADH
NAD
17
EtOH
MAL
12
PYR
13
HAc
NAD
NADH
Mitochondrion

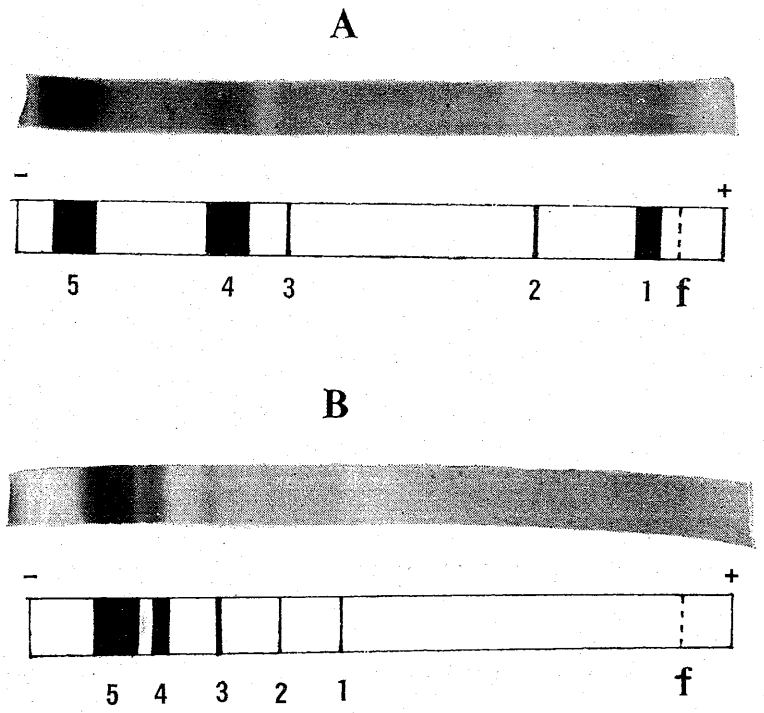


Fig. 2. Photograph of polyacrylamide gels stained for lactate dehydrogenase (LDH) activity after electrophoresis as in Materials and Methods. Gel A shows the distribution of LDH activity in a portion of the cytosolic fraction from *Clonorchis sinensis*, and Gel B shows that of the same fraction from rat liver. A diagram of each gel is shown besides the gels. The positive (+) and negative (−) electrodes are indicated. The numbers 1-5 refer to LDH-1 LDH-5 and the broken lines in the diagrams denote the front of the gel.

activity of pyruvate dehydrogenase, pyruvate decarboxylase (partial reaction of pyruvate dehydrogenase) and pyruvate oxidase (phosphate-linked) was detected in the crude extract, cytosolic fraction and mitrchondrial fraction of *C. sinensis*.

Isozymes of Lactate Dehydrogenase

Polyacrylamide disc gel electrophoresis analysis of lactate dehydrogenase (LDH) activity in cytosolic fraction of *C. sinensis* are presented in Fig. 2. The results show that the activity of LDH-1, LDH-4 and LDH-5 are prepónderant. In comparison, the electrophoretic pattern of LDH activity in rat liver is significantly different from that of *C. sinensis*. The distinct activity of LDH in rat liver was demonstrated in LDH-3, LDH-4 and LDH-5 (Fig. 2).

Cofactor Specificity of Malic Enzyme

The cofactor specificity of malic enzyme in *C. sinensis* was investigated in cytosol and mitochondrial fractions. The results show that the NAD-dependent specific activities from cytosolic and mitochondrial fractions are

TABLE 3
Cofactor specificity of malic enzyme from *Clonorchis sinensis*

Cofactor	Malic enzyme specific activity (U/mg protein)	
	Cytosolic	Mitochondrial
NAD^+	0.240	1.217
$NADP^+$	0.021	0

Each reading was obtained from 3 determinations. The assay conditions were described in the section of Materials and Methods.

0.240 and 1.217, respectively, whereas the NADP-dependent activities are 0.021 and 0.

