A MAGNETIC RESONANCE STUDY OF LIVER METABOLITES FROM MICE INFECTED WITH THE LARVAL CESTODE *TAENIA CRASSICEPS*

By

IAN CORBIN

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the degree of

MASTER OF SCIENCE

Department of Human Anatomy and Cell Science
Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba

December, 1997

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

A MAGNETIC RESONANCE STUDY OF LIVER METABOLITES FROM MICE INFECTED WITH THE LARVAL CESTODE TAENIA CRASSICEPS

BY

IAN CORBIN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TABLE OF CONTENTS

ABSTRACT

Magnetic resonance spectroscopy (MRS) was employed to investigate alterations in carbohydrate and lipid metabolism in livers of mice infected with the cysticerci of *Taenia crassiceps*. Livers of control and infected mice were removed and their perchloric extracts prepared. Carbon-13 decoupled proton spin-echo MRS analysis revealed that two hours after intraduodenal injection of [3-^{13}C]alanine, liver extracts from both control and infected groups of mice contained ^{13}C label in glycogen, glucose, succinate, glutamate, alanine and lactate. Livers of infected mice had a lower percentage of ^{13}C in alanine and lactate than uninfected controls. In addition, infected mice had a lower concentration of total hepatic glucose and glutamate, but higher concentration of betaine, acylcarnitine and β-hydroxybutyrate. *T. crassiceps* larvae also possessed ^{13}C label in glucose, acetate, alanine and lactate. When [3-^{13}C]lactate was used as the gluconeogenic substrate, a lower percentage of ^{13}C was present in glycogen, succinate, glutamate, acetate and lactate in livers of infected mice than in corresponding controls. Infected mice also had lower concentrations of hepatic glucose and phosphocholine, but higher concentrations of betaine, choline, acylcarnitine and β-hydroxybutyrate. Cysticerci contained ^{13}C label in glucose, alanine and lactate.

MRS analysis of chloroform/methanol extracts of livers from infected mice showed differences in the lipid profile when compared to those of normal livers. Infected mice had lower concentrations of total fatty acid (FA), phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, total glycerophospholipids and triacylglycerols than uninfected controls. Cholesterol concentrations remained unchanged. Furthermore, it

was found that the liver extracts of animals infected with cysticerci had higher ratios of the polyunsaturated moieties, docosahexaenoic acid, arachidonic acid and $CH{=}CH(CH_2CH{=}CH)_n$, to total FA, but lower ratios of the saturated moieties as indicated by the $-(CH_2)_n-$ and the $-CH_2COO^-$ values. The corresponding ratios of $-CH_2CH_2COO^-$, $CH{=}CH-$, $-CH_2CH{=}CHCHCH{=}CHCH_2-$, linoleic acid, and the average FA chain length did not differ between the infected and uninfected groups of mice. Extracts of *T. crassiceps* displayed a similar lipid profile to that of host liver but contained a lower lipid content and a shorter average FA chain length. It can be concluded that *T. crassiceps* indeed dramatically alters hepatic carbohydrate and lipid metabolism of its host.

ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the efforts of those individuals who contributed to the completion of my M.Sc degree.

I must first express my deep appreciation to my supervisors, Drs. Marie Novak and Barry J. Blackburn for their guidance and many hours of consultation throughout this project. Their vast knowledge of biology and chemistry that they generously shared with me and the many different techniques I learned under their instruction were invaluable. As well their critical and complete approach to science both in designing and performing experiments and writing scientific literature has taught me a great deal about research. Truly Drs. Novak and Blackburn are remarkable people and I am fortunate to have had them as mentors in my education.

I am grateful to Terry Wolowiec for his technical assistance in performing the MRS experiments. His friendship and support during this endeavour was very much appreciated. Special thanks also goes to Terry Durham for his technical assistance with the animal work.

A very special thank you goes out to Jodi Schoen, who has been a true friend over the years. Her positive attitude and jovial spirit always made work a pleasurable experience. I also enjoyed and valued our many long conversations.

I wish to acknowledge the department of Human Anatomy and Cell Science for their support and help throughout my degree. I am especially grateful to the Anatomical Research Fund which supplied financial support for travel to conferences.

I must also express a genuine thank you to the members of my advisory committee; Drs. Judy Anderson, Jim Thliveris and Jim Peeling. Their constructive suggestions and eagerness to act as advisors throughout my degree were greatly appreciated.

Finally, I acknowledge the support of my family. To my parents, Hugh and Wilma, my sister, Esther and my aunt, Miranda I thank you all because it is the result of your faithful support and consistent encouragement that I have been able to achieve such accomplishments.

LIST OF ABBREVIATIONS

AA	arachidonic acid
ACP	acyl carrier protein
ANOVA	analysis of variance
ATP	adenosine triphosphate
^{12}C	carbon-12
^{13}C	carbon-13
^{14}C	carbon-14
cAMP	cyclic adenosine 5'-monophosphate
CDP	cytidine 5'-diphosphate
CoA	coenzyme A
CNS	central nervous system
CT	cholesterol
DAG	diacylglycerol
DHA	docosahexaenoic acid
F-1,6-P_2ase	fructose-1,6-bisphosphatase
F-2,6-P_2ase	fructose-2,6-bisphosphatase
FA	fatty acid
FAC	fatty acid chains
FAD	flavin adenine dinucleotide (oxidized form)

$FADH_2$	flavin adenine dinucleotide (reduced form)
GPC	glycerophosphocholine
GPL	glycerophospholipid
^1H	proton
HDL	high density lipoprotein
HMG-CoA	β-hydroxy-β-methylglutaryl-CoA
IgG	immunoglobulin G
IL-4	interleukin-4
IL-2	interleukin-2
LA	linoleic acid
LnA	linolenic acid
LPL	lipoprotein lipase
MAG	monoacylglycerol
MHz	mega hertz
MR	magnetic resonance
MRS	magnetic resonance spectroscopy
NAD	nicotinamide adenine dinucelotide (oxidized form)
NADH	nicotinamide adenine dinuceotide (reduced form)
NADPH	nicotinamide adenine dinuceotide phosphate (reduced form)
NMR	nuclear magnetic resonance
^{31}P	phosphorus-31

PC	phosphocholine
PCA	perchloric acid
PD	pyruvate dehydrogenase
PDC	pyruvate dehydrogenase complex
PEP	phosphoenolpyruvate
PEPCK	phosphoenolpyruvate carboxykinase
PFK-1	phosphofructokinase
PFK-2	phosphofructokinase-2
PGI_2	prostaglandin GI_2
PGE_2	prostaglandin GE_2
PTC	phosphatidylcholine
PTE	phosphatidylethanolamine
PTI	phosphatidylinositol
SAM	S-adenosyl methionine
TCA	tricarboxylic acid cycle
T. crassiceps	*Taenia crassiceps*
TAG	triacylglycerol
T. solium	*Taenia solium*
TXA_2	thromboxane A_2
VLDL	very low- density lipoprotein

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

CYSTICERCOSIS

Human cysticercosis is a serious parasitic disease caused by the cysticercus larva of *Taenia solium*. This zoonotic infection afflicts both humans and pigs, often producing a grim prognosis (Gemmell *et al.*, 1983). Humans are the only definitive host for this parasite. They acquire infections with the adult tapeworm, taeniasis, by ingesting insufficiently cooked pork infected with *T. solium* cysticerci. In the intestine, the cysticerci attach to the intestinal mucosa and mature into adult tapeworms. These worms soon produce infective eggs which are released with human feces into the environment. The usual intermediate host, the pig, gets infected by consuming the parasite eggs. The eggs hatch in the small intestine and the released embryos penetrate into the intestinal wall, where they enter the blood circulation, and are disseminated throughout the body. There in tissues they develop into cysticerci. When humans accidentally consume *T. solium* eggs in contaminated food or water they may also serve as an intermediate host and the parasite embryos follow the same course of tissue invasion as in the porcine host, producing the dreaded condition of human cysticercosis. The disease is characterized by infections of variable intensity, where larvae lodged in different tissues evoke varying degrees of pathology, depending on the site of predilection. In humans, cysticerci are commonly found in the central nervous system (CNS), in parenchymal, subarachnoid, intraventricular and/or spinal sites (Craig *et al.*, 1996). In these sites the cysticerci often cause a number of severe CNS malfunctions, the most

common symptom being the sudden onset of epilepsy (Dumas *et al.*, 1989; White, 1997). Less common symptoms include intracranial hypertension and pychiatric disorders. These clinical manifestations result from localized cyticerci compromising neural tissue activity and obstructing cerebral spinal fluid flow and/or host inflammatory reactions. The inflammatory response often extends to neighbouring tissues surrounding the parasite causing significant lesions and scarring. If damage to neural tissue is allowed to continue and cerebral obstruction persists, the host's condition rapidly declines ultimately resulting in death. In pigs, the parasite is primarily found in large numbers in striated muscle, especially in the heart, tongue and limbs (Harrison and Swell, 1991), followed by sites in the CNS. Pigs usually tolerate intramusclular cysticercosis well. However, in the CNS cysticerci evoke similar pathological sequelae as in the CNS of humans, but because pigs are usually slaughtered within their first year of life, symptoms of this disease do not fully develop (Gemmell *et al.*, 1983).

Formerly a disease of western Europe, cysticercosis is now primarily a problem in the underdeveloped tropics (Grove,1990). Epidemiological studies have indicated that the endemic geographic distribution of this zoonosis includes Mexico, countries of Latin America, Asia and all the sub-Saharan countries of Africa (Harrison and Swells, 1991; White,1997). The alarming prevalence of this disease has significantly decreased public health and economic productivity in these regions (Craig *et al.*,1996). The high chronicity and significant morbidity of the disease, the estimated millions of dollars annually spent in hostipalization and treatment of patients (Roberts *et al.*, 1994), along with the financial losses resulting from condemned livestock has led international agencies such as the World

Health Organization and the International Task Force for Disease Eradication to commission special operations directed at the prevention, control and eradication of cysticercosis (Gemmell *et al.*, 1983; International Task Force for Disease Eradication, 1990).

In the strategic approach to combat cysticercosis, the area of experimental investigations of this host-parasite relationship has received a considerable amount of attention (Canedo *et al.*, 1976; McManus *et al.*, 1990; Terrazas *et al.*, 1994). Insight into the dynamics of the interactions between the host and parasite, whether immunological, molecular or biochemical in nature are viewed as an essential component to understanding this parasitic infection (Canedo *et al.*, 1976). Experimentation with *T. solium*, however, is hampered by the high costs, genetic variability and slow data retrieval when working with pigs (Sciutto *et al.*, 1990). In order to avoid these difficulties many investigators have adopted as a laboratory model for studying this disease, murine cysticercosis, caused by the cysticerci of *Taenia crassiceps* (Freeman, 1962). The mouse -*T. crassiceps* system is an excellent experimental model for studying cysticercosis, due to the similarities these cysticerci share with those of *T. solium* and because of the ease with which they can be maintained and reproduced in large numbers in the laboratory (Freeman, 1962; Larralde *et al.*, 1990; Sciutto *et al.*, 1990). Unlike *T. solium* the life cycle of *T. crassiceps* does not require humans as either a definitive or intermediate host. Although there have been a few reported cases of human cysticercosis caused by *T. crassiceps* (Shea *et al.*, 1972; Gemmell *et al.*, 1983), this species is primarily a parasite of wild rodents and canines.

