Biochemical studies on cysticercus fasciolaris: demonstration of certain hydrolytic and oxidative enzymes and isoenzymes

Fombotioh Ndifor

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BIOCHEMICAL STUDIES ON CYSTICERCUS FASCIOLARIS:
DEMONSTRATION OF CERTAIN HYDROLYTIC AND OXIDATIVE ENZYMES
AND ISOENZYMES

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY
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DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA
DECEMBER 1983
DOCTOR OF PHILOSOPHY THESIS

OF

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ABSTRACT

BIOLOGY

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Biochemical Studies on Cysticercus fasciolaris: Demonstration of Certain Hydrolytic and Oxidative Enzymes and Isoenzymes.

Advisor: Dr. W. B. LeFlore

Doctor of Philosophy Degree conferred May 1984
Thesis dated December 1983

Biochemical studies were carried out on fresh whole worm extracts of Cysticercus fasciolaris in order to demonstrate certain enzyme systems. Acid phosphatase (AcP), alkaline phosphatase (AlP), acetylcholinesterase (AChE), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) were determined quantitatively and demonstrated spectrophotometrically. Similar studies were conducted on the sera of the host. All the enzymes studied were found to be present in sera of infected and noninfected rats; however, the infected animals showed significantly higher levels of CPK, LDH and MDH. Changes involving the activities of AcP, AlP and AChE were insignificant. Elevations in levels of certain specific enzymes in sera of infected animals were attributed to the presence of C. fasciolaris.

Polyacrylamide gel electrophoresis was used to study isoenzymes and protein bands in fresh whole worm extracts and liver tissue homogenates in infected and uninfected rats. Isoenzymes for 6-phosphogluconate dehydrogenase (6-PGDH), glucose-6-phosphate dehydrogenase (G-6-PDH), α-glycerophosphate dehydrogenase (α-GPDH), lactate dehydrogenase
(LDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), glutamate dehydrogenase (GLDH), \( \beta \)-hydroxybutyrate dehydrogenase (\( \beta \)-HBDH), NADH diaphorase (NADHD), and NADPH diaphorase (NADPHD) were demonstrated. Hydrolytic isoenzymes of non-specific esterases (NSE), acid phosphatase (AcP) and alkaline phosphatase (AlP) were also demonstrated. Alcohol dehydrogenase (ALDH) was not detected in worm extracts, but was present in liver tissues homogenates of both uninfected and infected rats. It is suggested that significant alcoholic fermentation does not occur in \textit{C. fasciolaris} since ALDH is not present. The presence of \( \alpha \)-GPDH and LDH suggests a typical glycolytic pathway. Krebs citric acid cycle is indicated because of the presence of 6-PGDH and G-6-PDH. The presence of NADHD and NADPHD in \textit{C. fasciolaris} suggests that the electron transport system operates in this organism.

There were some significant variations in the number and pattern of isoenzymes in the parasite and in the liver homogenates of infected and uninfected animals. Molecular variations were observed between enzymes of \textit{C. fasciolaris} and similar enzymes in the intermediate host. The knowledge derived from this research could possibly be useful in the diagnosis of human cysticercosis by an evaluation of the presence of isoenzymes in the sera of infected hosts.
ACKNOWLEDGEMENTS

I wish to express my thanks and appreciation to Dr. W. B. LeFlore for suggesting this problem and for the valuable advice and assistance that I have received throughout the course of this investigation. I would also wish to thank the members of my Guidance Committee (Dr. W. B. LeFlore, Chairman, Dr. C. L. Moore and Dr. C. L. Parker) for reading the manuscript and for their very helpful suggestions and directions. My thanks are also due to Dr. E. L. Stevenson who assisted with the electrophoretic techniques.
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CHAPTER I

INTRODUCTION

Isoenzymes are multiple molecular forms of an enzyme. They have been recognized for over 20 years as an important part of enzymology and as a major characteristic of biochemical organization of living things. The use of the term isoenzyme was first introduced in the literature by Markert and Moller (1959). These investigators suggested that organisms commonly synthesized enzymes in several molecular forms — that is, as two or more isoenzymes.

Isoenzymes of oxidative and hydrolytic enzymes have been observed in tissue extracts of several parasites. These include malate dehydrogenase in *Ascaris suum* (Rhodes et al., 1964); lactate and malate dehydrogenases of *Schistosoma mansoni* adults and cercariae (Conde-del Pino et al., 1966); acid phosphatase, non-specific esterases, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase and malate dehydrogenase in *Schistosoma mansoni* and *S. haematobium* (Coles, 1970). Waitz (1963b) demonstrated biochemically glyceraldehyde 3-phosphate dehydrogenase, aldolase, 6-phosphofructokinase, phosphoglucoisomerase, glucose-6-phosphate dehydrogenase, glucokinase, 6-phosphogluconate dehydrogenase, galactokinase, galactose uridy1 transferase, phosphoglucomutase, glycogen phosphorylase, lactate dehydrogenase, glutamate oxaloacetic transaminase and malate dehydrogenase activities in the larval and adult stages of *Hydatigera taeniaeformis*. These studies, however, only involved enzymes
of the parasites, and host enzymes were not determined. Burke et al. (1972) have demonstrated lactate dehydrogenase (LDH) in Hymenolepis diminuta. Isoenzymes of L(+) LDH have been reported in H. diminuta by Walkey and Fairbairn (1973) and Logan et al. (1977).

The present investigation was undertaken to study specific hydrolytic and oxidative enzymes in Cysticercus fasciolaris and the rat intermediate host. Cysticercus fasciolaris is the larval form of the cat tapeworm, Hydatigera taeniaeformis (=Taenia taeniaeformis), that develops in the liver of rats and mice. In the early stages of development in the intermediate host, the cysticercus is a bladder containing a single inverted scolex. Later the scolex evaginates, segments into a young tapeworm and retains the bladder at its terminal end. This type of larva is known as a strobiilocercus. Spectrophotometric techniques were employed for enzyme studies and the isoenzymes were separated by polyacrylamide gel electrophoresis. The enzymes of C. fasciolaris and those of the host were compared.

The following hydrolytic isoenzymes were determined in extracts of the parasites and liver homogenates of infected and uninfected rats; acid phosphatase (AcP), alkaline phosphatase (AlP), acetylcholinesterase (AChE) and non-specific esterases (NSE). In addition, oxidative isoenzymes of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGDH), glucose-6-phosphate dehydrogenase (G-6-PDH), α-glycerophosphate dehydrogenase (α-GPDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), glutamate dehydrogenase (GLDH), β-hydroxybutyrate dehydrogenase (β-HBDH), NADH diaphorase (NADHD) and NADPH diaphorase have also been demonstrated in the parasites and host tissues. No activity
was observed for alcohol dehydrogenase (ALDH) in extracts of *C. fasciolaris*, but the enzyme was demonstrated in the liver homogenates of infected and uninfected rats.
CHAPTER II
REVIEW OF THE LITERATURE

Enzymatic studies on parasitic helminths have been generally conducted through histochemical, cytochemical or biochemical techniques. Most of these studies have been concerned with trematodes and very few investigations have been performed on the enzyme systems of cestodes. Early investigators studied some common protozoa in order to develop chemotherapeutic agents against these organisms. Bayer and Wense (1936) biochemically determined acetylcholine in a species of free-living protozoan Paramecium. Bulbring and Burn (1949) studied acetylcholine in Trypanosoma rhodesiense and Plasmodium gallinaceum. They observed that T. rhodesiense synthesized acetylcholine but P. gallinaceum was unable to produce acetylcholine.

Bueding (1950) reported that in Schistosoma mansoni glycolysis proceeds at an exceedingly rapid rate. He indicated that this rapid process was very critical for the survival of the organism. Bueding and Peters (1951) and Bueding et al. (1953) suggested that the process of glycolysis is more critical for the survival of S. mansoni than is respiration. Mansour and Bueding (1953) compared the kinetics of lactate dehydrogenase of S. mansoni with those of lactate dehydrogenase of rabbit muscle. Differences were found in the pH optima and in some dissociation constants. These investigators indicated that such differences would be of importance for the development of chemotherapeutic agents which will selectively inhibit enzymes of the parasites without injury to host enzymes. The nutritional requirements of C. fasciolaris
seem to be totally dependent on the tissues of the host because glycoproteins of infected tissues are altered and the glycogen of the liver is depleted (Lewert and Lee, 1956).

Goldberg and Nolf (1954) demonstrated the presence of succinic dehydrogenase in adults of *Hymenolepis nana* by colorimetric methods. Hyneman and Voge (1960) used 2,3,5-triphenyl tetrazolium chloride (TTC) as the reducing dye for succinic dehydrogenase in *H. nana* and *H. diminuta*. It was found that these two cestodes contained succinate dehydrogenase. *Hymenolepis nana* produced more of the enzyme than *H. diminuta*. They concluded that metabolic differences exist between larval tapeworms in the insect hemocoel and the corresponding adult worms in the vertebrate hosts.

Rothman and Lee (1963) demonstrated isocitrate dehydrogenase, glutamate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and lactate dehydrogenase histochemically in the larval forms of *Hymenolepis diminuta*, *H. citelli*, *H. microstoma*, and *Taenia taeniaeformis*. Enzyme activities were observed in the tegumental mitochondria of the parasites.

Lee et al. (1963) histochemically visualized nonspecific esterases and cholinesterase in *Hydatigera taeniaeformis*, *H. diminuta*, *H. citelli* and *H. microstoma*. Esterase activity was found in the tegument of all species studied. In *H. taeniaeformis*, the anterior part of the tegument of each proglottid in the mature and gravid regions stained intensely. Cholinesterase was also observed within the same region. In the various species of *Hymenolepis* studied this activity was much weaker than that observed in *H. taeniaeformis*. *H. citelli* and *H. microstoma* gave weaker results than *H. diminuta*. Nonspecific esterases were
localized in the excretory systems of all species studied.

Waitz (1963a) histochemically demonstrated the distribution of glycogen, lipid, mucostubstances and acid phosphatase in *Hydatigera taeniaeformis*. Later, Waitz and Schardein (1964) carried out histochemical studies on four cyclophyllidean cestodes and reported the presence of lipids, PAS-positive material, alkaline phosphatase, acid phosphatase, succinic dehydrogenase, β-glucuronidase, amylophosphorylase, transglucosidase and cytochrome oxidase in adults of *Hymenolepis nana*, *H. diminuta*, *Hydatigera taeniaeformis* and *Dipylidium caninum*. Differences were seen among the various species in the distribution of alkaline phosphatase, amylophosphorylase, lipids and polysaccharides. Meanwhile, the distribution of other substances was found to be similar in the species examined.

Hart (1967) demonstrated the nervous system of the intact tetrahyridium of *Mesocestoides corti* using the cholinesterase method. Ramisz (1967) localized cholinesterase in the nervous system of *Dilepis undula* and *Choanotaenia unicoronata*. Wilson and Schiller (1969) described the anatomy of the nervous system in whole mounts of two species of *Hymenolepis* through cholinesterase localization, and Shield (1969) histochemically demonstrated the nervous systems of *Dipylidium caninum*, *Echinococcus granulosus* and *Hydatigera taeniaeformis*.

Cestode biochemistry is very complex. Although very little work has been done along these lines, it is well known that metabolic and glycolytic enzymes are present in homogenates of these parasites. Read (1951) demonstrated the presence of several enzymes in homogenates and acetone powders of *Hymenolepis diminuta*. He determined glucose-6-phosphate dehydrogenase, glucose-1-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, acid phosphatase and alkaline phos-
phatase using densitometric techniques. Read (1952) determined manometrically the cytochrome oxidase and succinate dehydrogenase in *H. diminuta* and Read (1953) demonstrated the presence of malate dehydrogenase, fumarase, L-glutamic dehydrogenase and α-glycerophosphoric acid dehydrogenase which was cytochrome linked. He suggested that the dehydrogenases were NAD-linked enzymes. He was unable to demonstrate ethanol, glucose, glucose-6-phosphate, formate, acetate, isocitrate, citrate or choline dehydrogenases.

Erasmus (1957a,b) conducted biochemical and histochemical studies on *Taenia pisiformis* and *Monieza expansa*. Acid and alkaline phosphatases were found in the tegument of the parasites, and only alkaline phosphatase was detected in the subtegumental cells and membranes bounding the ovary, vitelline tubules, and walls of the uterus. Agosin and Aravena (1959) demonstrated enzymes of the glycolytic pathway in the hydatid cyst of *Echinococcus granulosus*. Agosin and Aravena (1960) observed enzymes of the pentose phosphate pathway of *E. granulosus*.

