Parasitology (1984), **89**, 229–244
With 1 figure in the text

Glucose and pyruvate catabolism in *Litomosoides carinii*

TH. RAMP *and* P. KÖHLER

Department of Parasitology, University of Zürich, Winterthurerstrasse 266a, 8057 *Zürich, Switzerland*

(*Accepted* 17 *January* 1984)

SUMMARY

The filarial worm *Litomosoides carinii* showed a rapid uptake of glucose during *in vitro* incubation. This uptake proceeded linearly with time, and was significantly higher under aerobic compared to anoxic conditions. Under an atmosphere of nitrogen the worms converted glucose almost quantitatively to lactate, whereas in the presence of oxygen appreciable quantities of acetate, acetoin and CO_2, in addition to lactate, were formed. Although aerobically only 73% of the carbohydrate carbon could be accounted for by the latter products as well as by a net glycogen synthesis, attempts to identify other compounds presumed to be derived from glucose metabolism have been unsuccessful. The complete sequence of the glycolytic enzymes was detected in particulate-free cytosolic extracts of the filarial worm. With the exception of 6-phosphofructokinase, all glycolytic enzyme activities were considerably higher than those reported for rat liver. In addition, *L. carinii* possesses the entire set of enzymes catalysing the eight successive reaction steps of the tricarboxylic acid cycle. On a mitochondrial protein basis, the specific activities of these enzymes were similar to those present in rat liver. Various enzymatic activities of the mitochondrial respiratory chain were detected in the parasite. These include low levels of NADH and cytochrome c oxidases, but a high activity value for NADH dehydrogenase. Cell-free extracts and the mitochondrial fraction of the worms were found to exhibit an enzyme capable of catalysing the decarboxylation of pyruvate. Since this activity was stimulated 5- to 20-fold by the cofactors known to be required by the pyruvate dehydrogenase complex of other animal cells, pyruvate decarboxylation and thus acetate formation in the parasite may be mediated by an enzyme similar to, or identical with, the pyruvate dehydrogenase system. Isotopic carbon balance studies and experiments in which substrates specifically labelled with ^{14}C were employed showed that substrate carbon can to some extent enter into respiratory CO_2. From these and the enzymatic analyses it is suggested that complete oxidation of carbon substrate may be of relevance as an energy-conserving pathway in the filarial worm.

INTRODUCTION

Unlike the majority of helminths studied so far, the adult stage of *Litomosoides carinii* shows an oxygen requirement for maintenance of motility, and possibly for survival (Bueding, 1949; Saz, 1981). In spite of this, the filariid was incapable of completely oxidizing substrates to carbon dioxide and water (Bueding, 1949;

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Wang & Saz, 1974). When glucose is employed as the sole carbon source, catabolism results primarily in the formation of a mixture of lactic and acetic acids (Bueding, 1949; Wang & Saz, 1974). Wang & Saz (1974) and Middleton & Saz (1979) have recently investigated the *in vitro* metabolism of *L. carinii* and reported only very little $^{14}CO_2$ production from glucose labelled in positions 1 and 6 and from [3-^{14}C]pyruvate. This observation is in agreement with the supposition that the tricarboxylic acid cycle is not functional to any significant extent and it has been suggested that the aerobic requirement of the parasite may be explained solely on the single step of pyruvate oxidation resulting primarily in the formation of equimolar amounts of acetate and CO_2 (Saz, 1981). In addition, small amounts of pyruvate are oxidized to acetoin and CO_2 (Middleton & Saz, 1979).

Although several glycolytic enzymes have been demonstrated in *L. carinii* (Srivastava, Chatterjee, Sen, Ghatak & Krishna Murti, 1970), virtually nothing is known of the enzymes of the tricarboxylic acid cycle and the mitochondrial respiratory chain. Also, only few attempts have been made to investigate the parasites' respiration process and enzymatic system responsible for the production of acetate (Bueding, 1949; Wang & Saz, 1974; Middleton & Saz, 1979). Bueding (1949) reported that oxygen uptake of *L. carinii* is inhibited by cyanide and cyanine dyes, and the same author and later on Middleton & Saz (1979), have demonstrated a pyruvate-dependent production of acetate and CO_2 by the adult filarial worm.

In order to assess the biological importance of the pathways presumed to be involved in the catabolism of carbohydrate in *L. carinii*, a systematic study was made on the enzymes of glycolysis, the tricarboxylic acid cycle and the mitochondrial respiratory chain. An attempt was also made to re-examine the worm's capabilities for complete substrate oxidation and to characterize the enzymatic process accomplishing pyruvate oxidation in the filarial worm.

MATERIALS AND METHODS

Parasites

Adult female and male *Litomosoides carinii* were obtained from the pleural cavity of cotton rats (*Sigmodon hispidus*) or jirds (*Meriones unguiculatus*). The host animals were killed in chloroform vapour and the worms were subsequently removed and transferred to basic filarial medium (BFM) (Bueding, 1949).

Glucose uptake and carbon balance studies

To determine glucose uptake, glycogen content and metabolic end-products, 40–200 mg of *L. carinii* were incubated at 37 °C in a Warburg apparatus under atmospheres of either air or high purity (99·995 %) nitrogen. Unless otherwise indicated, incubations were conducted for 6 h in 12 ml of BFM containing 25 mM glucose as initial substrate concentration. To attain at least nearly anaerobic incubation conditions, vessels were flushed with the nitrogen for 10 min prior to initiation of the reaction. Metabolic CO_2 was trapped in filter paper wicks moistened with 0·1 ml of 5 M KOH which were placed in the centre wells of the

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Warburg vessels. Incubations were terminated by removal and the filariids and incubation solutions were frozen at −80 °C and −20 °C, respectively, for subsequent glycogen and metabolite analyses. When $^{14}CO_2$ was to be determined, incubations were carried out in a similar manner, except that [U-^{14}C]glucose, [6-^{14}C]glucose, [1-^{14}C]pyruvate or [3-^{14}C]pyruvate were used as the substrates, respectively. These reactions were terminated by the addition of 0·2 ml of 3·5 M perchloric acid and the vessels were shaken for an additional 90 min. The worms were then removed and assayed for endogenous metabolic components (see below). The filter paper wicks were transferred to scintillation vials containing 20 ml of Aquassure (New England Nuclear), and incubated with constant shaking overnight. Radioactivity was determined in a model 460 CD Tri-carb liquid scintillation spectrometer (Packard Instrument Co.). Control incubations, in the absence of worms, were carried out simultaneously with the labelled glucose and pyruvate species, respectively. Other control measurements with ^{14}C-labelled acetate and propionate revealed that, under the reaction conditions employed, volatile acids were not trapped in the KOH.