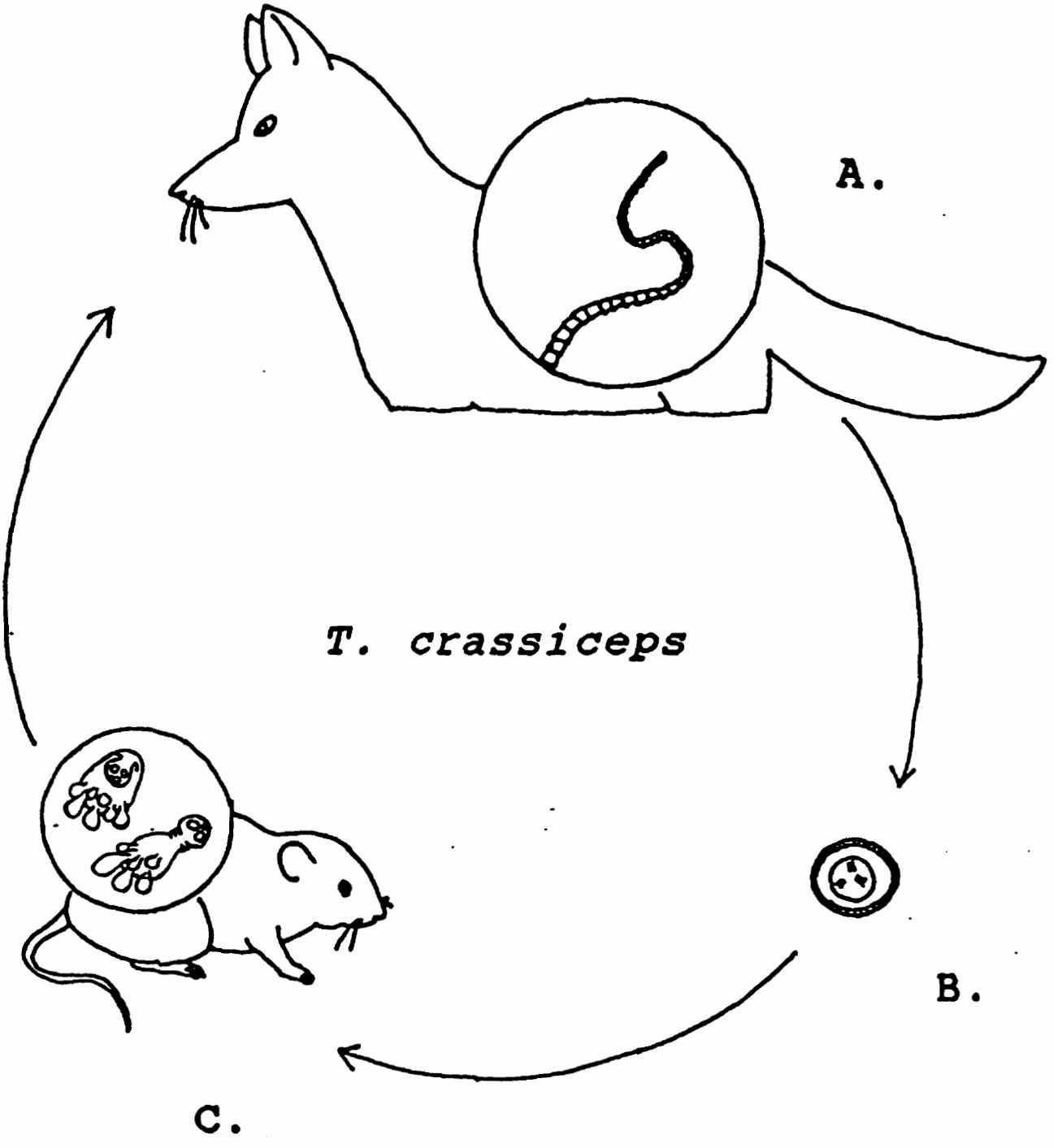
In the normal life cycle of *T. crassiceps* (Fig. 1) the adult tapeworm inhabits the small intestine of various canines, particularly the red fox, *Vulpes vulpes*. Situated in the

Figure 1: **The life cycle of *T. crassiceps***

A. Adult tapeworm in the small intestine of a fox.

B. Egg is passed with feces.

C. Egg is eaten by a rodent, the embryos hatches and migrates to body cavities, where it develops into cysticercus. The life cycle is complete when a fox eats the infected rodent.


A.
T. crassiceps
B.
C.

small intestine of the canine host the tapeworm continuously undergoes a process of strobilation, in which terminal gravid segments, proglottids, are ladden with infective eggs. These proglottids detach and pass with feces of the infected host. Eggs, when eaten by various small rodents, hatch under the influence of gastric secretions and the released embryos,oncospheres, quickly penetrate into the intestinal wall, enter the circulation and are delivered to subcutaneous tissues or body cavities, where they develop into cysticerci.

The cysticercus of *T. crassiceps*, formerly known as *Cysticercus longicollis*, is a spherical translucent fluid filled bladder containing a single, usually invaginated, head or scolex bearing four suckers and a crown of hooks. The external body covering of the cysticercus, is a syncitial epidermis referred to as tegument. Since tapeworms lack a functional digestive tract, their tegument serves as a sole site for the acquisition of nutrients and elimination of waste products. For this purpose the tegument is covered with numerous membranous extensions, microtriches, which increase the tegumental surface area and therefore contribute to increased absorptive and secretory activities. The outer surface of the tegument is coated with a glycocalyx. The components of the glycocalyx, a complex array of macromolecules such as glycoproteins, lipids and sphingolipids render the cysticerci highly immunogenic (Kunz *et al.*, 1991; Dennis *et al.*, 1992) and elicit a strong immune response from the host (Freeman, 1964; Siebert *et al.*, 1981). Although some cysticerci are overcome by the host's immune response (Siebert *et al.*, 1978), the majority of them manage to survive and get established in this microenvironment (Siebert *et al.*, 1978; Siebert *et al.*, 1981; McManus *et al.*, 1990; Kalinna *et al.*, 1993; Ambrosio *et al.*, 1994).

In addition to immune evasion, *T. crassiceps* cysticerci are also immunosupressive to their host by being able to alter the late events in the T cell receptor signalling pathway, thereby, depressing T cell activity (Villa *et al.*, 1996; Sciutto *et al.*, 1995). This depression selectively afflicts CD4+ Th1 cells, while CD4+ Th2 cells appear to be up regulated. Indeed, elevated levels of IgG1, eosinophils, basophils and IL-4 associated with the up regulation of Th2 cells and the marked absence of IgG2a and IL-2, related to the downregulation of Th1 cells, are consistent with the histochemical findings in murine cysticercosis (Freeman, 1962; 1964; Villa *et al.*, 1996). This imunosuppressive activity of *T. crassiceps* cysticerci is further supported by the differential susceptibility between male and female hosts to infection. Female mice, regardless of strain, are more prone than males to acute infections with *T. crassiceps* cysticerci (Freeman, 1962; 1964). However, once the infection reaches the chronic stage, sex associated differences tend to diminish. During the course of the infection several hormonal changes occur in both male and female hosts. Males experience a dramatic increase in serum estrogen to levels as high as 200 times their normal value, while testosterone concentrations may decrease by 90%. The hormonal changes in females are not as pronounced as they experience only a slight increase in estrogen levels (Larralde *et al.*, 1995). Immunological experiments have demonstrated that the estrogen 17-β-estadiol positively regulates parasite reproduction in the host of both genders, presumably by interfering with cellular immune mechanisms to promote Th2 cell secretions and inhibit those of Th1 cells (Terrazas *et al.*, 1994). Thus this immunoendocrine association allows cysticerci to thrive in the estrogen rich milieu of the female host, while in males the parasite resourcefully stimulates the host's endocrine system towards abnormal estrogen synthesis to

create a favourable, Th2 cell up regulated/ Th1 cell down regulated, environment (Larralde *et al.*, 1995).

Having overcome the immune response of the host, the majority of the cysticerci of *T. crassiceps* establish themselves, unlike *T. solium,* within the peritoneal cavity by undergoing asexual multiplication through exogenous budding. Multiple buds start to appear on the bladder of the cysticercus usually 3 weeks post infection (Freeman, 1962). They continue to increase in size and their tegument differentiates (Novak and Dowsett, 1983). Soon after, the daughter buds detach from the parent cysticercus and coexist as independent individuals. The enormous reproductive potential of each cysticercus soon creates a heavily populated environment within the abdominal cavity. In the end, the parasite wins and the host succumbs.

CARBOHYDRATE METABOLISM

PARASITE

The highly active reproductive nature of the cysticerci demands considerable quantities of nutrients. As obligate endoparasites, they acquire all their nutrients from the host. Efficient absorptive mechanisms such as active transport and pinocytosis allow them to absorb nutrients which may be at low concentrations, as well as prevent the leakage of nutrients from the parasite's tissue when their levels fall in the external environment (Howell, 1976). Cysticerci like all other cestodes utilize glucose as a primary source of energy. The hexose is taken up across the tegumental membrane by a Na^+ sensitive carrier-mediated

system (Craig, 1982). Once internalized, glucose may either be polymerized to form glycogen, or be shuttled down the degradative pathways for energy generation. Assuming cysticerci possess the same limited capacity to conduct aerobic metabolism as other cestodes, their anaerobic pathways will predominate (Barrett, 1984; Schmidt and Roberts, 1989; Tielens *et al.*, 1993). The anaerobic catabolism of glucose involves both cytosolic and mitochondrial events (Fig. 2). In the cytosol glucose is metabolized via the classical Embden-Meyerhof glycolytic pathway to phosphoenolpyruvate (PEP). At this point cytosolic PEP may be converted to pyruvate by the enzyme pyruvate kinase. Pyruvate may then be transaminated by alanine aminotransferase to alanine or reduced to lactate via lactate dehydrogenase. Alanine and lactate are then excreted. Two molecules of ATP are generated by this degradative pathway regardless of which end product is excreted. However, lactate is the preferred end product as it maintains the cytosolic redox balance disturbed earlier in the pathway by the glyceraldehyde-3-phosphate dehydrogenase reaction. Cytosolic PEP may also experience a different fate if it condenses with CO_2 to produce oxaloacetate, which is then reduced by malate dehydrogenase to malate. The cytosolic formation of malate is comparable to that of lactate in that both reactions re-establish the cytosolic redox balance. However, in contrast to lactate, malate is not excreted, instead it is transported to the mitochondria where it is further metabolized.