Biochemical studies on the common cat tapeworm *Hydatigera taeniaeformis* have been few. Read (1961) and Read and Simmons (1963) determined carbohydrate metabolic enzymes in both larval and adult stages of the parasite. Waitz (1963b) performed various biochemical assays on extracts of fresh worm material to determine glyceraldehyde-3-phosphate dehydrogenase, aldolase, 6-phosphofructokinase, phosphoglucoisomerase, glucose-6-phosphate dehydrogenase, galactokinase, galactose uridyl transferase, phosphoglucomutase, glycogen phosphorylase, lactate dehydrogenase, and glutamate-oxalacate transaminase. Malate dehydrogenase was found in larval extracts but not in adult extracts. Glutamate and aspartate dehydrogenases were not demonstrated in extracts of
both forms of C. fasciolaris.

Several investigators have shown through histochemical studies that parasites utilize exogenous supplies of purine and pyrimidine bases for the synthesis of nucleic acids or other cell components (Brown, 1953; Bolton and Reynard, 1954; Cowie and Bolton, 1957; Henderson, 1962). The mode of passage of these compounds appears to vary with the organism studied. Simple diffusion may be the only entry process involved for a given compound in some organisms whereas mediated processes may be partially or wholly responsible for the transport of the same compound in other cases.

MacInnis et al (1965) carried out various experiments on the uptake of purine and pyrimidine bases by the rat tapeworm Hymenolepis diminuta. It was suggested that H. diminuta possesses at least one locus for the mediated transport of purines and pyrimidines. Hypoxanthine showed a great affinity for the locus evidenced by its rate of uptake and activity as an inhibitor. Uracil and adenine had a mediated transport system at low concentrations. Thymine and cytosine apparently entered mainly by diffusion. There were alternating inhibitions of purines and pyrimidines. The presence of thymine stimulated uracil transport. The purine-pyrimidine locus appeared distinct from the loci for sugar and amino acid transport. Thus, results obtained suggest that although tapeworms may show evidence of loss of functions present in many other organisms, they have retained specified membrane systems involved with nutrition and concerned with differential absorption of sugars, amino acids, purines and pyrimidines and nucleosides.

Jennings and LeFlore (1972) applied histochemical methods for nonspecific esterases, cholinesterases and acid phosphatase to cercariae
of Himasthla quissetensis and Zoogonus lasius. Reactions for non-specific esterases were observed in the nervous system, alimentary and excretory system and in cells in the acetabulum and mesenchyme. Cholinesterases were found throughout the nervous system and were selectively by $10^{-4}$ M eserine. Positive reactions for acid phosphatase occurred throughout the alimentary system, in parts of the excretory system and in scattered mesenchymal cells. The most recent detailed studies on the histochemical localization of esterases in Cysticercus fasciolaris were done by LeFlore and Smith (1976). Nonspecific esterases were demonstrated in the lateral margin of the proglottids, calcareous corpuscles, excretory canal, rostellum and suckers. The nervous system was shown to contain cholinesterase activity and a network of ganglia with a complex system of commissures in the scolex was demonstrated. LeFlore (1978) localized malate dehydrogenase, isocitrate dehydrogenase, $\alpha$-glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase in the tegument, tail, caudal pocket, excretory bladder, acetabulum and oral sucker on the cercariae of Plagiorchis elegans. Only moderate activity was obtained for lactic dehydrogenase and 6-phosphogluconate dehydrogenase at these sites. Glutamate dehydrogenase was localized only in the tail of the cercariae and tests for alcohol dehydrogenase were completely negative. It was suggested that the pentose phosphate pathway, glycolysis and Krebs citric acid cycle energy-producing sequences are available to these cercariae. LeFlore (1979) reported on the histochemical localization of hydrolytic enzymes in cercariae of Plagiorchis elegans. Reactions for alkaline and acid phosphatases occurred at the same sites in the tail, acetabulum, oral sucker, excretory bladder, and caudal pocket. No reactions
were obtained for β-glucuronidase and leucine aminopeptidase. Non-specific esterases and acetylcholinesterase reactions occurred throughout the nervous system of the body and tail and were inhibited by $10^{-3}$ M eserine. When the substrate concentration of acetylthiocholine iodide was increased, a superficial network of longitudinal and circular fibers was visualized. Localization with both enzymes revealed interior nerve trunks, sensory nerve endings, transverse commissures and highly reactive areas around the stylet and in the caudal pocket.

LeFlore et al. (1980) reported on the histochemical distribution of twelve dehydrogenases in the cercariae of Cloacitrema michiganensis. Strong to moderate activity was demonstrated at various sites in the cercariae for 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, lactate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, NADH diaphorase and NADPH diaphorase. There was no activity observed for alcohol dehydrogenase, glutamate dehydrogenase and β-hydroxybutyrate dehydrogenase. The results obtained indicate that the pentose phosphate shunt, glycolysis, Krebs cycle and the electron transport system operate in these parasites during their free-living existence.

Until the recognition of isoenzymes, the dominant view was that each enzyme existed as a single molecular form in any species and should be subject to purification and crystallization. That enzymes from a single organism might exist in multiple molecular forms was not acceptable in the biochemical community. Occasionally, scientists obtained results that suggested molecular heterogeneity of enzyme preparations
and even a few enzymologists suggested that this heterogeneity might be real. But these were minor voices among a large majority proclaiming that each enzyme was a single kind of molecule.

This popular view was not seriously challenged until Warburg and Christian (1943) showed that aldolase from yeast exhibited different properties than that derived from animal tissues. Later Warburg (1948) suggested that enzymes catalyzing the same reaction might be organ specific. Supporting this premise was the demonstration that lactate dehydrogenase (LDH) isolated from rat skeletal muscle differed in electrophoretic behavior from that extracted from rat myocardium.

Biochemists soon became more aware of the existence of molecular heterogeneity of enzymes as described by Smithies (1955). He was the first to use the zone electrophoretic techniques, coupled with histochemical staining procedures, to demonstrate multiple molecular forms of an enzyme. This zymogram technique quickly led to the recognition that organisms commonly synthesize their enzymes in multiple molecular forms and in patterns that are specific for each kind of cell at each stage of differentiation. Smithies (1955) demonstrated that proteins in blood serum could be sharply resolved by electrophoresis in starch gels.

Hunter and Markert (1957) coupled starch gel electrophoresis with histochemical staining methods to develop the zymogram technique. This sensitive, accurate and reliable technique initiated the vast amount of research that has now occurred in the field of isoenzymes. They first applied the zymogram technique to esterases. Markert and Hunter (1959) reported that the specific pattern of occurrence and
relative abundance of esterases was different in characteristic for each tissue.

The development of the generalized concept of isoenzymes occurred when Markert and Moller (1959) reported on the multiple forms of different enzymes with reference to their tissue ontogenetic, and species-specific patterns. At the focus of their research was the enzyme lactate dehydrogenase (LDH). Biochemical data have revealed that lactate dehydrogenase isolated from different tissues exhibited different enzymatic properties when analyzed by conventional enzyme techniques (Kaplan et al., 1956). The molecular basis for this diversity in enzymatic properties was not known. The general expectation was that lactate dehydrogenase was a single molecular species and that heterogeneity occasionally observed could be attributed to artifacts in the preparation and measurement of the enzyme.

A study of MDH isoenzymes in *Ascaris suum* was carried out by Rhodes et al. (1964). Multiple molecular forms of the enzymes were outlined. Burke et al. (1972) purified lactate dehydrogenase (LDH) in *Hymenolepis diminuta*. They reported that the enzyme is a tetramer composed of subunits of molecular weight 36,000. Only one isoenzyme of LDH was reported in *H. diminuta*. Walkey and Fairbairn (1973) have studied the biochemical properties of L(+) lactate dehydrogenase from infective eggs, cysticercoids, immature proglottids and pre-patent worms of *H. diminuta*. Two isoenzymes were demonstrated by kinetic analysis. However, the investigators were unable to visualize LDH isoenzymes in *H. diminuta* by electrophoresis. Logan et al. (1977) reported the existence of two isoenzymes of LDH in *H. diminuta* by starch-gel electrophoresis.

Conde-del Pino et al. (1966) separated isoenzymes from *Schistosoma*
**mansonii** by electrophoresis. Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were studied. Mammalian MDH$_1$ migrated towards the anode while MDH$_2$ was more cathodic. The first fraction of *S. mansonii* MDH$_a$ was found close to the origin. The second MDH$_b$ moved a shorter distance towards the cathode. The males and the cercariae contained a greater amount of MDH$_b$ than the female worms. Comparison of MDH isoenzymes from infected and uninfected animal tissues showed no significant difference. One band of LDH activity, corresponding in migration to that of human LDH$_4$ isoenzyme, migrated as a streak and not as a compact band. No differences were obtained between isoenzymes of tissue of normal and infected animals. It was reported that plasma of infected mice revealed a striking difference in their LDH isoenzyme content. In infected animals there was a higher increase in the LDH$_3$ isoenzyme fraction of the plasma. The total LDH activity was reported to show a 10-fold increase in the infected mice.

Conde-del Pino et al. (1968) demonstrated electrophoretically isoenzyme patterns of alkaline phosphatase, isocitrate dehydrogenase, glutamate oxalacetate transaminase, and glucose-6-phosphate dehydrogenase in the homogenates of adults and cercariae of *Schistosoma mansonii*. Two bands of alkaline phosphatase activity were found. Enzyme activity was not observed in snail tissues. Isocitrate dehydrogenase activity was not demonstrated in adults and cercariae as well as in snail tissues. Cercaria and adult extracts yielded three bands of glutamate oxalacetic activity. Two of the bands were anodic and one was cathodic. Two bands of activity were observed in homogenates of mouse liver, one anodic and one cathodic. One band of glucose-6-phos-
Phosphate dehydrogenase activity was observed in cercariae tissues.

Coles (1969) observed nine dehydrogenases in the tissue extracts of *Schistosoma haematobium* and homogenates of *Biomphalaria sudanica*. Isoenzymes of lactate dehydrogenase, malate dehydrogenase, β-hydroxybutyrate dehydrogenase, α-glycerophosphate dehydrogenase, glutamate dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase and alanine dehydrogenase were reported. Few changes were detected in the isoenzyme patterns of snail homogenates. One new glucose-6-phosphate dehydrogenase isoenzyme was observed in the homogenate of the infected snail. Coles (1969) was unable to demonstrate isocitrate dehydrogenase isoenzymes in the extracts of *S. haematobium*, but the enzyme was observed in *Biomphalaria sudanica*. Coles (1970) compared isoenzymes of *S. mansoni* and *S. haematobium*. Differences between sexes were observed for esterase, lactate, malate and glucose-6-phosphate dehydrogenase isoenzymes in *S. mansoni* whereas in *S. haematobium* there were differences between esterase and lactate dehydrogenase. Clear quantitative differences between species were observed with esterase, lactate and glucose-6-phosphate dehydrogenase isoenzymes.
CHAPTER III

MATERIALS AND METHODS

Maintenance of Hydatigera taeniaeformis. -- Domestic cats 6-8 months old were infected orally with gelatin capsules which contained at least 10 mature living strobilocerci. Within 70-90 days the feces were examined for the presence of gravid proglottids. Segments were collected and macerated in a Teflon homogenizer in order to release viable onchospheres which were used to infect rats.

Infection and bleeding of animals. -- Male and female Long Evans rats 4-8 weeks old were infected with eggs of Cysticercus fasciolaris through a stomach tube attached to a 3cc syringe (LeFlore and Smith, 1976). Each rat received about 1,000 eggs contained in a 0.5cc suspension of 0.85% physiological saline.

Blood from infected and uninfected rats was drawn either by cardiac puncture or by decapitation. The blood was collected in test tubes and was allowed to stand at room temperature for 30 min. The clotted blood was ringed and centrifuged at 2600 rpm for 30 min at 4°C. Sera from infected and uninfected animals were immediately used for biochemical experiments.