To determine endogenous concentrations of glucose, glycogen and metabolic end-products, worms were homogenized in 5–10 volumes of 3·5 M perchloric acid and the resulting suspension was neutralized with KOH. After centrifugation, the supernatant solution was assayed for the above components. Zero-time controls were carried out with worms which were homogenized in perchloric acid immediately before the experimental incubations were initiated.

Protein-free, neutralized worm extracts and incubation media were assayed spectrophotometrically for the following components: glucose was analysed using hexokinase and glucose-6-phosphate dehydrogenase according to the method of Bergmeyer, Bernt, Schmidt & Stork (1974). Acetate was assayed with acetate kinase as described by Bergmeyer & Möllering (1974). Acetate was determined from a concomitantly prepared standard curve ranging from 10 to 150 μM acetate. Determination of L- and D-lactate was carried out employing the corresponding stereospecific lactate dehydrogenases and a glutamate–pyruvate transaminase trapping system to prevent pyruvate accumulation (Noll, 1974). Ethanol and propanol were quantitated with alcohol dehydrogenase according to Beutler & Michal (1977). Formate was analysed using formate dehydrogenase (Höpner & Knappe, 1974) and succinate was assayed with succinyl-CoA synthetase (Michal, Beutler, Lang & Güntner, 1976). Pyruvate was determined with lactate dehydrogenase as described by Czok & Lamprecht (1974). Quantitation of acetoin was achieved by the colorimetric procedure described by Westerfeld (1945). Acetoin concentrations were calculated from concomitantly prepared standard curves ranging from 10 to 30 μM acetoin. Glycogen was degraded with amyloglucosidase (Keppler & Decker, 1974) and the amount of glucose, and ultimately glycogen, was determined as described above.

Glucose and anionic end-products present in incubation media were also quantitated following fractionation by ion-exchange chromatography. Aliquots of 1 ml of incubations carried out in the presence of [U-^{14}C]glucose were adjusted to pH 11 (KOH) and applied to a Dowex 50 (1 × 8, 100–200 mesh) anion-exchange column (45 × 1·6 cm). Elution of the column was performed with water (100 ml) followed by 5 mM HCl (50 ml) and 25 mM HCl (100 ml) to separate glucose,

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

acetate, lactate and possible other, as yet unknown, acidic products. Fractions of 1 ml were collected in scintillation vials and aliquots of these solutions were assayed for radioactivity as described above. Glucose, acetate and lactate were identified by their relative retention times, which were obtained in control experiments using ^{14}C-labelled standard compounds, and quantitated enzymatically as described above.

Cell fractionation and enzyme activity determinations

Adult *L. carinii* were homogenized (0·2 g worms/ml) in a Teflon-glass Potter-Elvehjem homogenizer employing a medium composed of 220 mM mannitol, 70 mM sucrose, 5 mM Hepes and 0·1 mM EDTA, pH 7·4 (MSH medium). The resultant crude homogenate was centrifuged at 600 ***g*** for 15 min and the supernatant fraction obtained was recentrifuged at 12000 ***g*** for 15 min. The pellet, containing the mitochondria, was washed once with 5 ml of MSH medium/initial g of worm wet weight and was then resuspended in 3 ml of 0·1 M potassium phosphate (pH 7·4). This suspension was disintegrated thoroughly by treatment with a Polytron PT 10 homogenizer (Kinematica, Lucerne, Switzerland) 10 times for 10 sec each with 1-min cooling intervals. During disintegration, the samples were cooled by an ice–salt mixture. After centrifugation at 200000 ***g*** for 60 min a clear supernatant fluid was obtained which contained the activities of the soluble mitochondrial enzymes. The pellet, containing the mitochondrial structure-bound enzymes and the α-oxoacid dehydrogenase complexes, was resuspended in 3 ml of 50 mM potassium phosphate (pH 7·4)/g of initial worm wet weight. The cytosolic enzyme fraction was obtained from the initial 12000 ***g*** supernatant fraction by centrifugation at 200000 ***g*** for 60 min. All operations were carried out at 0–5 °C.

Enzyme activities were measured at 25 °C either spectrophotometrically or by isotope techniques in a buffer system composed of 100 mM Hepes and 0·1 mM EDTA. The activities of aconitate hydratase, fumarate hydratase, cytochrome c oxidase and isocitrate, oxoglutarate and malate dehydrogenases were assayed at 25 °C in potassium phosphate buffer containing 0·1 mM EDTA. Other conditions were essentially the same as described by the following published procedures: hexokinase (EC 2.7.1.1) according to Slein, Cori & Cori (1950); glucose-6-phosphate isomerase (EC 5.3.1.9), fructose*bis*phosphate aldolase (EC 4.1.2.13), triosephosphate isomerase (EC 5.3.1.1), glyceraldehydephosphate dehydrogenase (EC 1.2.1.12) and lactate dehydrogenase (EC 1.1.1.27) as described by Delbrück, Zebe & Bücher (1959); 6-phosphofructokinase (EC 2.7.1.11) according to Racker (1947), as modified by Köhler (1972); phosphoglycerate kinase (EC 2.7.2.3) and phosphoglyceromutase (EC 2.7.5.3) according to Vogell, Bishai, Bücher, Klingenberg, Pette & Zebe (1959) and enolase (EC 4.2.1.11) and pyruvate kinase (EC 2.7.1.40) as described by Bücher, Luh & Pette (1966).