Within the mitochondria malate is channelled down either an oxidative or a reductive pathway. In the former, malate is first oxidized to pyruvate via malic enzyme, followed by a second oxidation catalyzed by pyruvate dehydrogenase to produce acetyl-CoA. On conversion of acetyl-CoA to acetate one molecule of ATP is produced. In the reductive

FIGURE : 2 Glucose metabolism in cestodes

Enzymes:

1, glyceraldehyde- 3-phosphate dehydrogenase

2, pyruvate kinase

3, alanine aminotransferase

4, lactate dehydrogenase

5, phosphoenolpyruvate carboxykinase

6, malate dehydrogenase

7, malic enzyme

8, pyruvate dehydrogenase complex

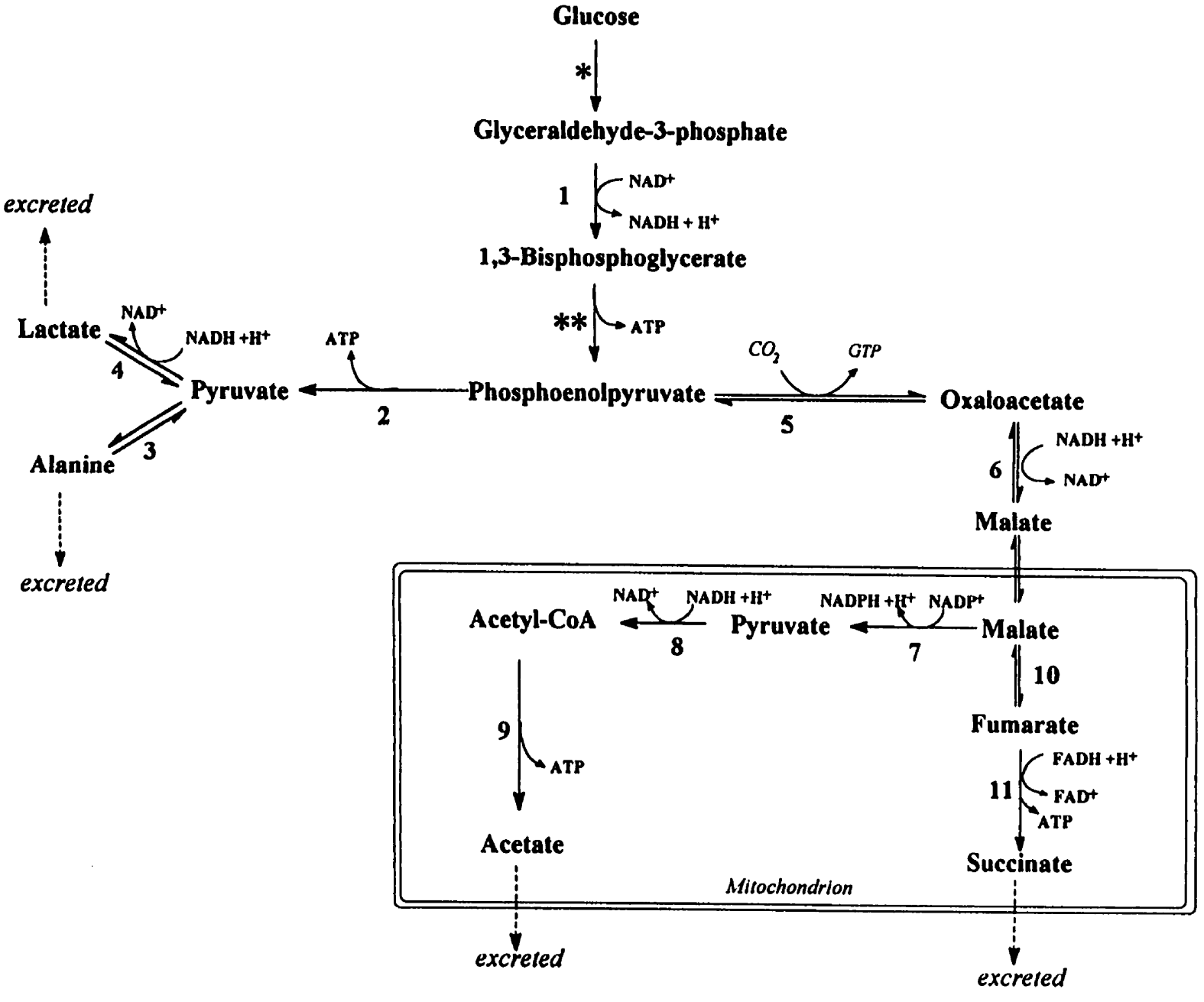
9, unknown

10, fumarase

11, fumarate reductase

* enzymes invovled in the degradation of glucose to glyceraldehyde-3-phosphate: hexokinase, phosphoglucoisomerase, phosphofructokinase and aldolase

** enzymes involved in the conversion of 1,3-bisphosphoglycerate to phosphoenolpyruvate: phosphoglycerate kinase, phosphoglyceromutase, and enolase.


Glucose
*
Glyceraldehyde-3-phosphate
1
NAD+
NADH + H+
1,3-Bisphosphoglycerate
**
ATP
excreted
Lactate
NAD+
NADH +H+
4
ATP
Pyruvate
2
Phosphoenolpyruvate
CO2
GTP
5
Oxaloacetate
3
Alanine
excreted
6
NADH +H+
NAD+
Malate
NAD+
NADH +H+
NADPH +H+
NADP+
Acetyl-CoA
8
Pyruvate
7
Malate
10
9
ATP
Fumarate
FADH +H+
11
FAD+
ATP
Acetate
Succinate
Mitochondrion
excreted
excreted

pathway, malate is dehydrated to fumarate by fumarase , which is then reduced via fumarate reductase to form succinate with the concomitant generation of another molecule of ATP. Both acetate and succinate are excreted as end products. The formation of acetate involves two oxidation reactions while that of succinate involves one reduction reaction. To maintain the mitochondrial redox balance in these mitochondrial reactions twice as much succinate as acetate must be formed.

In the parasite, the inability to further metabolize the end products acetate, succinate, alanine and lactate along energetically efficient oxidative or gluconeogenic pathways renders these compounds metabolically inert (Barrett, 1984). Therefore they accumulate and are excreted into the surrounding tissues of the host. Unlike cysticerci, host tissues do possess the ability to re-metabolize such compounds. The liver performs a vast array of both catabolic and anabolic functions and is a primary site for metabolism of parasite metabolic end products.

HOST LIVER

The mammalian liver serves as an intermediary control centre between the dietary sources of energy arriving from the gastrointestinal tract, and the extrahepatic tissues which require this source of energy. Central to the role the liver plays in energy metabolism is the maintenance of glucose homeostasis. Functioning in this capacity the liver is sensitive to the requirements of peripheral tissues through coordinated activities of hormones such as glucagon and insulin. During a state of excess glucose supply, as after the consumption of

a meal rich in carbohydrates, the liver channels glucose along various anabolic pathways. Large quantities of free glucose in the blood stream create osmotic disturbances, therefore, the majority of this hexose is polymerized in hepatocytes as the insoluble polysaccharide glycogen (Seifter and Englard, 1994). Also, once a sufficient amount of glycogen is stored, excess glucose may be used as a substrate for lipogenesis and/or shuttled down the pentose phosphate pathway to provide NADPH and ribose-5-phosphate for lipid and nucleic acid biosynthesis respectively.

In times of fasting or between meals when glucose levels are low, the liver liberates glucose via glycogen degradation. The majority of tissues in the mammalian body can use additional substrates like fatty acids and amino acids for energy generation. A few tissues, however, such as the renal medulla, retina and erythrocytes are obligate consumers of glucose. Thus even when glucose levels are low, the liver must ensure that a sufficient supply of glucose is available for the proper function and vitality of these tissues. Over a twelve hour period of fasting, hepatic glycogen stores become severely depleted, and tissues which are obligate consumers of glucose would soon approach a critical situation (Cahill, 1986). To avoid such potential disastrous scenarios the liver synthesizes glucose *de novo*, from three or four carbon noncarbohydrate precursors, through a process called gluconeogenesis.

The reactions of gluconeogenesis from pyruvate to glucose closely resemble those of glycolysis run in reverse (Fig. 3). However, due to the irreversibility of some glycolytic reactions three unique steps are incorporated into the gluconeogenic pathway allowing it to proceed in the direction of glucose synthesis. The first of these reactions involves the

Figure: 3 Reactions of glycolysis and gluconeogenesis

Enzymes:

1, pyruvate carboxylase

2, mitochondrial malate dehydrogenase

3, mitochondrial aspartate aminotransferase

4, cytosolic malate dehydrogenase

5, cytosolic aspartate aminotransferase

6, phosphoenolpyruvate carboxykinase

7, pyruvate kinase

8, fructose-1,6-bisphosphatase

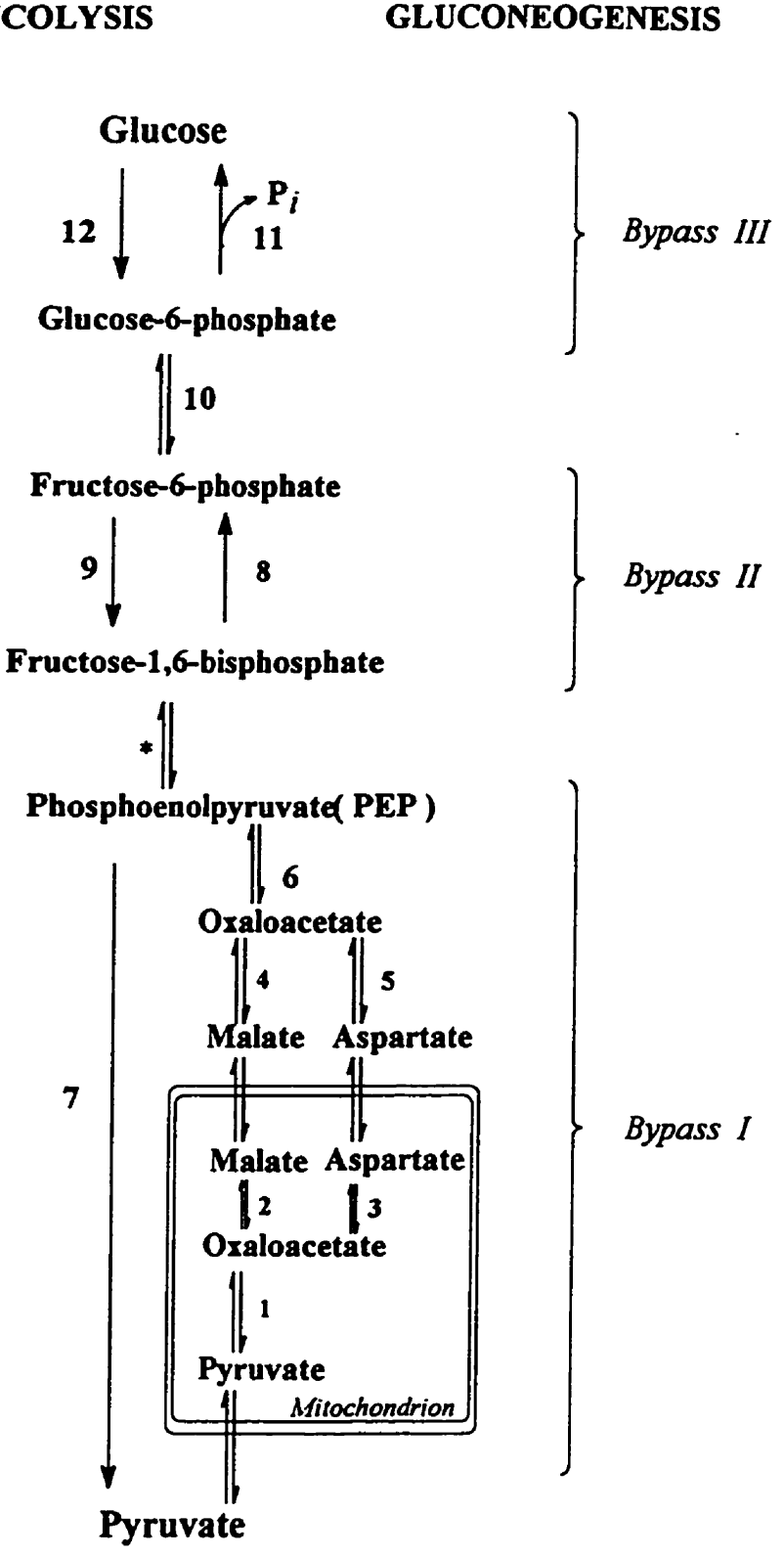
9, phosphofructokinase

10, phosphoglucoisomerase

11, glucose-6-phosphatase

12, glucokinase

* reversible glycolytic reactions between PEP and fructose-1,6-bisphosphate


GLYCOLYSIS
GLUCONEOGENESIS
Glucose
12
P_i
11
Glucose-6-phosphate
10
Fructose-6-phosphate
9
8
Fructose-1,6-bisphosphate
*
Phosphoenolpyruvate(PEP)
6
Oxaloacetate
4
5
Malate
Aspartate
7
Malate
Aspartate
2
3
Oxaloacetate
1
Pyruvate
Mitochondrion
Pyruvate
Bypass III
Bypass II
Bypass I

conversion of pyruvate to PEP. The glycolytic conversion of PEP to pyruvate is highly exergonic, making it thermodynamically an irreversible reaction. In order to bypass this step, pyruvate undergoes a series of reactions in the mitochondria and the cytosol to produce PEP. Cytosolic pyruvate is first transported into the mitochondrial matrix where by action of pyruvate carboxylase it is converted to oxaloacetate. Because the mitochondrial membrane is impermeable to oxaloacetate, it is reduced by mitochondrial malate dehydrogenase to malate. Malate is then able to be transported into the cytosol where, through the action of cytosolic malate dehydrogenase, oxaloacetate is regenerated. Alternatively, mitochondrial oxaloacetate may be transaminated by aspartate aminotransferase to aspartate, which can traverse the mitochondrial membrane. Once in the cytosol aspartate is reconverted to oxaloacetate by the cytosolic form of the same enzyme. Cytosolic oxaloacetate, whether generated from malate or aspartate, is acted upon by phosphoenolpyruvate carboxykinase to produce PEP. The overall conversion of pyruvate to PEP is energetically costly, as for every molecule of PEP synthesized an equivalent of 2 molecules of ATP must be invested into these reactions.

The reactions occurring between PEP and the production of fructose-1,6-bisphosphate proceed by glycolytic reactions acting in reverse. In glycolysis the formation of fructose-1,6-bisphosphate by phosphofructokinase is an irreversible reaction, therefore, a second bypass reaction must occur in gluconeogenesis to generate fructose-6-phosphate. This is accomplished by the enzyme fructose-1,6-bisphosphatase which catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate which then undergoes an isomerization reaction to glucose-6-phosphate by the reversible enzyme

phosphoglucoisomerase. In the final step of glucose synthesis the phosphate bond belonging to glucose-6-phosphate must be severed. Glucokinase can not catalyze this reaction due to thermodynamic constraints, hence the enzyme glucose-6-phosphatase is incorporated into the gluconeogenic pathway. Glucose-6-phosphatase catalyzes the hydrolysis of glucose-6-phosphate to free glucose and inorganic phosphate. This enzyme is found primarily in liver in the membranes of the endoplasmic reticulum. The kidney also possesses this enzyme enabling it to conduct gluconeogenesis, however, under most circumstances it is mainly the liver that supplies synthesized glucose to peripheral tissues (Owen *et al.*, 1969; Cherrington and Vranic, 1986). Other tissues of the body lack this enzyme, thus when glucose-6-phosphate is generated it is either degraded through glycolysis or isomerized to glucose-1-phosphate by phosphoglucomutase and incorporated into glycogen. Hence, this enzyme is essential to the liver's ability to synthesize free glucose.

Substrates available for gluconeogenesis arise from numerous non carbohydrate precursors originating from extra hepatic tissues. Precursors such as lactate, amino acids, propionate and glycerol can be channelled into the gluconeogenic cascade as oxaloacetate or pyruvate to synthesize glucose. Among these potential gluconeogenic precursors alanine and lactate are regarded as the primary substrates for glucose synthesis (Felig, 1973; Seifter and Englard, 1994; Kaloyianni and Freedland, 1990). Both alanine and lactate enter the gluconeogenic pathway as pyruvate through interorgan cycles called the glucose-alanine cycle and the Cori cycle respectively.

In the glucose-alanine cycle, skeletal muscle provides the liver with a supply of alanine. During the initial stages of starvation increased rates of skeletal muscle protein

degradation occur. While alanine comprises no more than 7%-10% of muscle protein and polyalanyl muscle proteins do not exist, a disproportionally large amount of alanine is released from muscle tissue during proteolysis (Felig *et al.*, 1970; Felig, 1973). It is believed that this alanine is synthesized during the events of protein degradation (Hellerstein and Munro, 1994). As muscle protein is degraded ammonia is released and keto acids are formed. At the same time skeletal muscle has adapted to utilizing fatty acids as their principle substrate for energy production. Therefore, glucose that is taken up by muscle tissue is diverted from energy generation to alanine synthesis. In this process glucose is converted to pyruvate, which then condenses with the generated ammonia in a transamination reaction to produce alanine. Pyruvate may also be generated from muscle amino acids as deaminated carbon skeletons of a variety of amino acids (keto acids) can be converted to pyruvate. Once alanine is formed it is transported to the liver where as its concentration increases, it produces an inhibitory effect on hepatic pyruvate kinase. This enzyme inhibition promotes gluconeogenesis as deposited alanine is transaminated back to pyruvate by alanine aminotransferase which then forms PEP. Under these conditions alanine derived PEP may freely continue through gluconeogenesis unopposed by the competitive pyruvate kinase reaction. During the process of intrahepatic conversion of alanine to pyruvate ammonia is released. This ammonia is then incorporated into the urea cycle to synthesize urea. Thus the transport of alanine to the liver not only provides a substrate for gluconeogenesis, but it also provides a means of disposing of excess ammonia formed during proteolysis.

In the Cori cycle, lactate produced by extra hepatic tissues is taken up and utilized

by the liver as a gluconeogenic substrate. As an obligate consumer of glucose, erythrocytes, which lack mitochondria, metabolize all their incoming glucose via glycolysis and produce considerable amounts of lactate (Seifter and Englard, 1994). In skeletal muscle, during intense exertion or upon stimulation by epinephrine, glycogen stores are rapidly mobilized, producing pyruvate far faster than the tricarboxylic acid cycle (TCA) can utilize it. Accumulated pyruvate is reduced to lactate by the enzyme lactate dehydrogenase and excreted into the blood (Hellerstein and Munro, 1994). Regardless of the source, lactate entering the liver is oxidized to pyruvate which then, depending on the energy status of the organ, is either further oxidized through the TCA cycle or directed towards glucose synthesis. The capability of the liver to re-metabolize the end products released by extrahepatic tissues suggest that it can play a similar role in the host-parasite relationship. End products excreted into the peritoneal cavity by the cysticerci of *T. crassiceps* must be disposed of as the accumulation of large quantities of such organic acids would soon pose severe pH and osmolarity problems for surrounding host tissues (Barrett, 1984). It is beleived that through the continuous movement of fluid across the peritoneal membrane parasite excretions are removed from this site via the blood and lymphatic systems. Once in the circulation, the excretory end products are delivered to the liver where as the need for energy in the parasitized host increases, they might be utilized in gluconeogenic and oxidative energy-producing pathways (Novak et al., 1995; Corbin, 1996).

To study what really happens during metabolic interactions between *T. crassiceps* and its mammalian host, the present study was initiated. In order to monitor biochemical changes of carbohydrate metabolism in the liver of the infected host, exogenous [3-^{13}C]

alanine or [3-^{13}C] lactate was introduced intraduodenally and the fate of the carbon-13 label was followed utilizing magnetic resonance spectroscopy (MRS). The distibution of label present in various metabolites and end products revealed information about the metabolic fate of alanine and lactate within the parasitized host.

High resolution carbon-13 MRS utilized in conjuction with ^{13}C-labelled substrates provides a useful approach to studying metabolism (Cohen,1983). Analogous in many ways to ^{14}C istope studies, both techniques observe the redistribution and fate of exogenous isotopes along cellular metabolite pathways. While both techniques are equally capable of detecting their respective isotopes (Cohen, 1981), ^{14}C analyses are restricted to carbon by carbon degradation of individual components. The ^{13}C MRS method offers the simultaneous detection of intact metabolites with adequate isotope enrichment, however difficulties associated with accurate quantification and low sensitivity hamper this technique.

Carbon-13 decoupled proton magnetic resonance spin echo spectroscopy provides an alternative method of measuring ^{13}C enrichment by MRS. In this proton-observed heteronuclear experiment, a sequence of spin echo and ^{13}C pulses are applied to selectively generate two proton MR spectra. In the first spectrum, in which a ^{13}C pulse is absent, protons attached to ^{12}C and ^{13}C are in phase. In the second spectrum, involving the ^{13}C pulse, protons attached to ^{13}C are 180° out of phase with respect to protons attached to ^{12}C and other nuclei. Hence selective observation of protons adjacent to either ^{12}C or ^{13}C labelled species may be obtained by computer addition or subtraction between the first and second spectra. In short this method allows the ^{13}C isotope to be monitored through ^{1}H MRS.