DISCUSSION

The present results showed that glycolytic sequence of adult *Clonorchis sinensis* is identical to that of *Faciola hepatica* (Prichard and Schofield 1968a) and mammals, as far as phosphoenolpyruvate was formed (Lloyd and Barret, 1983). Unlike the mammal host, however, significant higher activity of phosphoenolpyruvate (PEP) carboxykinase was obtained when compared with that of pyruvate kinase in the soluble extracts of adult *C. sinensis*. It is therefore suggested that most of phosphoenolpyruvate was metabolized via PEP carboxykinase to oxaloacetate in this fluke. This pathway should be the major route of energy metabolism for adult *C. sinensis*. The similar phenomenon was also observed in adult worm of *Ascaris suum* (Bueding and Saz, 1968), *Hymenolepsis diminuta* (Prescott and Campbell, 1965), *Fasciola hepatica* (Prichard and Schofield, 1968b) and *Ascaris lumbricoides* (Barrett and Beis, 1973).

Extremely high level of malate dehydrogenase in both cytosolic and mitochondrial fractions may rapidly remove oxaloacetate to form malate in *C. sinensis*. Low level of malic enzyme in the cytosolic fraction revealed that malate-pyruvate in extra-mitochondria of *C. sinensis* is minor significant. In contrast, this pathway in mitochondria is significantly important, because of the high level of malic enzyme in this faction. Seidman and Entner (1961) and Saz and Lescure (1969) also reported that malate is a mitochondrial substrate for *Ascaris lumbricoides*.

Kohler and Hanselman (1973) have pointed that malate entering the mitochondria would contribute to the formation of pyruvate and succinate. The occurrence of malate-succinate-propionate pathway needs further investigation.

Based on the above findings and the information showed in Tables 1-3, a scheme for carbohydrate metabolism of adult *C. sinensis* was therefore proposed as illustrated in Fig. 1. In this scheme, lactate, ethenol and acetate are supposedly produced in this parasite.

The preponderance of each type of lactate dehydrogenase (LDH) may revealed a distinctive metabolic pathway for parasite, Cahn *et al.* (1962), Kaplan *et al.* (1968) and Everse and Kaplan (1973) indicated that LDH-1, the heart type isoenzyme, favours the flow of glycolytic carbon away from lactate formation but into Krebs cycle, and of lactate into the Krebs cycle or fowards gluconeogenesis. LDH-5, muscle type isoenzyme, is the most active in channelling glycolytic carbon into lactate during anaerobic stress. The high level activity of LDH-1 and LDH-5 may demonstrate a preponderance of these metabolic functions in adult *C. sinensis*. The predominance of LDH-5 in this fluke further confirmed our proposed metabolic scheme on the production of lactate as one of its end products.

REFERENCES

BARRETT, J. (1976) Bioenergetics in helminths. In "2nd International Symposium on Biochemistry of Parasites and Host-Parasite Relationship" (H. van den bossche, ed.) Elsevier, Amsterdam.

BARRETT, J. (1981) In *Biochemistry of Parasitic Helminths*. Macmillan, New York.

BARRETT, J. and I. BEIS (1973) Studies on glycolysis in the muscle tissue of *Ascaris lumbricoides* (Nematoda). *Comp. Biochem. Physiol.* **44B**: 751-761.

BRADFORD, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.

BUEDING, E. and H. T. SAZ (1968) Pyruvate kinase and phosphoenolpyruvate carboxykinase activities of *Ascaris* muscle, *Hymenolepsis diminuta* and *Schistosoma mansoni*. *Comp. Biochem. Physiol.* **24**: 511-517.

CAHN, R. D., N. O. KAPLAN., L. LEVINE and E. ZWILLING (1962) Nature and development of lactate dehydrogenase. *Science* **136**: 962-969.

DAVIS, B. S. (1964) Disc electrophoresis. II Method and application to human serum protein. *Ann. N. Y. Acad. Sci.* **121**: 404-427.

EVERSE, J. and N. O. KAPLAN (1973) Lactate dehydrogenase: Structure and function. *Advances in enzymology* **37**: 61-148.

HAGER, L. P. and F. LIPMANN (1955) Phosphate-linked pyruvic acid oxidase from *Lactobacillus delbruckii*. *In Methods in enzymology*. (S. P. Colowick and N. O. Kaplan, ed.) Academic Press, New York and London. **1**: 482-486.

KAPLAN, N. O., J. EVERSE and J. ADMIRAL (1968) Significance of substrate inhibition of dehydrogenases. *Annals N. Y. Acade. Sci.* **151**: 400-412.

KOHLER, P. and K. HANRELMANN (1973) Intermediary metabolism in *Dicrocoelium dendriticus* (trematoda). *Comp. Biochem. Physiol.* **45B**: 825-845.