Citrate synthase (EC 4.1.3.7), aconitate hydratase (EC 4.2.1.3), isocitrate dehydrogenase (NAD^+) (EC 4.1.1.41), isocitrate dehydrogenase ($NADP^+$) (EC 4.1.1.42), fumarate hydratase (EC 4.2.1.2), malate dehydrogenase (EC 1.1.1.37) and malate dehydrogenases (decarboxylating) (EC 1.1.1.38 and 1.1.1.40) were assayed according to the methods of Köhler & Hanselmann (1973). The methods for the measurement of succinate dehydrogenase (EC 1.3.99.1),

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

NADH-fumarate reductase, NADH oxidase, NADH dehydrogenase (EC 1.6.99.3) and NADH-cytochrome c reductase have recently been published by Köhler & Bachmann (1978). Succinyl CoA synthetase (EC 6.2.1.4) was estimated according to Cha & Parks (1964*a*, *b*). Assay conditions for oxoglutarate dehydrogenase (EC 1.2.4.2) were the same as those described for pyruvate dehydrogenase (Linn, Pelley, Pettit, Hucho, Randall & Reed, 1972), except that pyruvate as substrate was replaced by 3 mM oxoglutarate.

Assay conditions and methodology for the measurement of pyruvate decarboxylation activities in intact worms were essentially the same as those used for the glucose carbon balance studies, except that glucose was replaced by either [1-^{14}C] or [3-^{14}C]pyruvate, each at 5 mM concentrations, and that the incubations were carried out in BFM in a final volume of 1 ml. Homogenates, extracts and mitochondrial fractions were prepared as described above for the other enzyme activity determinations, except that 1 mM mercaptoethanol was present throughout the fractionation procedure. These systems were incubated for 1 h at 37 °C in 50 mM potassium phosphate (pH 7·4) and half concentrated MSH either in the presence or absence of the various cofactors required for optimal activity of the pyruvate dehydrogenase complex (Linn *et al.* 1972, and Table 7). For these assays [1-^{14}C]pyruvate only was employed as the substrate.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using crystalline bovine serum albumin as a standard.

Reagents

Enzymes, cofactors and substrates were purchased from Boehringer Mannheim Co. (Mannheim, F.R.G.). All radiochemicals were obtained from The Radiochemical Centre (Amersham, England).

RESULTS

Glucose uptake and carbon balance studies

Adult *L. carinii* incubated in glucose-containing BFM showed a rapid uptake of glucose which remained linear over at least 6 h (Fig. 1). In these experiments, the worms removed significantly more glucose from the incubation medium when air was used as the gas phase than under anaerobic conditions ($P < 0{\cdot}001$, paired *t*-test).

In the carbon balance studies summarized in Table 1, incubation of worms in the presence of glucose resulted in a net synthesis of glycogen, which was much more pronounced in the presence of air than under an atmosphere of nitrogen. Independent of whether oxygen was present or not glucose was catabolized by the parasites to form L-lactate, acetate, acetoin and CO_2. However, in agreement with earlier findings (Bueding, 1949), considerably more lactate (i.e. 88 % of the glucose utilized) but only slight amounts of acetate and acetoin accumulated under anaerobic as compared to aerobic conditions. When oxygen was excluded from the incubations, nearly all (95 %) of the glucose removed was converted to lactate, acetate, acetoin and glycogen, whereas only 73 % of the carbohydrate carbon could be accounted for by these products when oxygen was available in the incubation

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

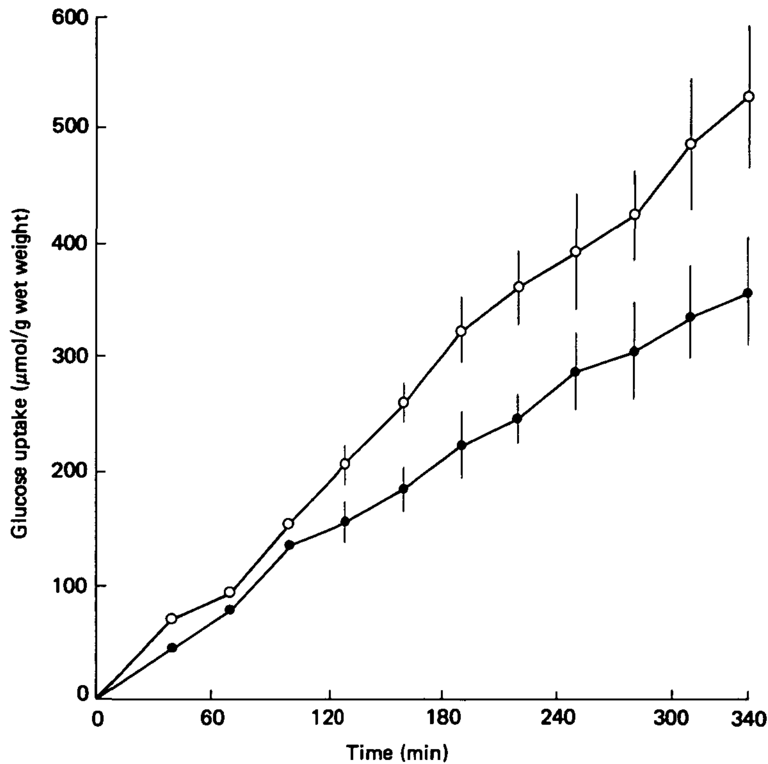

Fig. 1. Glucose uptake by adult *Litomosoides carinii* under aerobic and anaerobic incubation conditions. Incubations were carried out in the presence of glucose as described in the Materials and Methods section. Glucose uptake was determined in 30 min intervals and calculated from the decrease of glucose concentration in the incubation medium. After each sampling, the anaerobic vessels were flushed with nitrogen for 5 min. All values represent means ± S.D. of 4 experiments. (○), Aerobic; (●), anaerobic.

mixtures. Interestingly, when only male worms were employed in the carbon balance studies rates of glucose uptake and the pattern and quantitative proportions of end-products were essentially the same as observed in incubations which contained worms of mixed sexes (not shown).