The advantages of this technique over the conventional application of ^{13}C MRS are

three-fold. First and foremost the observation of the ^{1}H nuclei offers a far more sensitive method of metabolite detection. In principle the ^{1}H nucleus is 63 times more sensitive to MR detection than the ^{13}C nucleus (Silverstein *et al.*, 1991). This greater sensitivity permits metabolites at lower concentrations to be detected as well as significantly decreasing the acquisition time required to accumulate adequate spectra. Secondly, the percent of labelled ^{13}C is readily calculated with this method as both ^{12}C and ^{13}C-labelled species are observed. Also the problem of the nuclear Overhauser effect which makes quantitation with ^{13}C MRS difficult is not present in this technique.

LIPID METABOLISM

PARASITE

Lipids are a diverse and heterogenous group of water insoluble molecules which function in tissues primarily as structural component of membranes and as a storage form of metabolic fuel (Marinetti, 1990). Membrane renewal and energy generation are processes which are continually active and necessary for the integrity of all cells. Hence if vitality of the cell and the organism are to be maintained it is essential that the synthesis and/or acquistion of lipids must occur. High rates of membrane biosynthesis and renewal occur in *T. crassiceps* cysticerci due to their continuous growth and asexual reproduction. Cysticerci and other cestodes, however, are incapable of *de novo* long chain fatty acid (FA) and sterol synthesis (Barrett, 1983; Frayha and Smyth, 1983; Smyth and Mc Manus, 1989). Therefore, all the lipids they require must be obtained from the host. Lipids in the form of free fatty

acids (FFA), glycerophospholipids (GPL), sterols, mono-, di-, and triacylglycerols (MAG, DAG, and TAG) are taken up by the worms through a combination of diffusion and mediated transport processes (Smyth and McManus, 1989). Once internalized these lipids may be channelled along different metabolic paths.

In many organisms lipids can serve as an important fuel, as the oxidation of FA generates considerable quantities of energy. Although cestodes do possess numerous lipases and intramitochondrial β-oxidation enzymes, there is no evidence that the β-oxidation sequence in these parasites is functional (Mills *et al.*,1981; Barrett, 1983). The anaerobic life style adopted by these organisms render them incapable of utilizing sufficient quantities of oxygen to reoxidize the many reduced flavoproteins generated during the oxidation of fatty acids. Absorbed lipids, therefore, are sequestered for membrane biosynthesis and renewal. Cestodes do, however, possess a limited capacity to modify and combine incoming lipids into different and more complex lipids (Barrett, 1983). Through the process of chain elongation, where acetyl-CoA units are sequentially added to absorbed acyl chains, FA are modified to varying lengths. FFA, whether modified or not, may later be incorporated into absorbed MAG and DAG fractions to manufacture new lipid membrane components. Cestodes may also regulate the composition of their internalized FA by selectively absorbing different FA from the host (Barrett, 1983). The number of double bonds per chain of absorbed FA, however, cannot be altered as the worms can neither saturate olephinic carbons nor desaturate FA. Apart from FA, cestodes are also able to manipulate other classes of acquired lipids. Given the necessary precursors they can synthesize various GPL moieties through the incorporation of polar head groups into absorbed acylglycerols or through the

exchange of polar head groups between preformed GPL (Barrett, 1983; Smyth and McManus, 1989). In addition, steroids are commonly modified by these worms, as exogenous steroids and FA are readily incorporated into steroid esters.

HOST LIVER

The liver performs many central metabolic functions in various aspects of lipid and lipoprotein metabolism (Glickman and Sabesin, 1994). Under normal conditions the liver derives most of its energy from the metabolism of FA (Seifter and Englard, 1994). FFA, however, are normally present at low concentrations. Mostly they are found esterified to sterols or glycerol moieties in the form of acylglycerols and GPL or bound to proteins as lipoproteins and albumin. Under the action of specific lipases, FA are liberated from their glycerol or protein backbones and channelled, when needed, towards the production of energy. The process whereby energy is generated from the degradation of FA is called β-oxidation (Lehninger *et al.*, 1993). It occurs in the mitochondrial matrix and therefore FA that are to be degraded must first be activated and then transported from the cytosol to the mitochondrial matrix (Fig. 4). The activation process requires a chain length-specific fatty acyl-CoA ligase which catalyzes the esterification of the FA carboxyl group with the thiol group of CoA, producing a fatty acyl-CoA intermediate. This intermediate freely permeates the outer mitochondrial membrane, but requires a specific transporter to traverse the inner mitochondrial membrane. Through the action of the enzyme carnitine acyltransferase I, carnitine, the fatty acyl transporter, displaces the CoA group from the

Figure 4: Reactions of FA β-oxidation

Enzymes

1, fatty acyl-CoA ligase

2, carnitine acyltransferase I

3, carnitine acyltransferase II

4, enzymes involved the degradation of the fatty acyl chain to acetyl- CoA : fatty acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and β-ketothiolase.

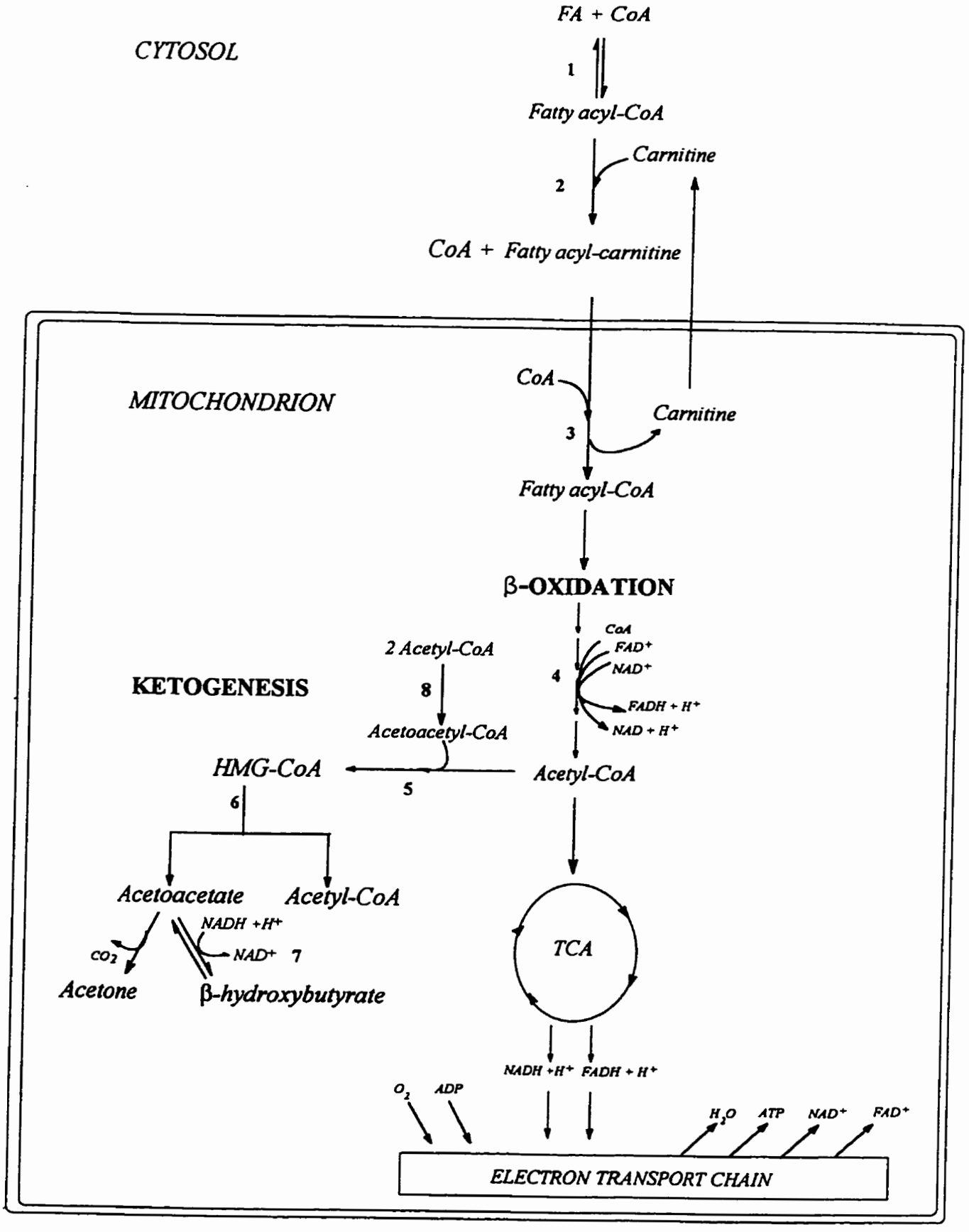
5, HMG-CoA synthase

6, HMG-CoA lyase

7, β-hydroxybutyrate dehydrogenase

8, β-ketothiolase.

FA OXIDATION



fatty acyl-CoA moiety producing free cytosolic CoA and the derivative, fatty acyl-carnitine, which is able to traverse the inner mitochondrial membrane. The inner mitochondrial membrane translocator operates as an antiport where the entry of fatty acyl-carnitine occurs in exchange for the export of free carnitine. Inside the mitochondrial matrix, carnitine acyltransferase II catalyzes the dissociation the fatty acyl-carnitine complex, generating free carnitine and the fatty acyl group which then binds with a CoA molecule from the intramitochondrial pool to regenerate the fatty acyl-CoA intermediate. In the β-oxidation sequence a series of catalyzed dehydrogenations and thiolytic attacks by CoA molecules release acetyl-CoA units from the fatty acyl chain. Successive repetitions of these reactions convert even-numbered FA to numerous free acetyl-CoA units. FA containing an odd number of carbon atoms are reduced to several molecules of acetyl-CoA and one molecule of propionyl-CoA per fatty acyl chain. As each acetyl-CoA unit is liberated it enters the TCA cycle to generate ATP and reduced flavoproteins, $NADH + H^+$ and $FADH + H^+$. These reduced flavoproteins are further shuttled into the electron transport chain to produce their oxidized counterparts and more ATP. Propionyl-CoA formed from the oxidation of odd numbered FA is enzymatically converted to succinyl-CoA which may also enter the TCA cycle. After the conversion of mono- and polyunsaturated FA to their saturated counterparts, via the enzymes enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase, they are also metabolized through β-oxidation.