LLOYD, G. M. and J. BARRETT (1983) *Fasciola hepatica*: Carbohydrate metabolism of the adult. *Exp. Parasitol.* **56**: 81-88.

MCMANUS, D. P. and B. L. JAMES (1975) Tricarboxylic acid cycle enzymes in the daughter sprocysts of *Microphallus similis* (Jag) (Digenea: Microphallidae). *Comp. Biochem. Physiol.* **50B**: 491-495.

PRESCOTT, L. M. and J. W. CAMPBELL (1965) Phosphoenol-pyruvate carboxylase activity and glycogenesis in flatworm, *Hymenolepsis diminuta*. *Comp. Biochem. Physiol.* **14**: 491-511.

PRICHARD, R. K. and P. J. SCHOFIELD (1968*a*) The glycolytic pathway in adult liver fluke *Fasciola hepatica*. *Comp. Biochem. Physiol.* **24**: 697-710.

PRICHARD, R. and P. SCHOFIELD (1968*b*) The metabolism of phosphoenolpyruvate and pyruvate in the adult liver fluke *Fasciola hepatica*. *Biochem. Biophys. Acta* **170**: 63-76.

READ, C. P. (1961) The carbohydrate metabolism of worms. In *Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals* (A. W. Martin, ed.) University of Washington Press, Seattle.

REED, L. J. and C. R. WILLMS (1966) Purification and resolution of the pyruvate dehydrogenase complex. In *Methods in enzymology* (*W. A. Wood, ed.*) Academic Press, New York and London. **9**: 247-265.

SAZ, H. J. and O. L. LESCURE (1969) The functions of phosphoenolpyruvate carboxykinase and malic enzyme in the anaerobic formation of succinate by *Ascaris lumbricoides*. *Comp. Biochem. Physiol.* **30**: 49-60.

SEIDMAN, I. and N. ENTNER (1961) Oxidative enzymes and their role in phosphorylation in sarcomes of adult *Ascaris lumbricodes*. *J. Biol. Chem.* **236**: 915-919.

SHONK, C. E. and G. E. BOXER (1964) Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Research* **24**: 709-721.

SICILIANO, M. J. and C. R. SHAW (1976) Separation and visualization of enzymes on gels. In *Chromatographic and electrophoretic techniques* 4th edition. (I. Smith, ed.) William Heinemann Medical Books Ltd., London. **2**: 185-209.

UEDA, T. and T. SAWADA (1968) *Jap. J. Exp. Med.*, **38**: 145-147.

WILLMS, F. R. and L. P. HAGER (1961) Pyruvic (cytochrome b.) dehydrogenase (crystalline) In *Methods in enzymology*. (W. A. Wood, ed.) Academic Press New York and London. **9**: 265-272.

中華肝吸蟲(*Clonorchis sinensis*)成蟲之醣類代謝

施秀惠　陳秀男　劉　新

本研究乃在探測中華肝吸蟲成蟲之所有醣解酵素及部分關鍵性呼吸酵素之活性，以推測其醣類代謝途徑。同時並分析部分酵素在細胞內之分布情形。

實驗結果顯示，中華肝吸蟲成蟲具有脊椎動物之典型 Embden-Meyerhof 醣解途徑的所有酵素活性，其中丙酮酸激酶之活性相對較低。經探測磷烯丙酮羧酸激酶之活性，發現在蟲體萃取液中此2酶之活性比值爲 0.274，說明此蟲之葡萄糖代謝，係沿着 E-M 途徑進行，直到磷烯醇丙酮酸生成爲止，此後則依已建立之寄生性蠕蟲之代謝模式進行，即由磷烯丙酮羧酸激酶催化，產生草酸，而後有蘋果酸生成。

探討中華肝吸蟲細胞內部分關鍵性呼吸酶之分布時發現：乳酸去氫酶、丙酮酸激酶和蘋果酸去氫酶主要分佈於細胞質內，而 Malic enzyme 和丙酮酸氧化酶主要出現於粒線體中。兩種胞器中皆有活性顯現的有丙酮酸脫羧酶及磷烯丙酮羧酸激酶。在粗萃取液、細胞質和粒線體中皆無法測得活性的有3種：丙酮酸去氫酶、丙酮酸脫羧酶（丙酮酸去氫酶之部分反應）和與磷酸相連之丙酮酸氧化酶。

另以圓盤聚丙烯醯胺膠體電泳分析乳酸去氫酶之同功酶時發現，中華肝吸蟲成蟲具有活性較强的1、4和5同功酶。