In order to account for the observed deficit in the aerobic carbon balance, several attempts were made to demonstrate further products presumed to be derived from glucose metabolism of the filarial parasite. As shown in Table 1, upon incubation of adult *L. carinii* in the presence of [U-^{14}C]glucose small quantities of $^{14}CO_2$, which theoretically could not be accounted for by the production of acetate and acetoin, did arise from the glucose carbon catabolized. In spite of the fact that this apparent additional CO_2 accumulation contributed only a small portion (2 %) of the total carbon dissimilated by the worms, further attempts were made to determine its metabolic origin and to substantiate the validity of this result. By employing substrates specifically labelled with ^{14}C it could be shown that both the 6 carbon of glucose and the 3 carbon of pyruvate can to some extent enter into respiratory CO_2 (Table 2). As this process of CO_2 formation is known to be solely dependent

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 1. *Glucose balance of adult* Litomosoides carinii *under aerobic and anaerobic incubation conditions*

(Incubation conditions and analytical procedures were as described in the Materials and Methods section. Product formation is expressed as μmol/6 h/g wet wt. Average amounts of 529·3±45·9 μmol and 357·6±48·4 μmol of glucose were removed from the incubation medium within 6 h/g wet wt under aerobic and anaerobic incubation conditions, respectively. All values represent means±S.D. of 7 (aerobic) and 4 (anaerobic) experiments, respectively. $^{14}CO_2$ formation was determined in 2 incubations.)

	Air		Nitrogen	
Products	μmol	%*	μmol	%
Lactate	495·2±233·4	46	643·1±209·8	88
Acetate	109·3±58·3	12	20·5±7·8	3
Acetoin	15·5±3·1	3	2·1±0·8	1
$^{14}CO_2$	235·7†	2‡	19·1	—
Glycogen	64·1±21·4	12	11·3±7·4	3
Glucose removed accounted for		75		95

* Mean percentage of μmol glucose removed in individual experiments.
† Total $^{14}CO_2$ produced.
‡ $^{14}CO_2$ formation which could not be accounted for by the production of acetate and acetoin.

Table 2. $^{14}CO_2$ *production from* [^{14}C]*glucose and* [^{14}C]*pyruvate by* Litomosoides carinii

($^{14}CO_2$ formation and substrate utilization are expressed as μmol/h/g wet wt at 37 °C. Values represent means of 2 determinations. Initial substrate concentrations were for glucose 25 mM (sp. act. 0·04 Ci/mol) and for pyruvate 5 mM (sp. act. 0·16 Ci/mol). Glucose-dependent incubations were run for 3 h and contained 127 mg worm wet wt in 2 ml of BFM and pyruvate-dependent incubations were run for 1 h and contained an average of 45 mg of worm wet wt in 1 ml of BFM. All other experimental conditions were described in the Materials and Methods section.)

	Anaerobic		Aerobic		
Substrate	Substrate removed	$^{14}CO_2$ formed	Substrate removed	$^{14}CO_2$ formed	Percentage of substrate removed
[U-^{14}C]Glucose	45·0	2·4	61·2	21·4	—
[6-^{14}C]Glucose	52·0	0·03	72·0	1·4	2
[1-^{14}C]Pyruvate	—	—	35·7	16·3	45
[3-^{14}C]Pyruvate	—	—	28·7	2·5	9

on the presence of an active tricarboxylic acid cycle it can be suggested that in *L. carinii* a small, but significant, portion of the substrates utilized can undergo complete oxidation by this metabolic event. When *L. carinii* was incubated either in the presence of [6-^{14}C]glucose or [3-^{14}C]pyruvate, 2 % of the added glucose and 9 % of the added pyruvate must have been degraded to CO_2 and water. In agreement with the basic biochemical mechanism of complete substrate oxidation, the observed $^{14}CO_2$ production was found to be dependent on the presence of

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 3. *Activities of the glycolytic enzymes in* Litomosoides carinii

(For conversion of the activity values obtained in the present study into μmol/h/g wet wt at 37 °C the units have to be multiplied by 12. This approximate factor corrects also for the amount of enzyme protein which escaped extraction during tissue homogenization.)

Enzyme	Specific activity*		
	Present study	Literature†	Rat liver‡
Hexokinase	54 ± 14	32	6
Glucosephosphate isomerase	1556 ± 182	585	630
6-Phosphofructokinase	8 ± 4	68	9
Fructose*bis*phosphate aldolase	112 ± 8	112	30
Glyceraldehyde-phosphate dehydrogenase	1394 ± 181	302	848
Triosephosphate isomerase	7761 ± 1160	—	2483
Phosphoglycerate kinase	3331 ± 466	—	1049
Phosphoglyceromutase	1396 ± 145	—	442
Enolase	1509 ± 195	204	224
Pyruvate kinase	497 ± 49	137	252
Lactate dehydrogenase	3371 ± 236	1365	1956

* Specific activities are expressed as nmol/min/mg protein at 25 °C and are given as means ± S.D. obtained from at least 5 experiments. Other experimental conditions were as described in the Materials and Methods section.

† From Srivastava *et al.* (1970).

‡ From Köhler (1972).

oxygen. The results summarized in Table 2 clearly show that in the presence of nitrogen $^{14}CO_2$ production from [6-^{14}C]glucose was negligible but was remarkably stimulated when air was used as the gas phase in the incubation experiments.

Attempts to identify additional end-products presumed to be formed during aerobic carbohydrate metabolism in the filarial worm have been unsuccessful. Neither pyruvate, D-lactate, ethanol, propanol, formate and succinate were found in the incubation mixtures. In a search for other acidic fermentation products the solution in which *L. carinii* had been incubated in the presence of ^{14}C-labelled glucose was analysed by anion-exchange chromatography (see Materials and Methods section). In the course of this study no acidic component other than lactic and acetic acids could be eluted from the column.

Enzymatic studies

The presence of a full glycolytic enzyme spectrum could be demonstrated in particulate-free cytosolic extracts of *L. carinii* (Table 3). Of these enzymes, triosephosphate isomerase, phosphoglycerate kinase and phosphoglyceromutase have to our knowledge not been reported previously in the filarial worm. All enzyme activity levels, with the exception of 6-phosphofructokinase, were considerably higher than those published earlier for *L. carinii* (Srivastava *et al.* 1970) and for rat liver tissue (Köhler, 1972). The finding that 6-phosphofructokinase is the glycolytic enzyme of lowest specific activity is in agreement with the situation in most other glycolytic systems so far investigated in detail.