A modified version of β-oxidation may also occur in peroxisomes. Peroxisomal β-oxidation serves to shorten very long chain FA, thereby facilitating mitochondrial oxidative activities (Lazarow, 1994). During this process long chain FA diffuse into this organelle,

where an alternative β-oxidation sequence liberates acetyl-CoA and shorter chain fatty acyl products. Because peroxisomes lack both the TCA cycle and the electron transport chain, acetyl-CoA units and shortened fatty acyl derivatives must be transported to the mitochondria in order for energy to be generated from these moieties.

In addition to β-oxidation two other pathways exist in which FA may be oxidized. Alpha-oxidation initiates oxidation of FA on the α carbon rather then the β carbon. FA containing alkyl substituents at the β position inhibit the initiation of β-oxidation, thus α-oxidation overcomes this hindrance permitting the metabolism of such FA. A third pathway in which FA may be degraded is called ω-oxidation. In this pathway long chain FA are converted to dicarboxylic acids in the endoplasmic reticulum cytochrome P450 system. Both α- and ω-oxidative pathways are minor contributors in the metabolism of FA (Marinetti, 1990).

Regardless of the manner in which FA are oxidized, the energetic returns generated from FA oxidation far outweigh those incurred from the oxidation of carbohydrates (Cahill, 1986; Marinetti, 1990). The energetic superiority of FA lies within the fact that the methylene carbons of FA are more highly reduced than the carbons of carbohydrates. Consequently the metabolic oxidation of FA generates considerably more energy. These high energy returns from FA metabolism become crucial to the organism during periods of metabolic stress such as prolonged starvation (Cahill, 1986; Hellerstein and Munro, 1994). During prolonged starvation increased lipolysis of adipose tissues occurs liberating large quantities of FA into tissues where energy may be generated from the oxidation of acetyl-CoA in the TCA cycle. The high influx of FA into the liver and their subsequent degradation

produces levels of acetyl-CoA too great for the TCA cycle to handle due to a limited supply of intermediates such as oxaloacetate which are necessary to incorporate the acetyl-CoA into the TCA cycle. In this case the excess acetyl-CoA is directed towards the production of an alternate, water soluble, energy source called ketone bodies.

The process whereby acetyl-CoA is converted to ketone bodies is called ketogenesis (Fig. 4). Like gluconeogenesis this biosynthetic process occurs in the liver. In the hepatocyte mitochondria two molecules of acetyl-CoA condense via β-keto-thiolase to produce acetoacetyl-CoA. This can react with another molecule of acetyl-CoA through the enzyme β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase to form HMG-CoA. This compound is then cleaved by HMG-CoA lyase to yield acetoacetate and acetyl-CoA. Acetoacetate may either be reduced to β-hydroxybutyrate or spontaneously decarboxylated to acetone. Collectively these three compounds acetoacetate, β-hydroxybutyrate and acetone, are referred to as ketone bodies. As liver lacks the enzymes acetoacetyl-CoA synthase and succinyl-CoA thiophorase which convert acetoacetate back to acetyl-CoA, ketone bodies are always exported from the liver and metabolized by extrahepatic tissues. During late stages of starvation ketone bodies are the major source of energy for many tissues especially, the brain (Cahill, 1986). Under such conditions when glucose availability is low and FA are excluded from the CNS due to the blood brain barrier, ketone bodies, which are water soluble forms of FA, are able to cross the barrier of the CNS and fuel the brain with the same energetic returns as short chain FA.

Acetyl-CoA is often viewed as a product of catabolic metabolism, however during the post prandial state, when the supply of glucose is in excess, acetyl-CoA generated from

the pyruvate dehydrogenase reaction or from FA oxidation may be used in the synthesis of FA (Fig. 5). Given that this biosynthetic process involves the condensation of numerous acetyl-CoA molecules, it was formerly believed that FA biosynthesis proceeded simply by reverse reactions of β-oxidation. However, it is now known that this biosynthetic process operates along a distinct pathway requiring a unique reaction site and set of enzymes (Wakil, 1970; Lehninger *et al.*, 1993). Intramitochondrial acetyl-CoA must first traverse the mitochondrial membrane and enter the cytosol where FA synthesis occurs. Within the mitochondria, acetyl-CoA combines with oxaloacetate to form citrate which is then transported through the mitochondrial membrane to the cytosol. There it is enzymatically cleaved by citrate lyase to regenerate acetyl-CoA and oxaloacetate. Oxaloacetate is reduced by the malate dehydrogenase to malate which may reenter the mitochondria to regenerate mitochondrial oxaloacetate by the mitochondrial form of the same enzyme. Alternatively cytosolic malate can also be converted by malic enzyme to pyruvate which is able to enter the mitochondria. The resultant mitochondrial pyruvate may then be reconverted to oxaloacetate by pyruvate carboxylase. In the first step of FA biosynthesis cytosolic acetyl-CoA is converted into a three carbon compound called malonyl-CoA by acetyl-CoA carboxylase. A second molecule of acetyl-CoA serves as a primer on which molecules of malonyl-CoA will be added to create the growing fatty acyl chain. The actual synthesis of fatty acids takes place on a multienzyme complex called FA synthase. Acetyl and malonyl-CoA first activate this complex by binding to a specific group of proteins called acyl carrier protein (ACP). During this binding process the enzymes acetyl-CoA-ACP transacylase and malonyl-CoA-ACP transacylase remove the CoA groups from the acyl moiety to form

Figure: 5 Reactions of FA synthesis

Enzymes

1, glycolysis and oxidation of pyruvate through the pyruvate dehydrogenase complex

2, citrate lyase

3, acetyl-CoA carboxylase

4, malonyl-CoA-ACP transacylase

5, acetyl-CoA-ACP transacylase

6, enzymes invovled in formation of butyryl-ACP from malonyl-ACP: β-ketoacyl-ACP synthase, β-ketoacyl-ACP reductase, 3-hydroxyacyl-ACP dehydrase, and enoyl-CoA reductase.

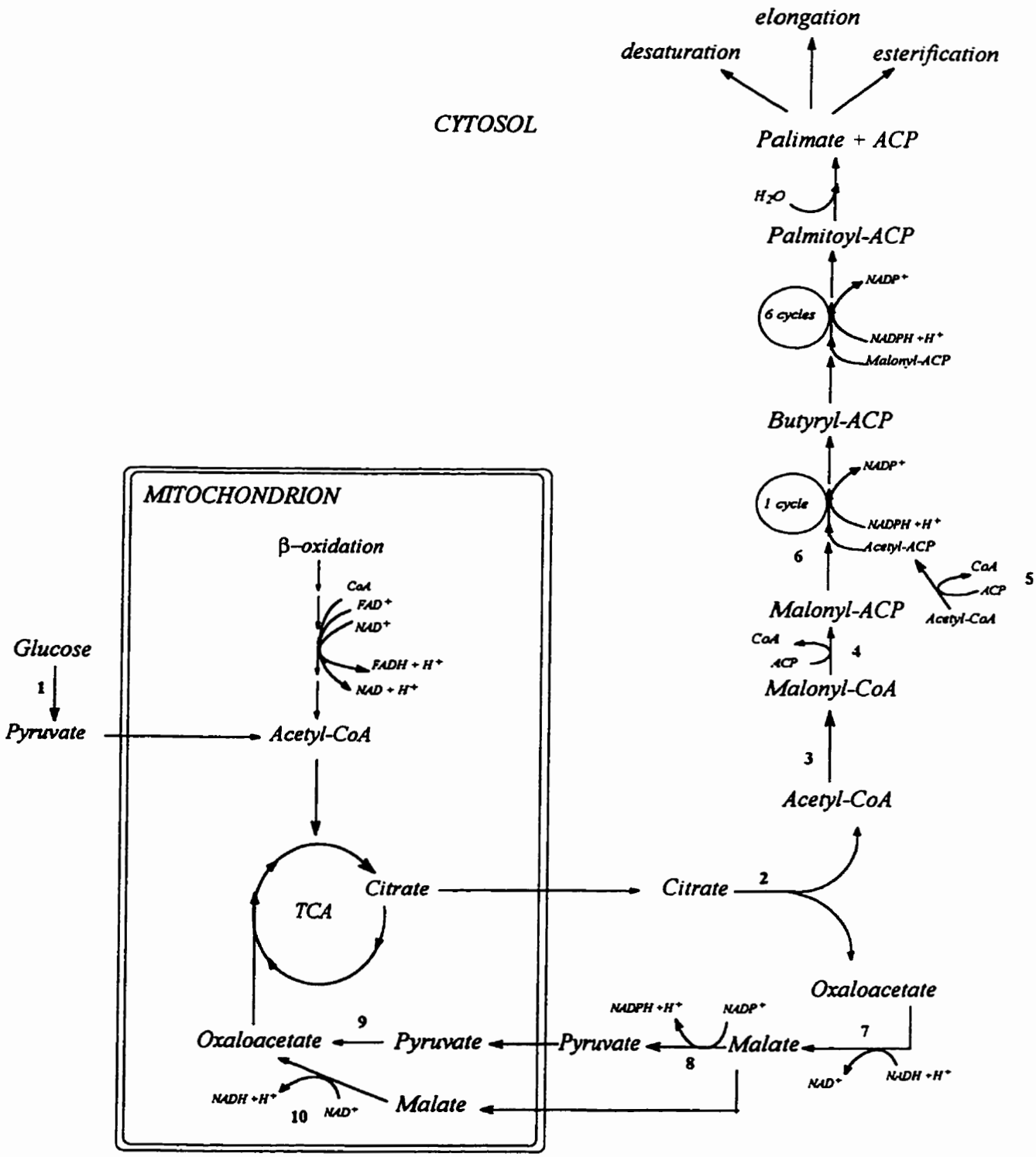
7, cytosolic malate dehydrogenase

8, malic enzyme

9, pyruvate carboxylase

10, mitochondrial malate dehydrogenase

FA SYNTHESIS



acetyl-ACP and malonyl-ACP derivatives respectively. These acyl-ACP derivatives then enter a condensation reaction and series of reactions requiring NADPH generated from malic enzyme and the pentose phosphate pathway to produce a four carbon FA derivative, butyryl-ACP. Butyryl-ACP remains attached to the multienzyme complex where it undergoes successive reactions with new molecules of malonyl-ACP to form the growing fatty acid chain (FAC). The repetitive addition of malonyl-ACP to the acyl-ACP moiety usually continues for seven cycles from which the 16 carbon FA derivative palmitoyl-ACP is formed. Palmitoyl-ACP undergoes hydrolysis to yield palmitate and free ACP. The FA synthase complex primarily produces palmitic acid. The synthesis of longer FA involves the process of chain elongation which occurs in the endoplasmic reticulum and mitochondria. In the former compartment, chain elongation occurs by successive additions of acetyl-CoA, while in the latter compartment malonyl-CoA is the donor of acetyl residues. Once formed, FA are esterified to phosphorylated glycerol derivatives (glycerol-3-phosphate or dihydroxyacetone phosphate) to synthesize TAG or GPL. Synthesized FA may also be desaturated to unsaturated FA; these can serve as persursors for other unsaturated FA. Hepatocytes are also able to synthesize sterols, the most common being cholesterol (CT) (Vlahcevic *et al.*, 1994). Through an elaborate pathway of 26 different reactions, acetyl-CoA molecules are used as a substrate to synthesize CT. This sterol can then either be incorporated into cell memebranes as an important structural constituents or modified and conjugated to the amino acids glycine or taurine to form bile salts, or liberated to other tissues to be used in production of steroid hormones.