Removal of Cysticerci. -- Rats were killed at intervals of 3-6 months postinfection. The cysticerci were dissected intact from infected livers, washed in cold 0.85% physiological saline, and rinsed in petri dishes which contained 0.85% physiological saline. Strobilocerci were liberated by gently rupturing the cyst walls with a pair of fine scissors or forceps.
Protein content of extracts and homogenates. — Protein was determined on extracts of *C. fasciolaris* and liver tissue homogenates according to the methods described by Layne (1957). The parasites were weighed and transferred to a Teflon homogenizer to which 0.85% physiological saline was added in the ratio of 1 mg/ml. The homogenizer containing the parasites and saline was placed in an ice bath and the contents were homogenized in the cold until no microscopic cells were seen. The homogenate was centrifuged for 1 h at 6000 rpm (4500g) in a Beckman L5-65 ultracentrifuge at 4°C and the clear supernatant solution was used for protein and enzyme assays. A Gilford 240 spectrophotometer was used to determine the amount of protein in the supernatant solution. Reading of the supernatant solution was done at 280 nm and 260 nm in the ultraviolet (UV) wavelength. The protein was quantitated using the formula of Warburg and Christian (1941), which is as follows: 

\[ 1.55 \frac{D_{280}}{D_{260}} - 0.76 \]

*D<sub>280</sub>* is the optical density (OD) at 280 nm and proteins are absorbed at this wavelength. *D<sub>260</sub>* is the OD reading at 260 nm for nucleic acids; 1.55 and 0.76 were statistically derived by Warburg and Christian (1941). All spectrophotometric readings were made with extracts versus 0.85% physiological saline as reference. In order to calculate the protein content of *C. fasciolaris* or liver tissue homogenates, 3 ml of the supernatant were placed in a cuvette; two readings were made at 280 nm and 260 nm. Calculations were done using the above formula.

Calculations of protein in *C. fasciolaris*: mg/ml
Calculations of protein content in the serum of uninfected rat:

\[
\begin{align*}
&1.55D_{280} - 0.76D_{260} \\
&= 1.55(2.129) - 0.76(2.437) \\
&= 3.2999 - 1.8521 \\
&= 1.4478 \text{ mg/ml protein}
\end{align*}
\]

Calculations of protein content in the serum of infected rat:

\[
\begin{align*}
&1.55D_{280} - 0.76D_{260} \\
&= 1.55(2.2334) - 0.76(1.501) \\
&= 3.6177 - 1.14076 \\
&= 2.4769 \text{ mg/ml protein}
\end{align*}
\]

Calculation of protein in the liver tissue homogenate of uninfected rat:

\[
\begin{align*}
&1.55D_{280} - 0.76D_{260} \\
&= 1.55(2.938) - 0.76(2.544) \\
&= 4.5539 - 1.9334 \\
&= 2.6205 \text{ mg/ml protein}
\end{align*}
\]

Calculation of protein in the liver tissue homogenate of infected rat:

\[
\begin{align*}
&1.55D_{280} - 0.76D_{260} \\
&= 1.55(1.235) - 0.76(0.956) \\
&= 1.9142 - 0.7265 \\
&= 1.1877 \text{ mg/ml protein}
\end{align*}
\]
Biochemical determination of hydrolytic enzymes. -- Cholinesterase (AChE), acid phosphatase (AcP), alkaline phosphatase (AlP), and creatine phosphokinase (CPK) were determined on extracts of fresh worm material. The parasites were placed in cold 0.85% physiological saline in the ratio of 2 mg/ml and homogenized in a Teflon homogenizer placed in an ice bath. The homogenate was centrifuged at 6000 rpm (4500g) for 1 h at 4°C in a Beckman L5-65 ultracentrifuge. The clear supernatant was used for all enzyme studies. The above enzymes were also determined in the sera of uninfected and infected rats. The blood was centrifuged at 2,600 rpm (1,940g) for 30 min at 4°C. The reaction mixture for each of the assays consisted of the worm extract or the serum, the enzyme substrate, the co-enzyme, a suitable mineral (when required) and a buffer to make 3 ml, modified after Waitz (1963b). The OD readings were done on a Gilford 240 spectrophotometer. Two controls were set up for each assay. One contained all the constituents except the worm extract or the serum and in the second, the substrate was omitted.

Cholinesterase activity. -- The method used for determining AChE was essentially that of Biggs et al. (1958) and Rappaport et al. (1959). The clinical procedure for this experiment is also outlined in Sigma Technical Bulletin No. 420 (1975). The substrate for AChE was 15% acetylcholine chloride, with a m-nitrophenol as the indicator. Tubes containing the control tissue extracts of C. fasciolaris were heated in a hot water bath at 90°C for 10 min to inactivate the enzyme. Control sera were similarly heated at 60°C for 10 min. The color change produced during the reaction was read at 410 nm. The equation for the reaction is as follows:
Acetylcholine + H$_2$O $\xrightarrow{\text{Cholinesterase}}$ acetic acid + choline

The reaction was conducted in the presence of an acid-base indicator, m-nitrophenol. The acetic acid produced lowered the pH, and caused a loss in the color of m-nitrophenol. This color change was proportional to the AChE activity present in the solution and the optical density (OD) was immediately read at 410 nm using a Gilford 240 spectrophotometer. Three assays were conducted: AChE activity in C. fasciolaris, AChE activity in normal rat serum and AChE activity in infected rat serum. The OD readings obtained from each experiment were used for the calibration curve following methods described in Sigma Technical Bulletin No. 420, p. 5 (1975).

The amount of AChE present in the tissue extracts of C. fasciolaris or sera of uninfected and infected rats was calculated using the following formula:

$$\Delta A = A^{\text{Blank}} - A^{\text{Test}}$$

$A^{\text{Blank}}$ is the absorbance of the Blank tubes at a wavelength of 410 nm.

The Blank tube contained the following solutions:

- 2.0 ml sodium chloride, Stock No. 150-3
- 2.0 ml fresh extract of C. fasciolaris or 2.0 ml serum
- 3.0 ml water
- 2.0 ml Nitrophenol solution, Stock No. 420-2
- 2.0 ml Acetylcholine chloride solution

The content of the "Blank" tube was heated to inactivate the enzyme, cholinesterase.
AChE activity in the serum of uninfected rat. --- The spectrophotometric determination of serum AChE of healthy animals was similar to that already described except that the control serum was heated at 60°C for 10 min to inactivate the enzyme.

AcP and AlP. --- The methods that were used to determine the activity of these enzymes in C. fasciolaris and in the sera of healthy and infected rats are similar to those described by Ohmori (1937) and the procedures are outlined in Sigma Technical Bulletin No. 104 (1978). The substrate for AcP was 0.5 ml p-nitrophenyl phosphate and the buffer solution was 0.5 ml citrate (pH 4.8). The same substrate was used for AlP and 0.5 ml alkaline buffer solution (pH 10.5) was employed instead of citrate buffer. The volume of fresh tissue extract of the parasite used for each experiment was 0.2 ml. The sera of healthy and infected rats employed were 0.2 ml for each respective assay. The activities of AcP and AlP were measured following the methods of Sigma Technical Bulletin No. 104 (1978) in a Gilford 240 spectrophotometer. The equation for both reactions is similar:

\[
\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{\text{Phosphatase}} \text{p-nitrophenol} + H_3PO_4
\]

(colorless in acid or alkali) (colorless in acid; yellow in alkali)
Table 1. Spectrophotometric method for detecting AChE activity in *C. fasciolaris*.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Distilled H₂O (ml)</th>
<th>Acetic Acid (ml)</th>
<th>Absorbance</th>
<th>Δ A</th>
<th>Cholines¬terase Activity (Rappaport units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>3.2</td>
<td>0</td>
<td>0.826</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
<td>0.2</td>
<td>0.745</td>
<td>0.081</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>0.4</td>
<td>0.670</td>
<td>0.156</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>0.6</td>
<td>0.556</td>
<td>0.270</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>0.8</td>
<td>0.533</td>
<td>0.293</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>1.0</td>
<td>0.414</td>
<td>0.352</td>
<td>100</td>
</tr>
</tbody>
</table>

Calibration was done with 2.0 ml pooled extract containing 1.5 mg protein/ml mixed in 2.0 ml sodium chloride, 0.15 mol/liter. Extract was inactivated at 90°C for 10 min (Sigma Technical Bulletin No. 420).

Calculation: AChE activity in extracts of *C. fasciolaris*

\[ \Delta A = A_{\text{Blank}} - A_{\text{Test}} \]

\[ A_{\text{Blank}} 0.914 = A_{\text{Test}} 0.821 = 0.093 \text{ Absorbance} \]
Table 2. Methods for determining AChE in serum of uninfected rat.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Contents</th>
<th></th>
<th>Absorbance (A)**</th>
<th>ΔA</th>
<th>Serum Cholinesterase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distilled H₂O (ml)</td>
<td>Acetic Acid Sol.*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>3.2</td>
<td>0</td>
<td>0.923</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
<td>0.2</td>
<td>0.811</td>
<td>0.112</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>0.4</td>
<td>0.688</td>
<td>0.235</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>0.6</td>
<td>0.553</td>
<td>0.370</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>0.8</td>
<td>0.423</td>
<td>0.501</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>1.0</td>
<td>0.378</td>
<td>0.545</td>
<td>100</td>
</tr>
</tbody>
</table>

*Stock #420-102

**Wavelength = 410 nm

Column 1 contains a Blank test tube and 5 test tubes of pooled serum. Columns 2 and 3 show the solutions used in each test tube. When the solutions were mixed, color formed. In Column 4 the absorbance of each tube is shown. Subtraction of (A) of each numbered tube from (A) of the Blank, resulted in ΔA found in Column 5. Column 6 contains values of cholinesterase activity.

Calculations (serum AChE of uninfected rat):

$$\Delta A = A_{Blank} - A_{Test}$$

Blank 1.500 - Test 1.235 = 0.265 Absorbance
### Table 3. Method for determining AChE activity in serum of infected rat.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Contents</th>
<th>Distilled H₂O (ml)</th>
<th>Acetic Acid Sol.*</th>
<th>Absorbance (A)**</th>
<th>Δ A</th>
<th>Serum Cholinesterase Activity (Rappaport Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>3.2</td>
<td>0</td>
<td>0.924</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>3.0</td>
<td>0.2</td>
<td>0.821</td>
<td>0.103</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2.8</td>
<td>0.4</td>
<td>0.688</td>
<td>0.236</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.6</td>
<td>0.6</td>
<td>0.554</td>
<td>0.370</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.4</td>
<td>0.8</td>
<td>0.423</td>
<td>0.501</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2.2</td>
<td>1.0</td>
<td>0.379</td>
<td>0.545</td>
<td>100</td>
</tr>
</tbody>
</table>

*Stock #420-102

**Wavelength = 410 nm

Columns 1, 2 and 3 contain solutions similar to those in the calibration data of uninfected rat previously discussed, except that pooled serum of infected rat was used in the above experiment.

Calculations: (Serum AChE of infected animal)

\[
\Delta A = A_{\text{Blank}} - A_{\text{Test}}
\]

\[
A = 1.509 - 1.235 = 0.274 \text{ Absorbance}
\]
In the above equation the measurement of phosphatase activity by optical density was based on the ability of the enzyme under alkaline or acid conditions to hydrolyze p-nitrophenyl phosphate. The cleaved product, p-nitrophenol, is yellow in alkaline solution and its color intensity measured at 410 nm is proportional to the phosphatase activity. Drops of concentrated hydrochloric acid (HCl) were used to eliminate the yellow color produced by the reaction of p-nitrophenol (Tables 4-6).

**Calculation of AcP activity in C. fasciolaris.** -- The test reagent consisted of 0.5 ml phosphatase substrate solution, Stock No. 104-40 (Sigma Technical Bulletin No. 104, 1978), 0.5 ml citrate buffer and 0.2 ml of the worm extract. The control reagent consisted of 6.0 ml 0.1 NaOH and 0.2 ml of the extract. Yellow color was produced within 30 min of the reaction.

\[
\text{p-nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{Phosphatase}} \text{p-nitrophenol} + \text{H}_3\text{PO}_4
\]

The absorbance (A) of the test reagent at 410 nm was 0.275.

\[ A_{410} \text{ for control reagent (Blank) was 0.046.} \]

Total AcP activity = \[ A_{410} \text{ Test} - A_{410} \text{ Control Blank} \]

\[ 0.275 - 0.046 = 0.229 \text{ Sigma Units} \]

**Calculation of AcP activity in serum of uninfected animal.** -- The reaction mixture was similar to the above but uninfected serum was used in place of the extract. Yellow color was produced within a period of 30 min.

\[ A_{410} \text{ for the Test reagent was 0.801} \]

\[ A_{410} \text{ for the uninfected serum blank was 0.402} \]

Total acid phosphatase activity = \[ 0.801 - 0.402 \]

\[ = 0.399 \text{ Sigma Units} \]
Calculation of AcP activity in serum of infected animal. - - The reaction mixture was the same as that already discussed except that serum of an infected animal was used. A yellow color was produced during the reaction of p-nitrophenyl phosphate to p-nitrophenol and \( \text{H}_3\text{PO}_4 \).

\[ A_{410} \text{ for the Test reagent was } 0.768 \]

\[ A_{410} \text{ for serum blank was } 0.381 \]

Total acid phosphatase activity:

\[ 0.768 - 0.381 = 0.387 \text{ Sigma Units} \]

CPK activity. - - The activity of CPK was measured according to techniques described by Oliver (1955), Rosalki (1967), and the procedures described in the Sigma Technical Bulletin No. 45-UV (1976). These methods are based on the increase in absorbance due to the reduction of \( \text{NADP}^+ \) which was measured using the ultraviolet spectrophotometer (Gilford 240). All measurements of optical density were made at 340 nm. The final reaction product includes the reduced form of NADPH which is significant in the estimation of CPK. The following reactions are involved in the process of CPK activity.

\[ \text{ADP} + \text{phosphocreatine} \xrightarrow{\text{CPK}} \text{ATP} + \text{creatinine} \]

\[ \text{ATP} + \text{glucose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{glucose-6-phosphate} \]

\[ \text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{Glucose-6-Phosphate Dehydrogenase}} 6\text{-phosphogluconate} + \text{NADPH} \]

The reduction of \( \text{NADP}^+ \) was proportional to the CPK activity.