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 4. *The activities of the tricarboxylic acid cycle enzymes in* Litomosoides carinii *mitochondria*

Enzymes	Specific activity*	
	L. carinii	Rat liver†
Citrate synthase	2090 ± 831	93
Aconitate hydratase	68 ± 48	5
Isocitrate dehydrogenase (NAD^+)	21 ± 11	12
Isocitrate dehydrogenase ($NADP^+$)	111 ± 51	71
Oxoglutarate dehydrogenase	16 ± 4	22
Succinyl CoA synthetase	26 ± 8	N.D.
Succinate dehydrogenase	51 ± 12	112‡
Fumarate hydratase	3423 ± 901	175
Malate dehydrogenase	3029 ± 1221	2684

* Specific activities are expressed as nmol/min/mg soluble mitochondrial protein at 25 °C and represent means ± S.D. obtained from at least 4 independent experiments. Oxoglutarate dehydrogenase and the membrane-bound succinate dehydrogenase activities refer to mg of protein obtained in the pellet fraction after high speed centrifugation of the disrupted mitochondrial preparations (see Materials and Methods section).

† Data were taken from Köhler & Hanselmann (1973).

‡ Reported by Köhler & Bachmann (1980).

N.D., Not determined.

Table 5. *Activities and intracellular distribution of the tricarboxylic acid cycle enzymes in* Litomosoides carinii

Enzyme	Specific activity*	
	Cytosol	Mitochondria
Citrate synthase	858 ± 212	1180 ± 430 (912)
Aconitate hydratase	5226 ± 1574	34 ± 16 (51)
Isocitrate dehydrogenase (NAD^+)	—	15 ± 10 (108)
Isocitrate dehydrogenase ($NADP^+$)	250 ± 93	68 ± 43 (708)
Oxoglutarate dehydrogenase	—	24 ± 8 (226)
Succinyl-CoA synthetase	—	15 ± 6 N.D.
Succinate dehydrogenase	—	57 ± 23 (1000)
Fumarate hydratase	2642 ± 705	1285 ± 564 (1620)
Malate dehydrogenase	103787 ± 21449	1861 ± 837 (25400)

* Specific activities are expressed as nmol/min/g of wet wt at 25 °C and are given as means ± S.D. obtained from at least 4 experiments. Extra- and intramitochondrial enzyme activities were separated by the fractionation technique described in the Materials and Methods section. The values in parentheses represent specific activities (nmol/min/g of wet wt) of the corresponding enzymes present in rat liver mitochondria (Köhler, unpublished observations).

N.D., Not determined.

The results summarized in Tables 4 and 5 indicate that *L. carinii* possesses also the entire set of enzymes catalysing the 8 successive reaction steps of the tricarboxylic acid cycle. Based on mitochondrial protein, the activity levels of most of these enzymes are comparable to those present in rat liver tissue (Table 4). Exceptions are the activities of the enzymes catalysing the first two reactions of

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 6. *Enzymatic activities of the mitochondrial respiratory chain in* Litomosoides carinii

(For conversion of the activity values into μmol/h/g wet wt at 37 °C the units have to be multiplied by 0·5.)

Enzyme	Specific activity*	
NADH oxidase	23 ± 5	(5)
NADH dehydrogenase	2811 ± 1000	(6)
NADH cytochrome c reductase	71 ± 32	(5)
Cytochrome c oxidase	9 ± 4	(5)

* Specific activities are expressed as nmol/min/mg of mitochondrial membrane protein at 25 °C as means ± S.D. obtained from the number of experiments shown in parentheses. Other experimental conditions were as described in the Materials and Methods section.

the cycle as well as for fumarate hydratase, which are all considerably higher than those of their mammalian counterparts. As a result of the low average amount of mitochondrial protein obtained (i.e. approximately 0·5–1·0 mg of soluble mitochondrial protein could be extracted from 1 g worm wet wt), the total activities of some of the Krebs cycle enzymes present in the worms are very low (Table 5). Compared with the corresponding rat liver enzymes the activities recorded for mitochondrial NAD^+- and $NADP^+$-linked isocitrate dehydrogenases, succinate and malate dehydrogenases are 7- to 18-fold lower in *L. carinii* than in the vertebrate tissue. Like in other eukaryotic systems, some of the enzymes of the cycle were found present in both cytosolic and mitochondrial fractions, whereas NAD^+-linked isocitrate dehydrogenase, oxoglutarate dehydrogenase, succinyl CoA synthetase and succinate dehydrogenase were completely confined to the mitochondrial compartment. From these activities, oxoglutarate and succinate dehydrogenases were primarily detectable in the pellet fraction obtained after high-speed centrifugation of the disrupted mitochondrial preparation. As in most other animal tissues, cytosolic malate dehydrogenase was present in large excess of the other activities. Interestingly, cytosolic aconitate hydratase levels were also very high but NADH fumarate reductase, an important enzyme of many helminth species, could not be demonstrated in the filarial worm. Also, NAD^+- and $NADP^+$-linked malate dehydrogenases (decarboxylating), two enzymes which are related to the tricarboxylate cycle, were only barely detectable (less than 5 nmol/min/mg protein) in both cytosolic and mitochondrial fractions of the parasite.

Various electron transport activities were analysed in the mitochondrial membrane fraction of the filarial worm. As shown in Table 6, a substantial activity was demonstrable for NADH dehydrogenase, as measured with ferricyanide as electron acceptor. Other activities of the mitochondrial respiratory chain, including those of NADH oxidase, NADH cytochrome c reductase and cytochrome c oxidase, were found to be rather low. Cyanide, at a 1 mM concentration, completely inhibited cytochrome c oxidase activity.