Hepatic lipids are constantly being synthesized, transformed and degraded so that

steady state levels are maintained within certain limits (Marinetti, 1990). They may be retained within hepatocytes for intracellular activities or packaged in a covering of apoproteins to be exported to peripheral tissues as lipoproteins. When one or more of these processes are disturbed by starvation or disease, various metabolic disorders can develop. In murine cysticercosis hepatic glucose metabolism is altered (Corbin *et al.*, 1996) and host body fat content is significantly reduced (Crompton et al., 1985). These observations indicate that the parasite exerts a systemic starvation effect on the host and that in addition to changes in carbohydrate metabolism, lipid metabolism of the host must also be severly impaired. Therefore in the second part of my study I investigated changes in the hepatic lipid profile of mice with experimental cysticercosis.

Several different techniques exist whereby lipids may be analyzed. Methods commonly employed include thin layer and column chromatographic techniques. While these methods provide the simultaneous detection and quantification of several lipids in a given sample, they are often tedious and time consuming, as GPL and FA are required to react with many reagents which sequentially degrade and hydrolyze the lipid molecules (Christie, 1982). In recent years MRS has emerged as an alternative technique where whole intact lipids can be analyzed without chemical perturbation. Many MRS studies have successfully employed ^{31}P MRS in the investigation of phosphorus containing lipids (Meneses and Glonek, 1988; Reddy et al., 1996). However, the low sensitivity of the ^{31}P nucleus detracts from this technique as large amounts of sample ($\geq$100 mg) are required to compensate for the low sensitivity of the technique. Observation of the ^{1}H nucleus by proton MRS provides a much more sensitive tool for lipid analysis. Second only to water, lipids

in tissues are highly detectable by proton MRS. Major GPL, sterols and neutral lipids, as well as FA are represented simultaneously in the proton MR spectrum and information about average chain length and degree of unsaturation of FA can also be obtained (Sze and Jardetzky, 1990b; Pollesello *et al.*, 1993). The increased sensitivity and spectral dispersion resulting from high field spectrometers (500 MHz and greater) has greatly facilitated both qualitative and quantitative analysis using proton MR spectra. In addition, quantitative MRS lipid data correlate well with traditional chromatographic results (Sparling *et al.*, 1989; Casu *et al.*, 1991). Although proton MRS lipid profiles are complex, one and two dimensional proton MRS experiments have assigned nearly all of the lipid resonances visible in the one dimensional proton MR spectrum (Sze and Jardetzky, 1990b; Casu *et al.*, 1991; Adrosraku *et al.*, 1994). Based on the above information it was decided to employ proton MRS for this experiment.

HYPOTHESIS

It is hypothesized that cysticerci of *Taenia crassiceps* will modify both lipid and carbohydrate metabolic activity of host liver and that these modifications can be detected by magnetic resonance spectroscopy.

MATERIALS AND METHODS

HOST AND PARASITE

Four month old female Swiss-Webster mice were used in this experiment. The mice were maintained in accordance with the principles of the Canadian Council on Animal Care as stated in " *Guide to the Care and Use of Experimental Animals*" and were allowed to feed on commercial pellets and water *ad libitum*.

Cysticerci of *T. crassiceps* (Manitoba strain) were maintained by serial inoculation into Swiss-Webster mice by the method described by Freeman (1962).

CARBOHYDRATE METABOLISM

INFECTION AND TISSUE COLLECTION

Forty-eight mice were randomly separated into four groups of twelve mice each. Two groups of mice were infected via intraperitoneal (i.p.) injection with 0.5 ml of packed *T. crassiceps* larvae each, while the remaining two groups of mice served as uninfected controls. Prior to injection of labelled compounds all animals were fasted for 16 hours. Then each day, between days 110 - 123 post infection (p.i.), several mice from each group were anesthetized intramuscularly with a mixture of ketamine and xylazine (87 mg/kg and 13 mg/kg respectively) and a subcostal incision was made to expose the proximal end of the

duodenum. Mice in one infected and one uninfected group each received a single intraduodenal injection of a L-[3-^{13}C] alanine (99 atom % from MSD Isotopes) solution. The [3-^{13}C] alanine, dissolved in 0.85 % NaCl at a concentration of 50 mg/ml of solution, was delivered as a 0.52 ml injection over a duration of five minutes. The mice in the other two groups each received, in a similar manner, an equimolar amount of L-[3-^{13}C] lactate (99.4 atom% from ISOTEC Inc.). The animals in all groups were left to metabolize the labelled compounds under anesthesia for two hours. At the end of the two hour period the livers of all animals were excised, rinsed with saline, immersed in liquid nitrogen (N_2) and weighed. After the liver samples were collected the animals were euthanized by cervical dislocation. For infected mice cysticerci were collected from the peritoneal cavity, rinsed several times in saline, strained, frozen in liquid N_2 and weighed. All samples were kept at -70° C until preparation of perchloric acid (PCA) extracts. The experimental procedures described above were performed between 9:00A.M. and 12:00 noon on each day the experiment was conducted.

PREPARATION OF PCA EXTRACTS AND MRS SAMPLES

Each frozen sample of liver or larvae was transferred to a precooled mortar where it was pulverized in liquid N_2 with a pestle. The pulverized sample was then placed into a precooled glass homogenizing tube with 4 ml of 0.5 M perchloric acid per gram of tissue and homogenized while surrounded by an ice bath. After homogenization the suspension was centrifuged at 15,000 rpm for 10 min at -2° C. The supernatant was then removed,

neutralized using KOH, centrifuged again to remove precipitated potassium perchlorate and frozen at -70° C until lyophilization. Following lyophilization each sample was prepared for MR analysis by resuspending the freeze dried residue in 1.3 ml of deuterium oxide (D_2O) (99.3 atom % D from MSD Isotopes) and 0.2 ml of a 3.15 x 10^{-2} M solution of sodium [2,2,3,3-$^2H_4$]-3-trimethylsilypropionate (TSP) (MSD Isotopes) in D_2O. TSP served as an intensity and chemical shift standard. Following one hour of stirring the sample was adjusted to a pH meter reading of 7.3, and then centrifuged at 12,000 rpm and 4° C for 25 min. The supernatant was then transferred to a 5 mm nuclear magnetic resonance (NMR) tube for analysis.

MR SPECTROSCOPY

MR spectra were obtained with a Bruker AMX-500 NMR spectrometer operating at 500.1 and 125.8 MHz for ^1H and ^{13}C, respectively. Proton spin-echo spectra were obtained with ^{13}C decoupling using the GARP sequence (Shaka *et al.*, 1985) and with presaturation of the solvent resonance. The assumed averaged carbon-proton coupling constant was 143 Hz. A spectral width of 6578.9 Hz, a recycle time of 13.3 sec, and 16K data points were used for acquisition. Scans acquired with, and without, ^{13}C population inversion were acquired alternatively in blocks of 16 scans and stored in sepatrate computer memory locations. Inverting the ^{13}C puts the attached protons 180° out of phase with respect to protons attached to ^{12}C and other nuclei. Consequently, resonances from protons attached to ^{13}C cancel when the separately stored spectra are added and all other resonances

cancel, leaving only resonances of protons attached to ^{13}C, when spectra are subtracted. Chemical shifts are given relative to TSP at 0 ppm. Peak assignments were based on published data (Evanochko *et al.*, 1984; Gilroy *et al.*, 1988; Desmoulin *et al.*, 1990; Sze and Jardetzky, 1990; Berners-Price *et al.*, 1991; Yacoe *et al.*, 1991) and the spectra of authentic compounds.

QUANTIFICATION OF METABOLITES

Quantitative measurements were made by integration of metabolite peaks relative to TSP as an intensity standard. Integration was performed with a sub-routine from the software on either a Bruker AMX-500 spectrometer or a Bruker X-32 data station. The integration routine used with this program permits separate adjustments of slope and bias for each peak, or groups of peaks, and is therefore particularly useful for determining the area of a peak or set of peaks, superimposed on a broad resonance. The areas were corrected for the number of protons contributing to each signal, and in the case of glucose, for the relative amounts of anomers. Concentrations, in μmoles/g wet weight, were calculated using the recorded weight of the tissue. The percent carbon-13 in metabolites was determined using the [^{12}C + ^{13}C] spectrum and the sum of the [^{12}C + ^{13}C] and [^{12}C - ^{13}C] spectra which generates a [^{12}C] spectrum. Given that ^{13}C has a 1.1% natural abundance, the following equation can be applied to calculate percent carbon-13.

$$100 - [\{ [^{12}C] (0.989) / [^{12}C + ^{13}C]\} \times 100] = \% \, ^{13}C$$

Statistical analysis was performed with the SAS statistical program. Data were

analyzed statistically using analysis of variance (ANOVA) where a P value of ≤ 0.05 was deemed significant.

LIPID METABOLISM

INFECTION AND TISSUE COLLECTON

Twenty-four mice were randomly separated into two groups of twelve mice each. The mice in one group were infected via i.p. injection with 0.5 ml of *T. crassiceps* cysticerci each. The remaining twelve mice served as uninfected controls. From 120 to 122 days p.i., between 9:00 A.M. and 12:00 noon, several mice from each group were anesthetized intramuscularly with a mixture of ketamine and xylazine (87 mg/kg and 13 mg/kg respectively), their abdomens opened and their livers excised. The organs were rinsed with saline, immersed in liquid N_2 and weighed. The animals were sacrificed by cervical dislocation. For infected mice cysticerci were collected from the peritoneal cavity, rinsed several times in saline, strained, weighed and frozen in liquid N_2. All samples were kept at -70° C until preparation of lipid extracts.

PREPARATION OF LIPID EXTRACTS AND MR SAMPLES

Each frozen sample of the liver or the larvae was transfered to a precooled mortar where it was pulverized in liquid N_2 with a pestle. The pulverized sample was then placed in a 50 ml beaker (precooled in an ice bath) with 20 ml of a mixture of chloroform (HPLC grade-hydrocarbon stabilized- from EM Inc.) and methanol (HPLC grade from BDH Inc.) (2:1, v/v) per gram of tissue as described by Folch *et al.* (1957) and homogenized with a Brinkman Polytron homogenizer while surrounded by an ice bath. The homogenized

suspension was then vaccum filtered using a sinter glass crucible and the filtrate retained. The residue was re-extracted with the same volume of fresh chloroform-methanol (C/M) mixture used initially and the filtrates were pooled and washed twice with equal volumes of 0.5 M KCl in 50% methanol to remove non-lipid substances. After washing, the lipid rich chloroform layer was rotary evaporated to dryness under N_2. The lipid residue was then dissolved in 5ml of benzene and again rotary evaporated to dryness under N_2 to azeotropically remove water. Resuspension of the residue with benzene and rotary evaporation was repeated a second time. The dried residue was then stored under N_2 at -20° C until preparation of MR samples.