This caused an increase in absorbance that was measured.

The reaction mixture for each assay consisted of the substrate (0.009 mol/liter) of phosphocreatine in 2.05 ml \( \text{H}_2\text{O} \) and 0.05 ml fresh
Table 4. AcP activity in *C. fasciolaris*. Removal of p-nitrophenol (yellow) from the test reagent.*

<table>
<thead>
<tr>
<th>HCl (ml)**</th>
<th>Absorbance***</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.135</td>
</tr>
<tr>
<td>0.2</td>
<td>0.085</td>
</tr>
<tr>
<td>0.3</td>
<td>0.039</td>
</tr>
<tr>
<td>0.4</td>
<td>0.020</td>
</tr>
<tr>
<td>0.5</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*Volume of reagent used for the test was 11.1 ml (10 ml NaOH, 0.05N; 0.5 ml alkaline buffer, pH 10.3; 0.5 ml p-nitrophenyl phosphate, and 0.1 ml extract of *C. fasciolaris*.

**Addition of concentrated HCl in the Test reagent eliminated the color that was produced in the reaction of p-nitrophenyl phosphate to p-nitrophenol and H$_3$PO$_4$. The absorbance gradually decreased in concomitance with the amount of HCl that was added.

The absorbance of the Test reagent was 0.275.

The absorbance of the Blank reagent was 0.035.

***Wave length = 410 nm
Table 5. AlP activity in serum of uninfected animal: Color removal from the reagent by the use of HCl.*

<table>
<thead>
<tr>
<th>Conc. HCl (ml)**</th>
<th>Absorbance***</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.220</td>
</tr>
<tr>
<td>0.2</td>
<td>0.190</td>
</tr>
<tr>
<td>0.3</td>
<td>0.168</td>
</tr>
<tr>
<td>0.4</td>
<td>0.106</td>
</tr>
<tr>
<td>0.5</td>
<td>0.095</td>
</tr>
</tbody>
</table>

*Volume of reagent used in the above test was 12 ml (10 ml NaOH, 0.05N; 0.1 ml uninfected serum; 0.5 ml alkaline buffer, pH 10.3, and 0.5 ml phosphatase substrate solution).

**The reaction mixture and the procedure were similar to that of C. fasciolaris, but 0.1 ml serum of uninfected rat was employed instead of the extract. Yellow color developed in the reaction after 15 min period following the addition of 10.0 ml 0.05N NaOH. The absorbance \((A_{410})\) was high at first. When concentrated HCl was added to the mixture the absorbance decreased. The decrease was proportional to the amount of HCl used.

The absorbance of the Test reagent was 0.315.

The absorbance of the Blank reagent was 0.065.

***Wavelength = 410 nm.

<table>
<thead>
<tr>
<th>Conc. HCl (ml)</th>
<th>Absorbance**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.232</td>
</tr>
<tr>
<td>0.2</td>
<td>0.187</td>
</tr>
<tr>
<td>0.3</td>
<td>0.166</td>
</tr>
<tr>
<td>0.4</td>
<td>0.108</td>
</tr>
<tr>
<td>0.5</td>
<td>0.087</td>
</tr>
</tbody>
</table>

*The reaction mixture contained all the constituents previously stated except that infected serum was used. The reaction of p-nitrophenyl phosphate and the serum was allowed to go on for a period of 15 min. After this period 10.0 ml of 0.05N NaOH was added. This caused the alkali to stop the reaction and a yellow color was developed. Both the test reagent and the blank reagent were read at a wavelength of 410 nm.

The absorbance of the test reagent was 0.289.

The absorbance of the blank reagent was 0.076.

**Wavelength = 410 nm.
tissue extract of C. fasciolaris or 0.05 ml of serum. There were
two controls for each assay. One control contained all the consti-
tuents except the worm extract or the serum, and in the second control
the substrate was omitted. Three experiments were performed as shown
in Tables 7-9.

Biochemical determination of oxidative enzymes. — LDH and MDH
were determined following procedures outlined in Sigma Technical Bulle-
tin No. 340-UV (1980). The method for determining LDH activity as
indicated in the bulletin was based on the spectrophotometric procedure
of Wroblewski and LaDue (1955) and techniques for MDH activity were
essentially those of Siegel and Bing (1956). In these procedures, the
activities of LDH and MDH were measured by monitoring the rate at which
the substrates, pyruvate and oxalacetate, were reduced to lactate and
malate, respectively. These reductions were coupled with the oxidation
of nicotinamide adenine dinucleotide, reduced form (NADH), which were
followed spectrophotometrically in terms of reduced absorbance (A) at
340 nm.

Equation for LDH:
\[
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \\
\text{(High A}_{340} \text{)} \quad \text{(Low A}_{340} \text{)}
\]

Equation for MDH:
\[
\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+ \\
\text{(High A}_{340} \text{)} \quad \text{(Low A}_{340} \text{)}
\]

Hence NADH has a higher absorbance at 340 nm than NAD\(^+\). The
reaction was measured in terms of the rate of decrease in absorbance
at the above mentioned wavelength. Controls were similar to those
previously described. Experiments were carried out in the tissue
Table 7. CPK activity in *C. fasciolaris*.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.530</td>
</tr>
<tr>
<td>6</td>
<td>0.534</td>
</tr>
<tr>
<td>7</td>
<td>0.536</td>
</tr>
<tr>
<td>8</td>
<td>0.538</td>
</tr>
<tr>
<td>9</td>
<td>0.541</td>
</tr>
<tr>
<td>10</td>
<td>0.543</td>
</tr>
</tbody>
</table>

The reaction mixture contained 0.009 mol/l of phosphocreatine solution, stock No. 45-1, 0.04 ml of the extract and 2.05 ml distilled H₂O. Activity of the enzyme was measured in terms of increase in absorbance due to the reduction reaction.

\[
\text{ADP + Phosphocreatine} \xrightarrow{\text{CPK}} \text{ATP + Creatine}
\]

\[
\text{ATP + Glucose} \xrightarrow{\text{Hexokinase}} \text{ADP + Glucose-6-Phosphate}
\]

\[
\text{G-6-P + NADP}^+ \xrightarrow{\text{Glucose-6-Phosphate Dehydrogenase}} \text{6-Phosphogluconate + NADPH}
\]

When NADP⁺ was reduced to NADPH the absorbance sharply increased and this increase in absorbance was proportional to CPK present in the tissue extract in the serum. Absorbance increased with increase in time.

Calculations of CPK activity in *C. fasciolaris*:

\[
\Delta A/5 \text{ min} = \text{Final A} - \text{Initial A. } 0.543 - 0.530 = 0.013
\]

\[
0.013 \times "F" \times \text{TCF (Sigma Technical Bulletin No. 45-UV, 1976)} = 0.013 \times 200 \times 1 = 2.6 \text{ Sigma Units/ml.}
\]

"F" = Factor = 200. TCF + Temperature Control Factor = 1
Table 8. CPK activity in serum of uninfected animal.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.270</td>
</tr>
<tr>
<td>6</td>
<td>2.715</td>
</tr>
<tr>
<td>7</td>
<td>2.750</td>
</tr>
<tr>
<td>8</td>
<td>2.763</td>
</tr>
<tr>
<td>9</td>
<td>2.782</td>
</tr>
<tr>
<td>10</td>
<td>2.810</td>
</tr>
</tbody>
</table>

Calculations:  \( \Delta A/5 \text{ min} = \text{Final A} - \text{Initial A} \)

\[
= \ 2.810 \ - \ 2.700 \ = \ 0.110
\]

Serum CPK (Sigma Units/ml) =  \( \Delta A/5 \text{ min} \times F \times TCF \)

\[
= \ 0.110 \times 200 \times 1
\]

\[
= \ 22 \text{ Sigma Units/ml}
\]

The reaction mixture was identical to the previous experiment except that 0.05 ml of uninfected serum was used instead of the extract. NADPH in the reaction mixture increased the absorbance as presented in the data above.
Table 9. CPK serum from infected animal.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.560</td>
</tr>
<tr>
<td>6</td>
<td>2.583</td>
</tr>
<tr>
<td>7</td>
<td>2.620</td>
</tr>
<tr>
<td>8</td>
<td>2.640</td>
</tr>
<tr>
<td>9</td>
<td>2.678</td>
</tr>
<tr>
<td>10</td>
<td>2.690</td>
</tr>
</tbody>
</table>

Calculations:  \[ \Delta A/5 \text{ min} = \text{Final A} - \text{Initial A} \]
\[ = 2.690 - 2.560 = 0.13 \]

Serum CPK (Sigma Units/ml) = \[ \Delta A/5 \text{ min} \times f \times \text{TCF} \]
\[ = 0.13 \times 200 \times 1 \]
\[ = 26 \text{ Sigma Units/ml} \]

The reacting constituents were similar to those already discussed except that 0.05 ml of serum from infected animal was used. There was increased absorbance as NADP was reduced to NADPH in the reaction.
extracts of *C. fasciolaris* and on the sera of uninfected and infected animals and are summarized in Tables 10-15.

**Tissue preparation for isoenzyme studies.** Liver tissues dissected from freshly killed animals were washed in several changes of physiological saline and suspended in saline, 1 gm/ml. The suspension was homogenized with a glass homogenizer placed in an ice bath. The homogenate was centrifuged at 2600 rpm for 30 min at 4°C. Two preparations, one from an infected animal and the other from an uninfected animal, were made.

*Cysticercus fasciolaris* parasites were obtained from the infected animal. The liberated strobilocerci were washed in physiological saline, homogenized and centrifuged at 2600 rpm for 30 min at 4°C. Total protein of homogenates was determined using techniques previously described. All extracts were diluted with saline to a concentration of 30 mg protein/ml (Conde-del Pino et al., 1968). The supernatant resulting from each of the three homogenates was placed in wells of graded acrylamide gel. High resolution electrophoresis was achieved following the techniques outlined in Ortec's Technical Bulletin (Ortec AN32A, 1973).

**Preparation of polyacrylamide gel for electrophoretic separation.** - Isoenzymes were separated by vertical flat-bed disc electrophoresis in a gradient pore polyacrylamide gel using an Ortec high voltage pulsing system. Gradient pore gels were prepared by layering of acrylamide solutions of different concentrations to form an electrophoretic matrix with discoid pores of various diameters.