The acetate-forming pathway in L. carinii

The results presented in Tables 1 and 2 and previous studies (Middleton & Saz, 1979) indicate that *L. carinii* contains substantial activity of an enzymatic system catalysing the glucose- or pyruvate-dependent formation of acetate. When adult

Downloaded from https://www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 7. *Decarboxylation of* [1-^{14}*C*]*pyruvate by various subcellular fractions of* Litomosoides carinii

System	Additions	$^{14}CO_2$ recovered*
Intact worms	None	4·38
Crude extract	None	0·04
Crude extract	Cofactors	0·86
Mitochondrial sonicate	None	0·05
Mitochondrial sonicate	Cofactors	0·33
Cytosol	None	0·05
Cytosol	Cofactors	0·23

* $^{14}CO_2$ evolution is expressed as μmol/h/g wet wt at 37 °C. Values represent means of duplicate examinations. [1-^{14}C]Pyruvate was used as the substrate at 5 mM concentration. The mixture of cofactors was composed of NAD^+, 2·5 mM; coenzyme A, 0·1 mM; thiamine pyrophosphate, 0·2 mM; dithiothreitol, 0·3 mM; magnesium chloride, 1 mM. For other conditions and experimental details see the Materials and Methods section and the legend to Table 2.

filarial worms were incubated in the presence of oxygen and glucose as substrate, 18 μmol of acetate and 2·6 μmol of acetoin were formed/h/g wet wt. As both products are presumed to be derived from pyruvate as the direct precursor, the pyruvate decarboxylating enzyme must have been operative in these experiments with an activity of more than 20 μmol/h/g wet wt. When pyruvate was used as the exogenous substrate intact worms were found to decarboxylate this compound at a similar rate to that obtained with glucose (Table 2). As illustrated in Table 7, such a pyruvate-dependent decarboxylation activity was also detected in cell-free systems, in particular within the mitochondrial fraction. These activities were found to vary considerably in the different experimental studies (not shown) and their values were very low. A marked stimulation of the rate of decarboxylation was, however, noted when a set of compounds known to be required as cofactors by the pyruvate dehydrogenase complex of other sources (Table 7) was added to the incubation systems. This observation and the fact that a substantial amount of the pyruvate decarboxylating activity of cell-free extracts was associated with the mitochondrial fraction (Table 7) suggests that pyruvate decarboxylation and thus acetate formation in *L. carinii* may be mediated by an enzyme similar to the pyruvate dehydrogenase complex of other organisms.

DISCUSSION

The data obtained in the present study confirm that *L. carinii* exhibits a rapid rate of both glucose consumption and lactate production, regardless of whether the worms were incubated under aerobic conditions or under an atmosphere of nitrogen (Bueding, 1949). When oxygen was present during incubation of worms utilizing glucose, the rate of lactate accumulation was slightly decreased, but significantly more glucose was consumed as compared to the anaerobic reactions (Tables 1 and 2). This latter observation, which was also made in earlier studies (Bueding, 1949), is not consistent with the classical Pasteur effect of most cells capable of both aerobic and anaerobic carbohydrate utilization, in which the onset

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

of oxygen consumption is known to slow down the rate of glucose utilization. The reason for this unusual metabolic behaviour of the parasite is unknown but is likely due to the specificities in the interlocking regulatory mechanisms that coordinate the various stages of the parasites' energy-yielding and biosynthetic pathways.

The profile of the glycolytic enzyme activities in *L. carinii* was found to be very similar to that of a typical vertebrate tissue, indicating that the organization of glycolysis in the parasite may resemble that in mammalian tissues. Except for 6-phosphofructokinase, the glycolytic enzyme activities in *L. carinii* were also comparable to those published recently for the homo-lactate fermenting helminth, *Schistosoma mansoni* (Shapiro & Talalay, 1982). As is the case in most animal cells, the 6-phosphofructokinase reaction appears to be the rate-limiting step in glycolysis of the filarial worm. In spite of the low activity of this enzyme detected in the worms' cytosolic fraction (Table 3) its total activity, as measured at 37 °C (approximately 100 μmol/h/g wet wt, see legend to Table 3), may be sufficient to explain the breakdown of glucose observed with intact worms, i.e. 45–90 μmol/h/g wet wt at 37 °C (Tables 1 and 2).

All the enzymes catalysing the 8 successive reaction steps of the tricarboxylic acid cycle have been demonstrated in the filarial worm. With the exception of citrate synthase, aconitate hydratase and fumarate hydratase, the activities of these enzymes did resemble those of rat liver tissue. However, this similarity was found to hold true only for the levels expressed on a mg of protein basis. As can be seen from the data in Table 5, total activities of a number of Krebs cycle enzymes in the parasite must be considered as very low. Two factors may account for this discrepancy. Firstly, the parasite may contain only a relatively small number of mitochondria. Secondly, a substantial portion of the tissue constituents may have escaped extraction into the homogenization medium. Evidence in support of the latter idea was provided by the results of an experiment in which more than 50% of the pyruvate decarboxylating activity of the worms has remained in the pellet fraction after low-speed centrifugation of the crude homogenate. Additional information was obtained by employing more intense homogenization techniques, which did result in the damage of the mitochondrial fraction, but significantly increased the amounts of various enzyme activities (e.g. lactate dehydrogenase) in the tissue extract. Considering this, and the loss of protein arising from the fractionation procedure, it can be assumed that the total content of mitochondrial enzymes in the parasite may be approximately 3 times higher than that indicated in Table 5. These activities appear to be sufficient to accomplish the observed rates of complete substrate oxidation.

Earlier studies have demonstrated that *L. carinii* requires oxygen for the maintenance of motility (Bueding, 1949) and it has been suggested that the oxidative component in the metabolism of the worm may supply the additional energy required for muscular activity (Middleton & Saz, 1979). However, very little is known about the nature of the oxygen-dependent pathways and the mechanism(s) involved in the assumed aerobic energy generating processes. Examination of the present results (Tables 1 and 2) and the data published by other authors (Bueding, 1949; Wang & Saz, 1974; Middleton & Saz, 1979) reveal a number of metabolic alterations that occur when incubations of *L. carinii*, in the presence of glucose as substrate, are changed from anaerobic to aerobic conditions.

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

(1) Lactate accumulation is slightly decreased but the production of acetate and acetoin is remarkably increased; (2) both glucose consumption and glycogen synthesis are stimulated; (3) small amounts of CO_2 are produced which cannot be accounted for by the production of acetate and acetoin; (4) $^{14}CO_2$ is produced from both, [6-^{14}C]glucose and [3-^{14}C]pyruvate. These findings suggest that during aerobic substrate catabolism of *L. carinii* oxygen may be involved in at least two different processes, i.e. the oxidative decarboxylation of pyruvate to acetate and acetoin, respectively, and in the complete substrate degradation to CO_2 and water.