The gel casting stand was placed in an upright position and a 59 mm layer of running gel was carefully poured in the casting compartment.
Table 10. Activity of LDH in C. fasciolaris.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.620</td>
</tr>
<tr>
<td>60</td>
<td>0.391</td>
</tr>
<tr>
<td>90</td>
<td>0.208</td>
</tr>
<tr>
<td>120</td>
<td>0.108</td>
</tr>
<tr>
<td>150</td>
<td>0.090</td>
</tr>
</tbody>
</table>

LDH activity (Unit/ml) = \( \Delta A/\text{min} \times 20,000 \times \text{TCF} \) (Temperature Correction Factor)

\[
\Delta A = \frac{0.108}{2} \times 20,000 \times 1 = 1080 \text{ Units/ml}
\]

The procedure for LDH determination was based on the decrease in the spectrophotometric absorption during the reaction of pyruvate to lactate. Reaction mixture included 0.1 ml extract (1.5 mg protein/ml).

\[
\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{Lactate + NAD}^+ \]

(High \( A_{340} \)) (Low \( A_{340} \))
Table 11. LDH activity in the serum of uninfected rat.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.488</td>
</tr>
<tr>
<td>60</td>
<td>0.257</td>
</tr>
<tr>
<td>90</td>
<td>0.247</td>
</tr>
<tr>
<td>120</td>
<td>0.201</td>
</tr>
<tr>
<td>150</td>
<td>0.120</td>
</tr>
</tbody>
</table>

LDH activity in serum of uninfected rat (Unit.ml).

\[
= \Delta A/\text{min} \times 20,000 \times \text{TCF}
\]

\[
= \frac{0.201}{2} \times 20,000 \times 1 = \underline{2010 \text{ Units/ml}}
\]

The reaction mixture contained 0.2 mg of stock No. 340-2, 2.85 ml potassium phosphate buffer, 0.05 ml serum and 0.1 ml pyruvate. Measurement of activity of LDH was by decreasing absorption due to the oxidation of NADH to NAD⁺.
Table 12. LDH activity in serum of infected rat.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.632</td>
</tr>
<tr>
<td>60</td>
<td>0.434</td>
</tr>
<tr>
<td>90</td>
<td>0.322</td>
</tr>
<tr>
<td>120</td>
<td>0.288</td>
</tr>
<tr>
<td>150</td>
<td>0.170</td>
</tr>
</tbody>
</table>

LDH activity = \( \Delta A/\text{min} \times 20,000 \times \text{TCF} \)

\[
= \frac{0.288}{2} \times 20,000 \times 1 = 2880 \text{ Units/ml}
\]

Reaction mixture was identical to that of uninfected rat.
Table 13. MDH activity in {C. fasciolaris}.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.336</td>
</tr>
<tr>
<td>60</td>
<td>0.186</td>
</tr>
<tr>
<td>90</td>
<td>0.124</td>
</tr>
<tr>
<td>120</td>
<td>0.104</td>
</tr>
<tr>
<td>150</td>
<td>0.094</td>
</tr>
<tr>
<td>180</td>
<td>0.094</td>
</tr>
</tbody>
</table>

$$\text{MDH activity (Units/ml)} = \Delta A/\text{min} \times 10,000 \times TCF$$

$$= \frac{0.104}{2} \times 10,000 \times 1$$

$$= 520 \text{ Units/ml}$$

Reaction mixture contained 0.2 mg of NADH Stock No. 340-2, 2.8 ml potassium phosphate buffer, 0.1 ml extract (1.5 mg protein/ml), and 0.1 ml oxalacetate solution.
Table 14. MDH activity in serum of uninfected rat.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.788</td>
</tr>
<tr>
<td>60</td>
<td>0.678</td>
</tr>
<tr>
<td>90</td>
<td>0.592</td>
</tr>
<tr>
<td>120</td>
<td>0.497</td>
</tr>
<tr>
<td>150</td>
<td>0.402</td>
</tr>
<tr>
<td>180</td>
<td>0.402</td>
</tr>
</tbody>
</table>

$$\text{MDH activity (Units/ml)} = \Delta A/\text{min} \times 10,000 \times \text{TCF}$$

$$= \frac{0.497 \times 10,000 \times 1}{2}$$

$$= 2485 \text{ Units/ml}$$

The reaction mixture contained NADH vial stock No. 340-2, 2.8 ml potassium phosphate buffer, 0.1 ml of serum and 0.1 ml oxalacetate solution.
Table 15. MDH activity in serum of infected rat.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance (340 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.970</td>
</tr>
<tr>
<td>60</td>
<td>0.791</td>
</tr>
<tr>
<td>90</td>
<td>0.662</td>
</tr>
<tr>
<td>120</td>
<td>0.622</td>
</tr>
<tr>
<td>150</td>
<td>0.601</td>
</tr>
<tr>
<td>180</td>
<td>0.601</td>
</tr>
</tbody>
</table>

MDH activity (Units/ml) = \( \Delta A/min \times 10,000 \times TCF \)

= \( \frac{0.622}{2} \times 10,000 \times 1 \)

= \(3110 \text{ Units/ml} \)

The constituents in the reaction mixture included 0.1 ml of infected serum. Other solutions were similar to those in the MDH reaction of uninfected rat. Absorbance decreased with increase in time.
The gel consisted of 8% acrylamide and 2.5% methylene bis-acrylamide. This first layer of gel had the smaller pores. Immediately, a second layer consisting of 9 mm intermediate pores was carefully layered over the first layer. The second layer consisted of 6% acrylamide and 2.5% methylene bis-acrylamide was cast. A twelve pronged well-former was inserted into this layer and allowed to polymerize for 20 min. The template was then removed and the wells rinsed with distilled water. The water was poured off and the twelve wells were carefully dried with absorbent paper. The citrate buffer employed in the gels was used for the separation of the isoenzymes of esterases and other isoenzymes.

Aliquots of extract (0.2 ml containing 3 mg of protein) were placed in each well using a microsyringe. Wells were capped with 8% gels and allowed to polymerize for 20 min and the gel cells were assembled for electrophoresis in an Ortec electrophoretic tank powered by the Model 4100 pulsed constant power supply (Ortec AN32A, 1973).

**Tank buffer for electrophoresis.** Tris-borate (pH 9, 0.65M) was used as the electrode buffer for the separation of hydrolytic iso-enzymes. The buffer was prepared with 62.88 g Tris and 8.74 g boric acid (H₃BO₃). Both compounds were diluted in distilled water to a final volume of 8 liters. Four liters were removed from the total volume and 0.1% bromophenol blue (tracking dye) was added to it. This colored buffer was used for the upper tank. The four liters of buffer without tracking dye were used for the lower tank. Oxidative isoenzymes were separated in 0.05 M Tris-glycine buffer, pH 8.4. Gels were electrophoresed for 20 min at 75 pulses per second (pps), 10 min at 100 pps, 15 min at 225 pps, and at 300 pps until the tracking dye migrated to about 2 mm from the bottom of the gels. The time required for electrophoresis...
phoretic separation of the isoenzymes was about 2 h. A current of 225 volts was applied for each separation. After electrophoresis, the cells were cooled in running tap water and the gels removed. They were then stained for hydrolytic and oxidative isoenzymes using the procedures described below.

Staining procedure for oxidative isoenzymes in gels. — Methods used were those of Lawrence et al. (1960), Conde-del Pino et al. (1966), and Pearse (1972). Substrates were calcium lactate or sodium-DL-lactate for lactate dehydrogenase (LDH), sodium-L-malate for malate dehydrogenase (MDH), disodium-DL-beta hydroxybutyric acid for beta-hydroxybutyrate dehydrogenase, (β-HBDH), sodium-L-glutarate for glutamate dehydrogenase (GLDH), sodium succinate for succinate dehydrogenase (SDH), barium-6-phosphogluconic acid for 6-phosphogluconate dehydrogenase (6-PGDH), disodium glucose-6-phosphate for glucose-6-phosphate dehydrogenase (G-6-PDH), disodium-DL-alpha-glycerophosphate for alpha-glycerophosphate dehydrogenase (α-GPDH), trisodium-DL-isocitrate for isocitrate dehydrogenase (ICDH), ethanol for alcohol dehydrogenase (ALDH), reduced beta-nicotinamide adenine dinucleotide phosphate for NADH diaphorase (NADHD), and reduced beta-nicotinamide adenine dinucleotide phosphate for NADPH diaphorase (NADPHD). Nicotinamide adenine dinucleotide (NAD) was used as the co-enzyme in the methods for LDH, MDH, ICDH, GLDH, α-GPDH, and β-HBDH, while beta-nicotinamide dinucleotide phosphate (NADP) was used for G-6-PDH and 6-PGDH. In all cases the tetrazolium salt used was Nitro-BT. Phenazine methosulfate and magnesium chloride were also used in all experiments. Following incubation, the gels were stored in 10% acetic acid at 4°C. Two controls were set up for each assay: One contained all the constituents
except the worm extract or liver homogenates or uninfected or infected rats, and in the second the substrate was omitted.

**Methods for staining hydrolytic isoenzymes in polyacrylamide gels.**

- The substrate for acid phosphatase (AcP) was 50 mg sodium alpha-naphthylphosphate. The reaction mixture consisted of 50 ml of acetate buffer (0.1 M, pH 5.0), 50 mg Fast Garnet GBC salt as a post-coupler, 5 drops of 10% magnesium chloride, 1 g sodium chloride, and 0.25% polyvinylpyrrolidone. The gels were incubated for 24 h at room temperature. The substrate for AlP was also 50 mg sodium alpha-naphthyl phosphate, but the salts used were 50 mg Fast Blue RR salt, and 5 drops of 10% aqueous solution of magnesium chloride. The constituents were incubated in 50 ml of Tris-buffer, (0.1 M, pH 8.8) at room temperature for 6 h.

Before staining for NSE, the gels were post-equilibrated in 50 ml of 0.04 M Tris-chloride buffer (pH 6.6) at 37°C for 5 min. The buffer was poured off and a solution of the substrate and complexing agent added to the slabs. The complexing agent contained 50 mg of Fast Blue RR salt in 50 ml of 0.04 M Tris-chloride buffer (pH 6.6). The substrate consisted of 2 ml of 1% alpha-naphthyl acetate (1 g/100 ml H2O from which 2 ml was obtained). Gels were incubated in the staining mixture at 37°C for 15 min with intermittent agitation. The reaction mixture was poured off and the reaction stopped with 50 ml of an acetic alcohol mix (20 ml of 95% ethyl alcohol and 80 ml of 10% aqueous glacial acetic acid). Storage and control techniques were identical to those already discussed for oxidative isoenzymes.

**Demonstration of various proteins in C. fasciolaris, uninfected rat liver and infected liver.** Fresh tissue extracts of the parasites and liver homogenates were prepared using methods previously described.
Electrophoretic separation of the proteins was identical to that of isoenzymes but the staining techniques were different. Coomassie blue solution which was made up of 45 ml of 0.2% aqueous Coomassie brilliant blue, 45 ml of absolute ethanol and 10 ml of glacial acetic acid was applied for staining of various proteins. Before staining, the slabs were equilibrated in 12% trichloroacetic acid for 30 min at room temperature. The trichloroacetic acid was poured off and the gels were rinsed 5 times with distilled water and then stained at 65°C for 15 min with agitation every 5 min.

The gels were destained in a solution which was made up of 25 ml absolute ethanol, 10 ml glacial acetic acid, and 60 ml of distilled H₂O for 20 min at 65°C. This solution was discarded and a fresh solution was used after 30 min. The gels were rinsed in several changes of 10% acetic acid. All controls and storage methods were similar to those described for isoenzymes.

Gels were photographed and schematic representation prepared according to the methods of Lawrence et al., (1960). Isoenzyme patterns for the various enzymes were identified following the methods of Conde-del Pino et al. (1966,1968). Quantitative scans of some of the isoenzyme patterns were made in a Gilford densitometer and in a Beckman DU 8 spectrophotometer.
CHAPTER IV

EXPERIMENTAL RESULTS

Development of Hydatigera taeniaeformis. -- Cats that were infected with living strobilocerci produced gravid proglottids 95 days postinfection. The cats served as the definitive hosts (Fig. 1) and adult tapeworms developed in their intestines without any serious harm to these animals. Meanwhile, rats that were infected with onchospheres developed Cysticercus fasciolaris in the livers within five weeks (Fig. 2). The cysts removed from the liver of the rat intermediate host contained strobilocerci that measured from 25 mm to a maximum of 42 mm long (Fig. 3). Some of the rats had only a few cysts, while others had several. No cysts were observed in control animals.

Determination of protein. -- The protein content in tissue extracts of C. fasciolaris was 1.4478 mg/ml. Protein content in the serum of uninfected rat was 2.4769 mg/ml and the protein of infected rat serum was 2.4692 mg/ml. The protein content in liver tissue homogenates of the uninfected rat was 2.6205 mg/ml while the protein of the infected liver homogenates was 1.1877 mg/ml.

Biochemical determination of hydrolytic enzymes. -- AChE activity in C. fasciolaris measured 20 Rappaport Units/ml (Fig. 4). The maximum absorbance was 0.10. The activity of AChE in the serum of uninfected rats was 40 Rappaport Units/ml, with the maximum absorption at 0.265 (Fig. 5). AChE of infected rats was also 49 Rappaport Units/ml, with an absorbance of 0.275 (Fig. 6).

Positive reactions were obtained for AcP, AlP and CPK in the tissue
Fig. 1. Life cycle of Hydatigera taeniaeformis (=Taenia taeniaeformis). The rat serves as the intermediate host in which Cysticercus fasciolaris develops. The life cycle of the organism is completed in the cat which serves as the definitive host.
Oncosphere is liberated in intestine, bores into blood vessels and is carried to the liver of the rat where it develops to a cysticercus.

When the rat is eaten the cysticerci are digested out, evaginate their heads and attach to intestinal wall.

Cysticercus with head invaginated.

Strobilocercus develops in cat intestine.

Gravid proglottids are passed in feces.

Gravid proglottid disintegrates, liberating eggs. Some proglottic rupture before being passed and thus eggs also may occur in feces.

EGG

the shell disintegrates short after eggs are liberated and the embryophore is freed.

Embryophore with onchosphere is ingested by rat.

Fig. 1. Life cycle of Hydatigera taeniaeformis (= Taenia taeniaeformis)
Fig. 2. Photograph of an infected rat liver. Onchospheres developed into *Cysticercus fasciolaris*. This infection was five months old.

Fig. 3. Photograph of strobilocerci.

\[
\begin{align*}
A &= 25 \text{ mm long} \\
B &= 32 \text{ mm long} \\
C &= 35 \text{ mm long} \\
D &= 42 \text{ mm long}
\end{align*}
\]
Fig. 4. Graph of acetylcholinesterase activity in *Cysticercus fasciolaris*.

Maximum absorbance = 0.10

AChE activity = 20 Rappaport Units/ml.
Fig. 4
Fig. 5. Graph of acetylcholinesterase activity in serum of uninfected rat.

Maximum absorbance = 0.265

AChE activity = 40 Rappaport Units/ml.
Fig. 5

\[ \lambda = 410 \text{ nm} \]

Absorbance vs. Rappaport Units/ml
Fig. 6. Graph of acetylcholinesterase activity in serum of the infected rat.

Maximum absorbance = 0.275

AChE activity = 49 Rappaport Units/ml.
Fig. 6

$\lambda = 410 \text{ nm}$

Absorbance

Rappaport Units/ml
extracts of *C. fasciolaris* and in the sera of uninfected and infected rats. The quantitative results of the experiments are summarized in Table 16. CPK of the infected rat serum increased by 4 Sigma Units/ml. There were no reactions in tissue extracts that were heated at 90°C for 10 min. Reactions were also absent in sera that were heated at 60°C for 10 min. Negative reactions were obtained in experiments that were without the substrates.

**Biochemical determination of oxidative enzymes.** There were positive reactions for LDH and MDH in the sera of uninfected animals and infected animals. LDH and MDH were also found in *C. fasciolaris*.

The optical density (OD) of LDH in the serum of the uninfected rat reached a maximum of 0.480 during the first 30 sec of reduction of pyruvate to lactate (Fig. 7). The lowest OD reading was observed at about 0.150 during a period of 150 sec of reaction. The OD reading for LDH of infected rat serum peaked at about 0.630 within the first 30 sec of reduction of pyruvate to lactate (Fig. 8). LDH in *C. fasciolaris* showed a maximum absorbance of 0.590 within 30 sec of reaction (Fig. 9). The OD of MDH in the serum of the uninfected rat was 0.790, as shown in the histogram (Fig. 10), while infected rat serum measured 0.980 (Fig. 11) within the first 30 sec of reduction of oxalacetate to malate. The OD reading of MDH reaction in *C. fasciolaris* was 0.350 (Fig. 12). This was the smallest reading obtained. The activity of these enzymes is presented in Table 16. LDH in the serum of infected rats increased by 870 Units/ml. MDH of the infected rat increased by 625 Units/ml. The qualitative results of hydrolytic and oxidative enzymes are summarized in Table 17. The control experiments showed negative reactions.
Table 16. Summary of quantitative enzyme activity in C. fasciolaris and sera of infected and uninfected rats

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Cholinesterase</th>
<th>Acid Phosphatase</th>
<th>Alkaline Phosphatase</th>
<th>Creatine Phosphokinase</th>
<th>Lactate Dehydrogenase</th>
<th>Malate Dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysticercus fasciolaris</td>
<td>20</td>
<td>0.229</td>
<td>3.0</td>
<td>2.60</td>
<td>1080</td>
<td>520</td>
</tr>
<tr>
<td>Uninfected rat serum</td>
<td>49</td>
<td>0.399</td>
<td>3.2</td>
<td>22.0</td>
<td>2010</td>
<td>2485</td>
</tr>
<tr>
<td>Infected rat serum</td>
<td>49</td>
<td>0.387</td>
<td>3.2</td>
<td>26.0</td>
<td>2880</td>
<td>3110</td>
</tr>
</tbody>
</table>

*Enzyme units of measurement: Cholinesterase - Rappaport Units/ml; Lactate Dehydrogenase and Malate Dehydrogenase - Units/ml; Acid Phosphatase, Alkaline Phosphatase, and Creatine Phosphokinase - Sigma Units/ml.

There was no significant increase or decrease in the activity of Cholinesterase or Acid and Alkaline Phosphatases. Creatine Phosphokinase of the infected rat serum increased by 4 Sigma Units/ml; Lactate Dehydrogenase of infected rat serum showed an increase of 870 Units/ml; Malate Dehydrogenase also increased by 625 Units/ml.
Fig. 7. Histogram of LDH in the serum of uninfected rat. Pyruvate was reduced to lactate. The reduction was coupled with oxidation of NADH reduced form. Maximum absorbance was 0.480 during a period of 30 sec.

Fig. 8. Histogram of LDH in serum of infected rat. The reduction of pyruvate to lactate is shown. Maximum absorbance was over 0.600 in 30 sec of the reaction.

Fig. 9. Histogram of LDH in fresh tissue extracts of Cysticercus fasciolaris. Pyruvate was reduced to lactate. Maximum absorbance was 0.590 within 30 sec of the reaction.
Fig. 7.

Fig. 8.

Fig. 9.
Fig. 10. Histogram of MDH in the serum of the uninfected rat. Oxalacetate was reduced to malate in the reaction with concomitant oxidation of NADH to NAD⁺. Maximum absorbance was 0.790 during the first 30 sec of the reaction.

Fig. 11. Histogram of MDH in the serum of infected rat. This is the reduction of oxalacetate to malate. The maximum absorbance was 0.980 within the first 30 sec.

Fig. 12. Histogram of MDH in the fresh tissue extracts of *Cysticercus fasciolaris*. The reduction of oxalacetate to malate in demonstrated in the histogram and the maximum absorbance was 0.350 in the first sec.
Fig. 10.

Fig. 11.

Fig. 12.
Table 17. Summary of enzymatic activity in *Cysticercus fasciolaris* and in both infected and uninfected rats.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th><em>C. fasciolaris</em> extract</th>
<th>Serum of uninfected rat</th>
<th>Serum of infected rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Creatine Phosphokinase</td>
<td>+</td>
<td>++</td>
<td>++ +</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>+</td>
<td>++</td>
<td>++ +</td>
</tr>
<tr>
<td>Malate Dehydrogenase</td>
<td>+</td>
<td>++</td>
<td>++ +</td>
</tr>
</tbody>
</table>

+ = positive; ++ = moderately positive; +++ = strongly positive
Description of oxidative isoenzymes. -- Electrophoresis of tissue extracts of *C. fasciolaris* and rat liver homogenates were performed and the isoenzymes were compared with each other. Five distinct LDH isoenzymes were separated in extracts of *C. fasciolaris* (Fig. 13). The heterotetramers are LDH-1, LDH-2 and LDH-5. The intermediate isoenzymes, LDH-3 and LDH-4, are homotetramers. Five LDH isoenzymes were demonstrated in the uninfected rat liver but only one LDH band was observed in the infected liver (Fig. 14). The LDH-1, LDH-2 and LDH-3 are heterotetramers, while LDH-4 and LDH-5 are homotetramers.

There were strong MDH reactions observed in extracts of *C. fasciolaris* (Fig. 15). The anodic bands, MDH-1 and MDH-2 are homotetramers, while MDH-3 and MDH-4 are heterotetramers. Three MDH isoenzymes were separated in the liver homogenate of the infected rat, while four MDH isoenzymes were separated in the uninfected liver homogenate (Fig. 16). The MDH-1 and MDH-4 of the uninfected liver are homotetramers. The MDH-3 isoenzyme is missing in the infected liver but MDH-3 appears as a strong anodic band in extracts of *C. fasciolaris*.

Three bands of β-HBDH isoenzymes were observed in tissue extracts of *C. fasciolaris* (Fig. 17). One strong anodic isoenzyme, β-HBDH-1, is localized near the original zone. The β-HBDH-2 and β-HBDH-3 are homotetramers. These isoenzymes are weak bands. The number of bands of β-HBDH isoenzymes found in the uninfected and infected liver homogenates was identical (Fig. 18). In both animals, β-HBDH-2 and β-HBDH-3 isoenzymes are localized near the cathodic region (front), while β-HBDH-2 and β-HBDH-3 isoenzymes of the parasite appear within the vicinity of the anode (origin). The heterotetramers are β-HBDH-1, β-HBDH-2 and β-HBDH-3 of the uninfected and the infected rats. In
Fig. 13. Diagrammatic representation of lactate dehydrogenase isoenzymes in C. fasciolaris. One large band appeared in the zone of origin (O). It was followed by three small bands and one band in the front (f). Thus, a total of five bands was localized. Bands 1, 2, 3, and 4 were moderately strong and the fifth band was very strong.

Fig. 14. Diagrammatic representation of lactate dehydrogenase isoenzymes of liver homogenates. A total of five bands was localized in the uninfected rat liver (UL). Three of the bands were found in the zone of origin, while two of the bands migrated toward the front. In the infected rat liver (IL), only one anodic band was demonstrated.

Fig. 15. Diagrammatic representation of malate dehydrogenase isoenzymes in C. fasciolaris. Three strong bands were localized near the original zone (O). One small band appeared to the front (f). Four major bands were observed.

Fig. 16. Diagram of malate dehydrogenase isoenzymes of liver homogenates of rats. Four bands as shown in the diagram were localized. Three bands were very close together and a third band was further to the front. Only three bands were separated from the infected liver homogenate.
both animals $\beta$-HBDH-3 isoenzyme is the strongest band.

Two heterotetrameric isoenzymes of GLDH were observed in extracts of C. fasciolaris (Fig. 19). The GLDH-1 isoenzyme is very strong and GLDH-2 is a weak isoenzyme. The two isoenzymes are anodic. The migration pattern of isoenzymes in the gel of uninfected liver and infected liver (Fig. 20) differ significantly from patterns of isoenzymes in the parasite. GLDH-1 isoenzyme is anodally located in the gel of C. fasciolaris but the same isoenzyme is more cathodic in infected liver homogenate. The GLDH-1 and GLDH-2 isoenzymes of the infected liver are heterotetramers. The GLDH-1, GLDH-2 and GLDH-3 of the uninfected liver are also heterotetrameric isoenzymes.

Two bands of SDH isoenzymes were observed in C. fasciolaris (Fig. 21). The isoenzymes are anodic in nature and the bands are moderately strong. The SDH-1 and SDH-2 isoenzymes are homotetramers. Three bands of SDH isoenzymes were observed in the infected and the uninfected liver homogenates (Fig. 22). The SDH-1 and SDH-2 isoenzymes are homotetramers but SDH-2 migrated to the cathode while SDH-2 of the parasite migrated to the anode. The SDH-3 isoenzyme was localized in the gel of uninfected and infected liver homogenates but not in tissue extracts of C. fasciolaris.

The strong bands of 6-PGDH isoenzymes were detected in tissue extracts of the parasites (Fig. 23). The homotetramers are 6-PGDH-1 and 6-PGDH-2. These two isoenzymes stain strongly and migrated to the anode. Three bands of 6-PGDH isoenzymes were localized in the uninfected liver as well as in the infected liver homogenates (Fig. 24). The homotetramers are 6-PGDH-1 and 6-PGDH-3. In both animals the
Fig. 17. Diagram of beta-hydroxybutyrate dehydrogenase iso¬
zymes in C. fasciolaris. Three bands were sepa¬
rated. One moderately strong band followed by a
small band and a third band that appeared very weak.

Fig. 18. Diagram of beta-hydroxybutyrate dehydrogenase iso¬
zymes in liver homogenates of rats. The uninfe¬
ted as well as the infected liver homogenates had
the same number of bands. One band was localized in
the "0" region; one small band was found in the
middle and a third band migrated to the front. Three
bands of isoenzymes were detected in each animal.
All of the bands were weak.

Fig. 19. Diagram of glutamate dehydrogenase isoenzymes in
C. fasciolaris. One thick band was localized in the
original zone (0), and a light band was found in
front of the strong band within the same vicinity.

Fig. 20. Diagram of glutamate dehydrogenase isoenzymes in
liver homogenates of rats. In the uninfected liver,
one band was localized in the "0" region and the other
two appeared in the "f" region. In the infected liver
one band of GLDH isoenzyme was found in the "0" region
and a second band was localized in the front region.
Fig. 21. Diagram of succinate dehydrogenase isoenzymes in *Cysticercus fasciolaris*. Band number one lies in the original zone (0). The other band lies in the same vicinity. Both bands seem to be more anodic in nature. This was found in most extracts of the parasite.