A relationship between acetate production and oxygen utilization by *L. carinii* was observed by Bueding (1949) who found that under aerobic conditions glucose-dependent acetate production was considerably higher than anaerobically and that the amounts of oxygen utilized by the worms were similar, on a molar basis, to the rates of acetate formed. Recently, Middleton & Saz (1979) postulated the presence in *L. carinii* of a pyruvate oxidase system capable of catalysing the oxidative decarboxylation of pyruvate to acetate and CO_2. However, these results did not provide evidence indicating that this decarboxylation process can be assigned to a system similar to or identical with the pyruvate dehydrogenase complex, which is the predominant catalyst for oxidative pyruvate decarboxylation in most animal cells. In our experiments, such an enzymatic activity was demonstrated in cell-free worm extracts, including the mitochondrial fraction. This activity was found to be dependent on a mixture of cofactors known to be required by the pyruvate dehydrogenase complex isolated from mammalian sources (Table 7), indicating the possible similarity of the worm enzyme to its mammalian counterpart. The total activity of this enzymatic reaction in cell-free worm systems could, however, not account for the rate of pyruvate decarboxylation observed with intact parasites. The reason for this discrepancy may be due to rapid enzyme inactivation upon disruption of the worm tissue. Evidence in support of this suggestion was the finding that the rates of pyruvate decarboxylation in cell-free fractions showed considerable variation in different experimental studies. An alternative explanation for the above observation may be that the assay conditions employed for the worm enzyme had been far from optimal.

The results obtained from three different and independent experiments have revealed that the adult *L. carinii* are capable of oxidizing substrates completely to CO_2 and water using molecular oxygen. The evidence is as follows: (1) upon incubation of the filariids in the presence of glucose aerobic CO_2 production derived from the 6-carbon of the glucose molecule greatly exceeded that obtained under anaerobic conditions; (2) incubation of worms in the presence of pyruvate radioactively labelled in its methyl group gave rise to the formation of labelled CO_2; (3) carbon balance studies have shown that the rate of glucose dependent CO_2 production exceeded that which could be accounted for by the formation of acetate and acetoin, respectively. The actual data shown in Tables 1 and 2 suggest that approximately 2% of the total carbohydrate utilized by the worms may have undergone complete combustion. This is in agreement with the enzymatic analyses, since all of the enzymes required for the reactions of the tricarboxylic acid cycle are present in the parasite and their activities seem to be high enough (Tables 4 and 5) to account for the overall rate of complete substrate oxidation observed in the intact organisms (1–2 μmol/h/g wet wt). On the other hand, the activities

Downloaded from https://www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

Table 8. *Possible rates of ATP synthesis during aerobic and anaerobic glucose utilization in* Litomosoides carinii*

Pathway	Aerobic	Anaerobic
Lactate fermentation	81	105
Acetate and acetoin formation	106	19†
Complete oxidation	67	—
Total	254	124

* ATP formation is expressed as μmol/h/g worm wet wt. All values were calculated from the results shown in Table 1 considering the assumptions made in the text.

† This value is obviously too high, since under strictly anaerobic incubation conditions oxidative phosphorylation via the conventional mitochondrial respiratory system is not possible.

of NADH-dependent mitochondrial respiration and cytochrome c oxidase observed in the parasite (Table 6) may be not sufficient to account for the oxidation of the amount of substrate presumed to be catabolized to CO_2 and water. Preliminary experiments in our laboratory and the data published by Bueding (1949) have shown, however, that the respiratory rate of intact worms greatly exceeded that obtained for mitochondrial NADH oxidase (approximately 100 μatoms O/h/g wet wt versus approximately 10 μmol NADH h/g wet wt at 37 °C). The question to what extent the respiration activity of intact organisms is due to the action of the mitochondrial respiratory chain remains to be clarified. With the exception that a higher degree of complete substrate degradation was observed in the present study, the rates and proportions of glucose- and pyruvate-dependent product formation are in substantial agreement with the results reported previously by other authors (Bueding, 1949; Wang & Saz, 1974; Middleton & Saz, 1979). Primarily as a consequence of the low amounts of $^{14}CO_2$ liberated from [1-^{14}C] and [6-^{14}C]pyruvate (approximately 0·5 % of the amount of substrate removed by the worms), Saz (1981) assumed that the tricarboxylic acid cycle is not operative in *L. carinii* and that the aerobic requirement of this parasite may reside solely in the respiration-linked decarboxylation of pyruvate to yield acetate and acetoin, respectively.

An approximate calculation of the possible recovery of chemical energy as glucose is oxidized to the various products listed in Table 1 will show the quantitative importance of complete substrate oxidation, even if this process constitutes only a small percentage of the overall catabolic activity. For these calculations the following assumptions have been made: (1) fermentation of 1 mol of glucose to lactate yields a net production of 2 mol of ATP; (2) provided that the reducing equivalents arising from the oxidative decarboxylation steps involved in acetate and acetoin formation, respectively, enter into a conventional type of mitochondrial respiratory chain, the breakdown of 1 mol of glucose to these products will give 8 mol of ATP; (3) on the assumption that complete substrate degradation in *L. carinii* can be coupled to energy generation in the same way as in free-living aerobic organisms, for each mol of glucose undergoing complete oxidation a maximum of 38 mol of ATP can be generated. On the basis of these considerations the actual amounts of ATP likely to be synthesized in the filarial

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

worm under the experimental conditions described in Table 1 are presented in Table 8. If the assumptions made above are correct, then aerobic glucose utilization in *L. carinii* would result in an approximately 2-fold increase in the rate of ATP generation over that obtained in the absence of oxygen. Our calculations also show that the relatively small percentage of substrate flow presumed to be coupled to oxidative phosphorylation can make a substantial contribution to the overall energy budget of the parasite. This additional energy obtained in the presence of oxygen may be vital for the maintenance of motility and for survival of these organisms.

This work was supported by the Swiss National Science Foundation (Grant No. 3.429-078). The authors would like to thank Professor J. Eckert and Dr E. Peterhans for critical reading and Miss M. Bloch for typing the manuscript. Also, generous support of experimental animals by Hoffmann-LaRoche Ltd, Basle is gratefully acknowledged.

REFERENCES

Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1974). D-Glucose. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1196–1201. New York and London: Verlag Chemie Weinheim and Academic Press.