Fig. 22. Diagram of succinate dehydrogenase isoenzymes in liver homogenates of rats. In the uninfected liver (UL), one band was localized in the "O" region; a second but light band migrated to the middle and a third band was seen in the "f" region. Right half of the gel shows a similar diagram of three bands of the same isoenzymes in the infected animal (IL). The bands were moderately strong.

Fig. 23. Diagram of 6-phosphogluconate dehydrogenase isoenzymes in *C. fasciolaris*. Two strong bands were found near the "O" zone.

Fig. 24. Diagram of 6-phosphogluconate dehydrogenase isoenzymes in uninfected and infected animals. The uninfected liver (UL) demonstrated three bands and similar isoenzymes are seen in the infected liver (IL). One big band was located in the "O" zone. One small but distinct band was visualized near the middle of the gel. Finally, a big band migrated to the "f" region. Each animal had three bands of isoenzymes.
anodally migrating 6-PGDH-1 is identical to the 6-PGDH-1 of *C. fasciolaris* which is also visualized in the vicinity of the anode. However, 6-PGDH-2 of host animals is not identical to that of the parasite. The cathodic 6-PGDH-3 isoenzyme observed in the gel of host rats is absent in the gel of *C. fasciolaris*.

Two bands of G-6-PDH isoenzymes were separated from extracts of *C. fasciolaris* (Fig. 25). The heterotetramers are G-6-PDH-1 and G-6-PDH-2. The G-6-PDH-1 isoenzyme is a weak band while G-6-PDH-2 is a very strong band. Three bands of isoenzymes are demonstrated in the gel of uninfected and infected liver homogenates (Fig. 26). Both liver homogenates have identical patterns of isoenzymes. The G-6-PDH-1 and G-6-PDH-2 isoenzymes are homotetramers, but G-6-PDH-3 isoenzyme is not present in the parasite. The G-6-PDH-2 isoenzyme of the host is very weak as compared to the strong G-6-PDH-2 isoenzyme of the parasite.

One isoenzyme of α-GPDH occurred in the extracts of *C. fasciolaris*. This faint band of isoenzyme migrated to the middle of the gel (Fig. 27). Three bands of isoenzymes were demonstrated in the uninfected and infected liver homogenates. The α-GPDH-1 occurred near the anodal zone; a second but weak band, α-GPDH-2 was observed in the middle and a third band, α-GPDH-3, migrated to the cathode (Fig. 28). Among these, α-GPDH-1 and α-GPDH-3 are homotetramers. The pattern and number of isoenzymes observed from liver extracts of host animals are not identical to the single isoenzyme visualized in the gel of *C. fasciolaris* extracts.

Strong bands of ICDH isoenzymes are demonstrated in the gel of *C. fasciolaris* (Fig. 29). The anodic ICDH-1 isoenzyme is moderately
Fig. 25. Diagram of glucose-6-phosphate dehydrogenase isoenzymes in *C. fasciolaris*. Two bands migrated from the "0" zone to the "f".

Fig. 26. Diagrammatic representation of glucose-6-phosphate dehydrogenase isoenzymes in uninfected and infected rats. Three pairs of bands of isoenzymes were observed in the uninfected and infected livers (UL and IL). The light bands appeared in the middle while moderately strong bands were observed on either zones.

Fig. 27. Diagram of alpha-glycerophosphate dehydrogenase isoenzyme in *C. fasciolaris*. One detectable band was observed almost midway between the "0" and "f" regions in gels containing extract of *C. fasciolaris*.

Fig. 28. Diagram of alpha-glycerophosphate dehydrogenase isoenzymes in uninfected and infected animals. In the uninfected and infected livers (UL and IL), similar number of bands are demonstrated. One band was localized in the "0" region of each gel; a second, but very light band was visualized in the middle, and a third band migrated to the front.
strong, whereas ICDH-2 and ICDH-3 isoenzymes are very strong and are homotetramers. The two moderately strong isoenzymes present in the gel of uninfected and infected liver homogenates are homotetramers (Fig. 30). The ICDH-2 isoenzyme of the parasite stained intensely but ICDH-2 isoenzyme of the infected host did not. A third isoenzyme, ICDH-3, visualized in the gel of C. fasciolaris was not localized in the gel of uninfected and infected hosts (Fig. 30).

Three bands of ALDH isoenzymes were observed in either the uninfected or the infected liver homogenates (Fig. 31). However, there was no ALDH isoenzyme observed in extracts of C. fasciolaris. The pattern and number of isoenzymes in host animals are similar in that ALDH-1 and ALDH-3 isoenzymes are homotetramers. The ALDH-2 isoenzyme is a faint band and appears in the middle of the gel.

Two strong bands of NADHD isoenzymes were observed in extracts of C. fasciolaris (Fig. 32). The isoenzymes are anodic. In the uninfected and infected rat livers, moderately strong bands of NADHD isoenzymes were observed (Fig. 33). The homotetrameric isoenzymes are NADH-1 and NADH-2.

_Cysticercus fasciolaris_ had two bands of NADPHD isoenzymes (Fig. 34). One big band was observed near the original zone and a second but smaller band migrated to the front. The two isoenzymes, NADPHD-1 and NADPHD-2 are heterotetramers. In the gel of uninfected and infected liver extracts, similar NADPHD isoenzymes are localized (Fig. 35). The NADPHD-1 and NADPHD-2 isoenzymes are homotetramers. The infected liver had two homotetrameric isoenzymes whereas the parasite had two heterotetrameric isoenzymes.
Fig. 29. Diagram of isocitrate dehydrogenase isoenzymes in C. fasciolaris. A total of three bands was demonstrated. A moderately strong band appeared in the "O" region and two strong bands migrated toward the "f" region.

Fig. 30. Diagram of isocitrate dehydrogenase isoenzymes in uninfected and infected livers. In each of the rat liver homogenates, two bands were localized: one near the "O" region and the other near the front. Right half of the gel shows homotetrameric isoenzymes as well as the left half of the gel.

Fig. 31. Diagram of alcohol dehydrogenase isoenzymes in uninfected and infected livers. Two large bands and one small band were localized in each of the rat liver homogenates.

Fig. 32. Diagram of NADHD isoenzymes in C. fasciolaris. Two bands were localized in the anodic region (O).
Fig. 33. Diagram of NADHD isoenzymes in liver homogenates of rats. One band of isoenzyme was localized in the "0" zone and a second band was found in the "f" region of the uninfected rat liver (UL). Right half of the gel presents the same isoenzymes in the infected rat liver (IL). One band was localized near the "0" region and a second band migrated to the front. All the bands were moderately strong.

Fig. 34. Diagram of NADPHD isoenzymes in C. fasciolaris. One large band appeared in the original zone and a small band was localized toward the front.

Fig. 35. Diagram of NADPHD isoenzymes in liver homogenates of rats. In either animals, one band of isoenzyme was found in the "0" region and a second band near the front. Right half of the gel shows similar isoenzymes in the "0" region and another band was detected near the front (f).

Fig. 36. Diagram of acid phosphatase isoenzymes in C. fasciolaris. One band appeared to be more anodic and was located near the zone of origin (0). The second band moved further to the front (f).
Description of hydrolytic isoenzymes. - - Positive reactions were obtained for AcP, AlP and NSE. In extracts of *C. fasciolaris*, two bands of AcP isoenzymes were observed (Fig. 36). The anodic isoenzyme is AcP-1 and the cathodic isoenzyme is AcP-2. These isoenzymes are weak and they are heterotetramers. There is significant difference in the pattern of AcP isoenzymes in the uninfected and infected liver extracts (Fig. 37). The AcP-1 and AcP-2 isoenzymes in host livers are homotetramers and are moderately strong. The AcP-1 and AcP-2 isoenzymes of the parasite are different from those of the infected host liver.

The reaction for AlP isoenzymes in *C. fasciolaris* was weak (Fig. 38). The AlP-1 isoenzyme is anodic and AlP-2 isoenzyme migrated to the middle of the gel, and are heterotetramers. In the uninfected and infected rats, three faint bands of AlP isoenzymes were observed (Fig. 39). In host animals the patterns of the isoenzymes are identical. The AlP-1 and AlP-2 isoenzymes are homotetramers. The AlP-3 isoenzyme observed in the liver extracts of host animals is missing in the extracts of *C. fasciolaris*. The AlP-1 isoenzyme of the host and AlP-1 isoenzyme of the parasite migrated to regions within the anode.

Three bands of NSE isoenzymes were observed in extracts of *C. fasciolaris* (Fig. 40). Two of the bands migrated to the front while NSE-1 isoenzyme remained in the anodic region. The homotetramers are NSE-2 and NSE-3 isoenzymes. Figure 41 shows that eight and ten bands of NSE isoenzymes were observed in liver extracts of the uninfected and the infected rats. In the uninfected liver, NSE-2 and NSE-3 isoenzymes are homotetramers while NSE-1, NSE-4, NSE-5,
Fig. 37. Diagram of acid phosphatase isoenzymes in rat livers.
Two bands were localized. One band migrated to the front but the other band remained in the "0" region. Similar bands were found in both the infected and uninfected animals (IL and UL).

Fig. 38. Diagram of alkaline phosphatase isoenzymes in C. fasciolaris. The diagram shows a cathodic streak and a small band toward the anode.

Fig. 39. Diagram of alkaline phosphatase isoenzymes in rat livers. Three bands were localized near the original zone. In both the infected and the uninfected animals, these isoenzymes were identical. There were apparently no change in their mobilities.

Fig. 40. Diagrammatic representation of non-specific esterase isoenzymes in C. fasciolaris. Three bands were localized. One large band was situated in the "0" region. Two small bands were found very close to each other and laid within the vicinity of the original zone.
NSE-6, NSE-7 and NSE-8 are heterotetramers. In the infected liver homogenate, NSE-2, NSE-3, NSE-4 and NSE-10 isoenzymes are homotetramers while NSE-1, NSE-5, NSE-6, NSE-7, NSE-8 and NSE-9 isoenzymes are heterotetramers. The NSE-1, NSE-2 and NSE-3 isoenzymes of the infected rat stained as strong bands, but the same isoenzymes in the parasite stained as weak bands.

Electrophoresis of protein and gel scan. — Figure 42 shows the protein bands of C. fasciolaris stained with Coomassie blue. The bands may be called isoenzymes. There are thirteen isoenzymes of C. fasciolaris extracts. Isoenzymes one, two, three, five, ten and thirteen are homotetramers and they stained intensely. Meanwhile, the intermediate isoenzymes, four through twelve, are heterotetramers. Eleven bands of isoenzymes were observed in the liver extracts of the uninfected rat while twelve bands of isoenzymes were observed in the infected liver extracts (Fig. 43). There were more protein isoenzymes localized in the parasite than in the host liver homogenates. The anodally migrating isoenzymes, one, two and three, of the parasite were localized within a region identical to that of one, two and three isoenzymes of the host. The thirteenth isoenzyme separated from extracts of C. fasciolaris is not observed in the infected liver homogenate of the rat.

Densitometric scan of the gel containing protein isoenzymes of C. fasciolaris produced thirteen peaks (Fig. 44). These peaks correspond to the thirteen protein isoenzymes of the parasite. The first peak appeared at a distance of 5mm on the anodal region of the gel and the OD reading is 0.150. The thirteenth peak appeared at a distance of
Fig. 41. Diagrammatic representation of non-specific esterase isoenzymes in uninfected and infected livers. The uninfected animal (UL) had three strong bands in the original zone (0) and one small band in the front (f). There were four smaller bands. A total of eight bands was detected. The infected animal (IL) had a total of ten bands. Three big bands were found in the "0" region, one big band in the middle, followed by other smaller bands in the front region (f).

Fig. 42. Diagram of electrophoretic separation of proteins in *C. fasciolaris*. A total of thirteen proteins was identified. The strongest bands were located near the anodic and cathodic regions (0 and f). Smaller bands appeared between these two regions (0 and f). The picture is presented above.

Fig. 43. Diagram of electrophoretic separation of proteins in uninfected and infected livers. In the uninfected liver homogenate (UL), one strong band was localized in the "0" zone and four other bands were found in this vicinity. Two bands were located in the middle and four bands were detected in the front. Eleven different bands were localized; however, twelve bands were demonstrated in the infected liver (IL). Photographs are shown above.
90mm from the anode at 0.250 OD. This is the cathodal region of the gel.

The scan of protein bands in the uninfected liver produced eleven fractions (Fig. 45). The first peak was recorded at a distance of 3mm in the region of the anode. This peak is the highest of all the eleven peaks recorded at an OD reading of 0.230. The eleventh peak appeared at 85mm away from the anode with an OD reading of about 0.110. Twelve peaks were observed in the densitometric scan of liver extracts of the infected rat (Fig. 46). Peak number three of the infected rat appeared at a distance of about 30mm while that of the uninfected liver appeared at a distance of only 15mm from the anode. Comparisons of the protein bands of host animals and those of *C. fasciolaris* show significant differences in the number and height of peaks observed.

The scan of LDH of *C. fasciolaris* extracts showed five peaks that correspond to five isoenzymes. The first peak is anodic and appeared at a distance of 10mm with the OD reading of 0.300. The intermediate peaks, two, three and four, are not as high as peak number one and peak number five. The fifth peak is cathodic and appears at a distance of 90mm from the origin at an OD reading of approximately 0.290.

Densitometric scan of MDH isoenzymes produced four peaks (Fig. 48). Peaks one, two, and three appeared in the anodic region. Peak number four is cathodic and appeared at a distance of 75mm from the anode. Peaks one and four are the highest with OD readings of about 0.250 and 0.240, respectively. The four peaks observed correspond to four MDH isoenzymes.
Fig. 44. Densitometric scan of protein bands of *C. fasciolaris*. The gel contained various protein bands of *C. fasciolaris* which were stained with Coomassie blue. A total of 13 protein fractions was detected. For all the densitometric scans performed, the anode or original zone (0) begins at a distance of 0 millimeter and the cathode or front (f) ends at 100 millimeters.
Fig. 44.
Fig. 45. Densitometric scan of protein bands of uninfected liver.
Protein fractions in the liver homogenate of the uninfected rat showed eleven peaks.

Fig. 46. Densitometric scan of protein bands of infected liver.
The gel contained protein fractions of the infected liver homogenate. Twelve protein bands were observed.
Fig. 47. Densitometric scan of lactate dehydrogenase isoenzymes in C. fasciolaris. Densitometric scan of the isoenzymes showed five peaks.

Fig. 48. Densitometric scan of malate dehydrogenase in C. fasciolaris. The gel scan demonstrated four fractions.
The gel scan of NSE isoenzyme of the uninfected liver extracts revealed eight peaks (Fig. 49). Peaks one, two, and three are anodic with OD readings of about 0.350, 0.570 and 0.480, respectively. Peaks four and five appear in the middle of the profile. Peaks, six, seven and eight are cathodic and the highest peak within the cathodic region is seven. The gel scan of the infected liver extracts produced ten peaks (Fig. 50). Peaks one, two and three are anodic and they are the highest peaks observed in this profile. The intermediate peaks are four, five and six, while the cathodic peaks are seven, eight, nine and ten. There are significant differences in these peaks when compared with those of the uninfected liver extracts.
Fig. 49. Scan of non-specific esterase isoenzymes of uninfected liver homogenate. In the original zone, one small peak appeared at a distance of less than ten millimeters. The eighth peak was observed at an OD reading of about 0.200 within a distance of approximately 87 millimeters.

Fig. 50. Scan of non-specific esterase isoenzymes of infected liver homogenate. The first peak was observed at an OD reading of about 0.320 at a distance of over ten millimeters. Meanwhile, the final peak to the front appeared at an OD reading of approximately 0.100 and at a distance of about 90 millimeters.
CHAPTER V

DISCUSSION

The results obtained in the biochemical studies suggest that the protein content of C. fasciolaris is higher than that of the infected liver extracts. These findings suggest that developing cysticerci obtain nutrients from host liver probably depleting it as they grow. The presence of AChE in C. fasciolaris was demonstrated biochemically. Cholinesterase has been localized histochemically in the nervous system of C. fasciolaris by LeFlore and Smith (1976), in the nervous systems of Dipylidium caninum and Echinococcus granulosus by Shield (1969), and in Hydatigera taeniaeformis by Lee et al. (1963). The AChE level of infected rat serum showed no significant increase or decrease when compared with the uninfected rat serum. Apparently AChE level of the host is unaffected by the presence of the parasite. It appears therefore that the enzyme cannot be used for the serum diagnosis of cysticercosis.

The activities of AcP and AlP were demonstrated biochemically in tissue extracts of C. fasciolaris and in the sera of infected and uninfected rats. These enzymes have been reported histochemically in Taenia pisiformis, Cysticercus tenuicollis and Monieza expansa by Erasmus (1957 a,b). The possible significance of AcP is associated with lysosomal activity while AlP may be correlated with energy transfer mechanism and carbohydrate metabolism. The concentration of AlP was higher than that for AcP in tissue extracts of C. fasciolaris.
Waitz (1963a) observed higher concentrations of AlP than AcP in *Hydatigera taeniaeformis*. Although AcP and AlP were observed in the sera of infected and uninfected rats, there were no significant differences in the concentrations of these enzymes in both animals.

The occurrence of CPK, LDH, and MDH was demonstrated biochemically in *C. fasciolaris* as well as in the uninfected and infected rat sera. However, there were significant increases in the levels of these enzymes in the sera of infected animals. The consistent increase in the levels of LDH, MDH and CPK in the serum of the infected rat as compared to that of the uninfected animal may possibly prove fruitful in the serum diagnosis of cysticercosis. This is perhaps the first report in which elevated levels of these enzymes in the sera of infected animals may be associated with the presence of cysticerci. This observation suggests that extensive tissue damage causes leakage of the enzymes into the blood stream. Conde-del Pino et al. (1966) observed a 10-fold elevated level of LDH activity in the plasma of mice infected with *Schistosoma mansoni* which was attributed to tissue damage.

As a result of the electrophoresis of LDH in tissue extracts of *C. fasciolaris*, five distinct isoenzymes were demonstrated which may be attributed to the presence of five gene loci encoding LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5 subunits. Burke et al. (1972) observed only one LDH isoenzyme in *H. diminuta*. Walkey and Fairbairn (1973) reported the presence of two LDH isoenzymes in *H. diminuta* and Logan et al. (1977) observed two distinct L(+) LDH isoenzymes in *H. diminuta*. The LDH isoenzymes in *C. fasciolaris* were difference in electrophoretic mobility from those of the host liver extracts. Conde-del Pino et al. (1966) made similar observations on mouse liver tissue LDH isoenzymes...
when compared to the same isoenzymes in S. mansoni. The presence of α-GPDH and LDH isoenzymes in C. fasciolaris suggests that a typical glycolytic sequence from glycogen to lactic acid may be available. Enzymes in the glycolytic pathway including α-GPDH have also been demonstrated in the hydatid cysts of *Echinococcus granulosus* by Agosín and Aravena (1959).

The presence of MDH, ICDH and SDH isoenzymes in tissue extracts of *C. fasciolaris* was demonstrated by electrophoresis. However, Rothman and Lee (1963) have localized histochemically SDH in the tegument of *C. fasciolaris*. The observation of MDH, ICDH and SDH isoenzymes may indicate that the Krebs citric acid cycle operates in this organism. Although these enzymes were present in liver extracts of host animals, the patterns of their isoenzymes were different.

The presence of 6-PGDH and G-6-PDH isoenzymes in *C. fasciolaris* may suggest that the pentose-phosphate shunt operates in this organism. One of the functions of the pentose-phosphate cycle is to convert hexose into pentose for the synthesis of nucleic acids. Although only two isoenzymes of 6-PCDH and two isoenzymes of G-6-PDH were demonstrated in tissue extracts of the parasite, three isoenzymes of each of these dehydrogenases were observed in liver extracts of infected and uninfected rats. Waitz (1963b) demonstrated the pentose phosphate shunt biochemically in the larval and adult forms of *Hydatigera taeniaformis*. The pentose-phosphate cycle has been suggested for the trematode *cerchariae* of *Plagiorchis elegans* (LeFlore 1978) and *Cloacitrema michiganensis* (LeFlore et al. 1980); and in *S. mansoni* and *S. haematobium* (Coles 1970).

Three bands of β-HBDH isoenzymes and two bands of GLDH isoenzymes were demonstrated in tissue extracts of *C. fasciolaris*. 
although LeFlore et al. (1980) were unable to localize these dehydrogenases histochemically in the cercariae of Cloacitrema michiganensis. Rothman and Lee (1963) localized histochemically GLDH in C. fasciolaris. Although ALDH was observed in the liver extracts of the infected and the uninfected rats, it was not demonstrated in tissue extracts of C. fasciolaris. The two NADHD isoenzymes and two NADPHD isoenzymes demonstrated in extracts of C. fasciolaris indicate that the electron transport system is present in this organism, and it further supports the possibility of the Krebs cycle.

The electrophoresis of the hydrolytic enzymes, AcP, AlP and NSE demonstrated distinct isoenzymes in tissue extracts of C. fasciolaris and in the liver extracts of infected and uninfected rats. The patterns of bands of AcP and AlP isoenzymes in the rat tissue homogenates were similar, but they were not identical to the isoenzymes of C. fasciolaris. The similarities observed in the phosphate isoenzymes of both infected and uninfected rats probably suggest that hosts AcP and AlP are not significantly altered during cysticercus infection.

There were eight bands of NSE isoenzymes in tissue extracts of C. fasciolaris. This enzyme has been demonstrated histochemically in the muscles of adult worms and in sections of the nervous system by Lee et al. (1963), and in C. fasciolaris by LeFlore and Smith (1976). Some of the significant functions of NSE are concerned with the hydrolysis of esters. There was an increase in the number of NSE isoenzymes in the infected liver extracts as compared to the uninfected liver extracts. This increase may have been due to the presence of the parasite.

The presence of thirteen protein bands in tissue extracts of C. fasciolaris suggests that this tapeworm is enzymatically complex.
These bands appear to be correlated with the various isoenzymes that have been demonstrated in the parasite. Moreover, this may also indicate that at least thirteen gene loci operate in *C. fasciolaris* which are probably responsible for encoding the isoenzymes. Distinct protein bands were also observed in the extracts of the uninfected and the infected liver extracts, although they were significantly different from those of the parasite. When the scans of the protein gel of *C. fasciolaris* were compared with those of infected and uninfected liver extracts, the peaks were numerically identical to the number of isoenzymes observed in electrophoresis. These observations were also true for the gel scans of LDH, MDH, and NSE of the parasite and the liver extracts of rat intermediate host.
CHAPTER VI

SUMMARY AND CONCLUSIONS

The occurrence of AcP, A1P, AChE, CPK, LDH, MDH, 6-PGDH, G-6-PDH, α-GPDH, ICDH, SDH, CLDH, β-HBDH, ALDH, NADHD, NADPHD, and NSE have been studied biochemically and electrophoretically from fresh tissue extracts of C. fasciolaris and in the sera and liver homogenates of normal and infected rats. There were some variations in the quantities of enzymes obtained from the sera of the normal and infected animals. LDH from the serum of the infected rat was 870 units/ml higher than that of the uninfected rat. MDH from the serum of the infected rat also increased by 625 units/ml when compared with that from the uninfected animal. CPK from the serum of the infected rats was 4 Sigma units/ml higher than that from the serum of the normal rat. It is suggested that the increase in the levels of these enzymes may be attributed to the presence of C. fasciolaris in the livers of infected animals. Increases in the level of specific enzymes in the infected rat are significant because this may lead to the understanding, treatment and control of cysticercosis. AcP, A1P, and AChE activities remained essentially unaltered. Thus it is probable that the presence of C. fasciolaris in the liver may not seriously affect the levels of these three enzymes in the blood of infected animals. The results obtained from isoenzyme studies indicate the presence of a glycolytic pathway in C. fasciolaris because strong reactions of LDH and α-GPDH were observed. In addition, enzymes associated with the Krebs cycle
(MDH, ICDH, and SDH) were also localized. The presence of G-6-PDH and 6-PGDH isoenzymes demonstrates the possibility of a functional pentose-phosphate shunt. The absence of ALDH implies that alcoholic fermentation may not occur in this organism or if it does, it functions at relatively low levels. Similar studies were conducted on the liver homogenates of normal and infected rats. All the above enzymes, including ALDH, were demonstrated in liver homogenates of the rats. Isoenzymes of NSE and protein fractions were studied in tissue extracts of C. fasciolaris and in the liver homogenates of infected and uninfected rats. Significant differences were observed in isoenzyme patterns of esterases from the infected and the uninfected liver homogenates. Quantitative and qualitative differences were observed in protein bands of the parasites and host tissue homogenates.

These studies suggest that similar enzymes obtained from C. fasciolaris and the rat intermediate host may exhibit molecular variation. In addition, C. fasciolaris is a complex organism consisting of hydrolytic and oxidative enzymes. It is worth noting that increases in the levels of LDH, MDH and CPK in the sera of the infected rats are significant because this may possibly provide a useful tool in the understanding and diagnosis of cysticercosis in man and animal.
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