Bergmeyer, H. U. & Möllering, H. (1974). Acetate. Determination with preceding indicator reaction. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1520–8. New York and London: Verlag Chemie Weinheim and Academic Press.

Beutler, H.-O. & Michal, G.(1977). Neue Methoden zur enzymatischen Bestimmung von Aethanol in Lebensmitteln. *Fresenius' Zeitschrift für analytische Chemie* **284**, 113–17.

Bücher, T., Luh, W. & Pette, D. (1966). Einfache und zusammengesetzte optische Tests mit Pyridinnucleotiden. In *Hoppe-Seyler/Thierfelder. Handbuch der physiologisch- und pathologisch-chemischen Analyse*, vol. 6A (ed. K. Lang, E. Lehnartz, O. Hoffmann-Ostenhoff & G. Siebert), pp. 292–323. Berlin: Springer-Verlag.

Bueding, E. (1949). Studies on the metabolism of the filarial worm, *Litomosoides carinii*. *Journal of Experimental Medicine* **89**, 107–30.

Cha, S. & Parks, R. E. Jr. (1964*a*). Succinic thiokinase. I. Purification of the enzyme from pig heart. *Journal of Biological Chemistry* **239**, 1961–7.

Cha, S. & Parks, R. E. Jr. (1964*b*). Succinic thiokinase. II. Kinetic studies: initial velocity, product inhibition, and effect of arsenate. *Journal of Biological Chemistry* **239**, 1968–77.

Czok, R. & Lamprecht, W. (1974). Pyruvate, phosphoenolpyruvate and D-glycerate-2-phosphate. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1446–51. New York and London: Verlag Chemie Weinheim and Academic Press.

Delbrück, A., Zebe, E. & Bücher, T. (1959). Ueber Verteilungsmuster von Enzymen des energieliefernden Stoffwechsels im Flugmuskel, Sprungmuskel und Fettkörper von *Locusta migratoria* und ihre cytologische Zuordnung. *Biochemische Zeitschrift* **331**, 273–96.

Höpner, T. & Knappe, J. (1974). Formate. Determination with formate dehydrogenase. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1551–5. New York and London: Verlag Chemie Weinheim and Academic Press.

Keppler, D. & Decker, K. (1974). Glycogen. Determination with amyloglucosidase. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1127–31. New York and London: Verlag Chemie Weinheim and Academic Press.

Köhler, P. (1972). Vergleichende Untersuchungen der Glykolyse-Enzyme von *Dicrocoelium dendriticum* (Trematoda) und Rattenleber. *Zeitschrift für Parasitenkunde* **38**, 54–65.

Köhler, P. & Bachmann, R. (1978). The effects of the antiparasitic drugs levamisole, thiabendazole, praziquantel, and chloroquine on mitochondrial electron transport in muscle tissue from *Ascaris suum*. *Molecular Pharmacology* **14**, 155–63.

Köhler, P. & Bachmann, R. (1980). Mechanisms of respiration and phosphorylation in *Ascaris* muscle mitochondria. *Molecular and Biochemical Parasitology* **1**, 75–90.

Downloaded from https://www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268

KÖHLER, P. & HANSELMANN, K. (1973). Intermediary metabolism in *Dicrocoelium dendriticum* (Trematoda). *Comparative Biochemistry and Physiology* **45B**, 825–45.

LINN, T. C., PELLEY, J. W., PETTIT, F. H., HUCHO, F., RANDALL, D. D. & REED, L. J. (1972). α-Keto acid dehydrogenase complexes. XV. Purification and properties of the component enzymes of pyruvate dehydrogenase complexes from bovine kidney and heart. *Archives of Biochemistry and Biophysics* **148**, 327–42.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–75.

MICHAL, G., BEUTLER, H.-O., LANG, G. & GÜNTNER, U. (1976). Enzymatic determination of succinic acid in foodstuffs. *Fresenius' Zeitschrift für analytische Chemie* **279**, 137–8.

MIDDLETON, K. R. & SAZ, H. J. (1979). Comparative utilization of pyruvate by *Brugia pahangi*, *Dipetalonema viteae*, and *Litomosoides carinii*. *Journal of Parasitology* **65**, 1–7.

NOLL, F. (1974). L-(+)-Lactate. Determination with LDH, GPT and NAD. In *Methods of Enzymatic Analysis*, vol. 3 (ed. H. U. Bergmeyer), pp. 1475–9. New York and London: Verlag Chemie Weinheim and Academic Press.

RACKER, E. (1947). Spectrophotometric measurement of hexokinase and phosphohexokinase activities. *Journal of Biological Chemistry* **167**, 843–54.

SAZ, H. J. (1981). Biochemical aspects of filarial parasites. *Trends in Biochemical Sciences* **6**, 117–19.

SHAPIRO, T. A. & TALALAY, P. (1982). *Schistosoma mansoni*: mechanisms in regulation of glycolysis. *Experimental Parasitology* **54**, 379–90.

SLEIN, M. W., CORI, G. T. & CORI, C. F. (1950). A comparative study of hexokinase from yeast and animal tissue. *Journal of Biological Chemistry* **186**, 763–80.

SRIVASTAVA, V. M. L., CHATTERJEE, R. K., SEN, A. B., GHATAK, S. & KRISHNA MURTI, C. R. (1970). Glycolysis in *Litomosoides carinii*, the filarial parasite of the cotton rat. *Experimental Parasitology* **28**, 176–85.

VOGELL, W., BISHAI, F. R., BÜCHER, T., KLINGENBERG, M., PETTE, D. & ZEBE, E. (1959). Ueber strukturelle und enzymatische Muster in Muskeln von *Locusta migratoria*. *Biochemische Zeitschrift* **332**, 81–117.

WANG, E. J. & SAZ, H. J. (1974). Comparative biochemical studies of *Litomosoides carinii*, *Dipetalonema viteae*, and *Brugia pahangi* adults. *Journal of Parasitology* **60**, 316–21.

WESTERFELD, W. W. (1945). A colorimetric determination of blood acetoin. *Journal of Biological Chemistry* **161**, 495–502.

Printed in Great Britain

Downloaded from https:/www.cambridge.org/core. University of Basel Library, on 30 May 2017 at 21:07:54, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182000001268