Each sample was prepared for MR analysis by resuspending the dried residue in 2.5 ml of chloroform per gram of tissue with 2.5 $x10^{-3}$ g of tetrakis(trimethylsilyl)silane (TMSS) (Aldrich Chem. Company Inc.) added as an intensity standard. The suspension was then centrifuged at 2,500 rpm for 10 minutes. For liver samples, 0.20 ml of supernatant was added to a 1.0 ml mixture of $CDCl_3$ (99.8 atom % D from CDN Isotopes) and CD_3OD (99.8 atom % D from CDN Isotopes) (2:3, v/v). To increase the concentrations of lipids in the final MR sample of parasite tissue, 0.4 ml of the supernatant was added to 0.6 ml of CD_3OD. All samples were transferred to 5mm NMR tubes for proton MR analysis.

MR SPECTROSCOPY

The ^{1}H MR spectra of the tissue lipid extracts were obtained at a temperature of 27°C using a Bruker AMX-500 spectrometer operating at 500.14 Mhz in the quadrature detection mode. Spectra were accumulated locked on the methyl deuterons of CD_3OD, with a pulse interval of 10.2 sec at a flip angle of 78° for 160 scans. Off resonance presaturation, using a shaped gauss 1024 pulse at 59 dB, was applied to reduce the chloroform peak. The spectral width was 4504.50 Hz with 32 K data points used for acquisition. Chemical shifts are given relative to the C-18 methyl of cholesterol at 0.68 ppm. Peak assignments were based on published data (Sparling *et al.*, 1989; Sze and Jardetzky, 1990; Casu *et al.*, 1991; Adosaraku *et al.*, 1993; Choi *et al.*, 1993; Adosarku *et al.*, 1994) and the spectra of authentic compounds.

QUANTIFICATION OF LIPIDS

Quantitative measurements were made by integration of lipid peaks relative to TMSS as an intensity standard. Integration was performed with a sub-routine from the software on either a Bruker AMX-500 spectrometer or a Bruker X-32 data station. Peak areas were corrected for the number of protons contributing to each signal.

Concentrations of individual lipids, total FA and various FA components, in μmol/g of wet weight, were calculated using the recorded weight of the tissue. The FAC composition was also determined, the sum of the ω-CH_3 proton resonances at 0.86 ppm and

0.95 ppm was used as a measure of total FA content. The values for FA components are presented as ratios relative to total FA content. The average FAC length was calculated using the following equation...

Average FAC length = $1_a + 1_b$ + (-(CH_2)n- : total FA) + (-$\underline{CH_2}CH_2COO$: total FA) + (-CH_2COO : total FA) + 2(-CH=CH- : total FA) + (-$\underline{CH_2}CH{=}CHCH{=}CH\underline{CH_2}$: total FA) +(-CH=CH($\underline{CH_2}$CH=CH)$_n$: total FA) + (-CH=CH$\underline{CH_2}\underline{CH_2}COO^-$ of docosahexaenoic acid: total FA) + (-CH=CH$\underline{CH_2}$CH=CH- of linoleic acid : total FA) + (-CH=CH$\underline{CH_2}CH_2CH_2COO^-$ of arachidonic acid : total FA)

where $1_a + 1_b$ are equal to one terminal methyl group (ω-CH_3) and one carbonyl group (C=O) per FA chain, respectively.

Statistical analysis was performed using the SAS statistical computer program. Data was analyzed using ANOVA where a value of $P \leq 0.05$ was deemed significant.

RESULTS

CARBOHYDRATE METABOLISM

In the [3-^{13}C]alanine experiment, the infected mice had on average 9.72 ± 1.65g of larvae whereas those in [3-^{13}C]lactate experiment, had on average 8.49± 2.20g of larvae (Table. 1). In both experiments the livers from these infected mice were lighter than those of the corresponding uninfected control mice.

Representative ^{13}C-decoupled ^{1}H MR spin echo spectra, without ^{13}C population inversion, of PCA liver extracts from uninfected (control) and infected mice and of parasite extracts after the introduction of exogenous [3- ^{13}C]alanine or [3- ^{13}C]lactate are presented in Fig. 6 - 11. Only major, well resolved resonances were quantitatively evaluated due to the complex overlap between resonances of several compounds and the low concentration of many metabolites present in the spectra. The peaks which were evaluated were numbered as follows: 1, H-1 of glucose units having a 1-4 linkage in glycogen; 2, H-1 of α-glucose; 3, CH_2 of glycine; 4, CH_2S of taurine; 5, $(CH_3)_3N$ of betaine; 6, $(CH_3)_3N$ of glycerophosphocholine (GPC); 7, $(CH_3)_3N$ of phosphocholine (PC); 8, $(CH_3)_3N$ of choline; 9, $(CH_3)_3N$ of acylcarnitine; 10, CH_2N of lysine; 11, CH_2 of citrate; 12, CH_2CH_2 of succinate; 13, γ-CH_2 of glutamate; 14, CH_3 of acetate; 15, CH_3 of alanine; 16, CH_3 of lactate; 17, CH_3 of β-hydroxybutyrate; 18, CH_2 of FAC and peak 19, CH_3 of FAC. The spectra of liver extracts from mice injected with [3-^{13}C]alanine or [3-^{13}C]lactate showed

Table 1. Masses of mouse liver and *T. crassiceps* cysticerci .

Group (n=12)	liver mass (g)	cysticerci mass (g)
	3-^{13}C alanine experiment	
uninfected	1.18 ± 0.11	___
infected	1.00 ± 0.15*	9.72 ±1.65
	3-^{13}C lactate experiment	
uninfected	1.10 ± 0.09	___
infected	0.96 ± 0.13*	8.49 ±2.20

All values are given as means ± S.D.
* Indicates significant difference ($P \leq 0.05$) from corresponding uninfected liver mass.

Fig. 6. A ^{13}C-decoupled ^{1}H MR spectrum of liver from an uninfected mouse after the introduction of [3-^{13}C]alanine.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

4, CH_2S of taurine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

12, CH_2CH_2 of succinate

13, γCH_2 of glutamate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate

17, CH_3 of β-hydroxybutyrate

✢ Indicates water

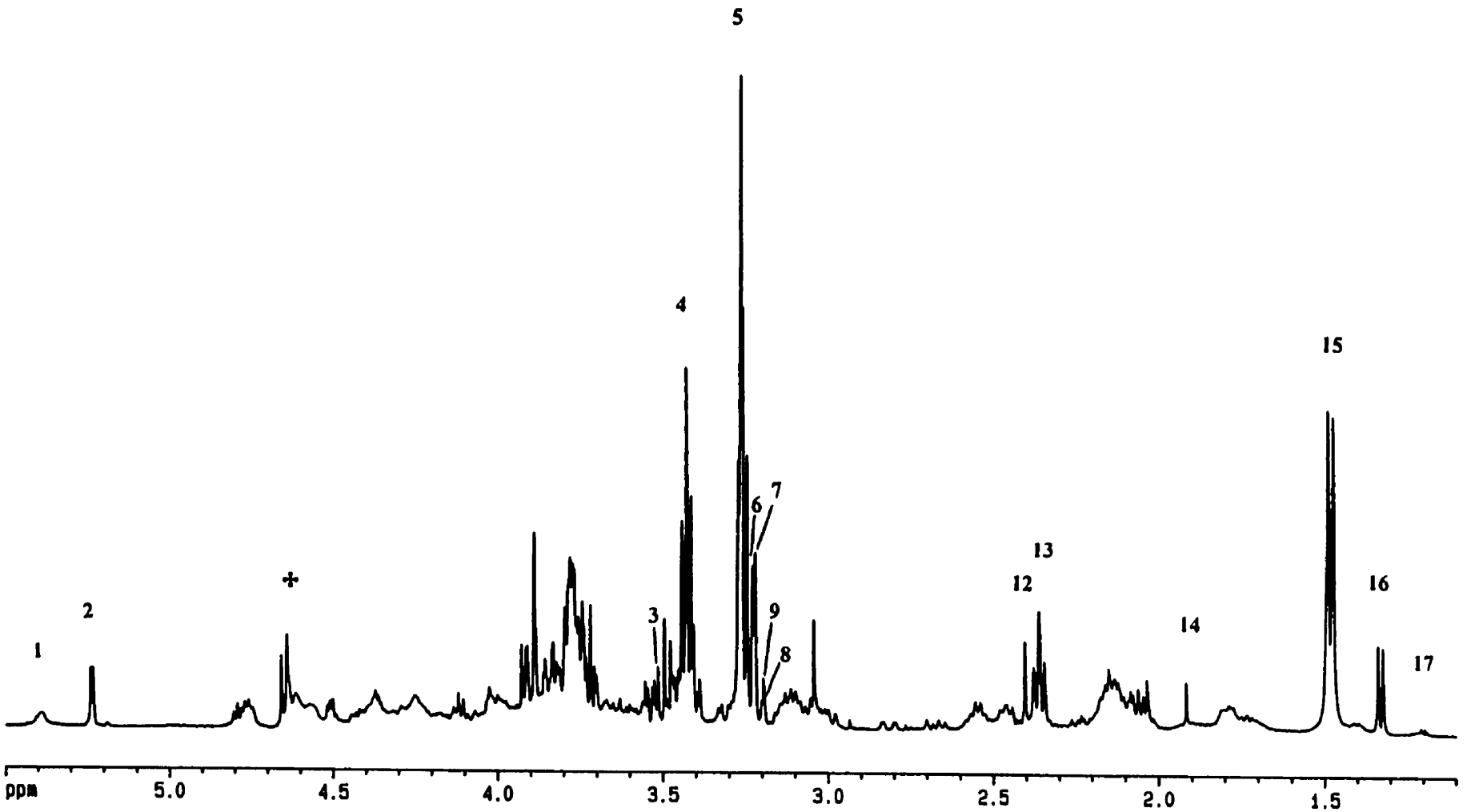

5
4
15
6
7
13
12
16
2
3
9
14
1
8
17
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Fig. 7. A ^{13}C-decoupled ^{1}H MR spectrum of liver from an infected mouse after the introduction of [3-^{13}C]alanine.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

4, CH_2S of taurine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

12, CH_2CH_2 of succinate

13, γCH_2 of glutamate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate

17, CH_3 of β-hydroxybutyrate

+ Indicates water

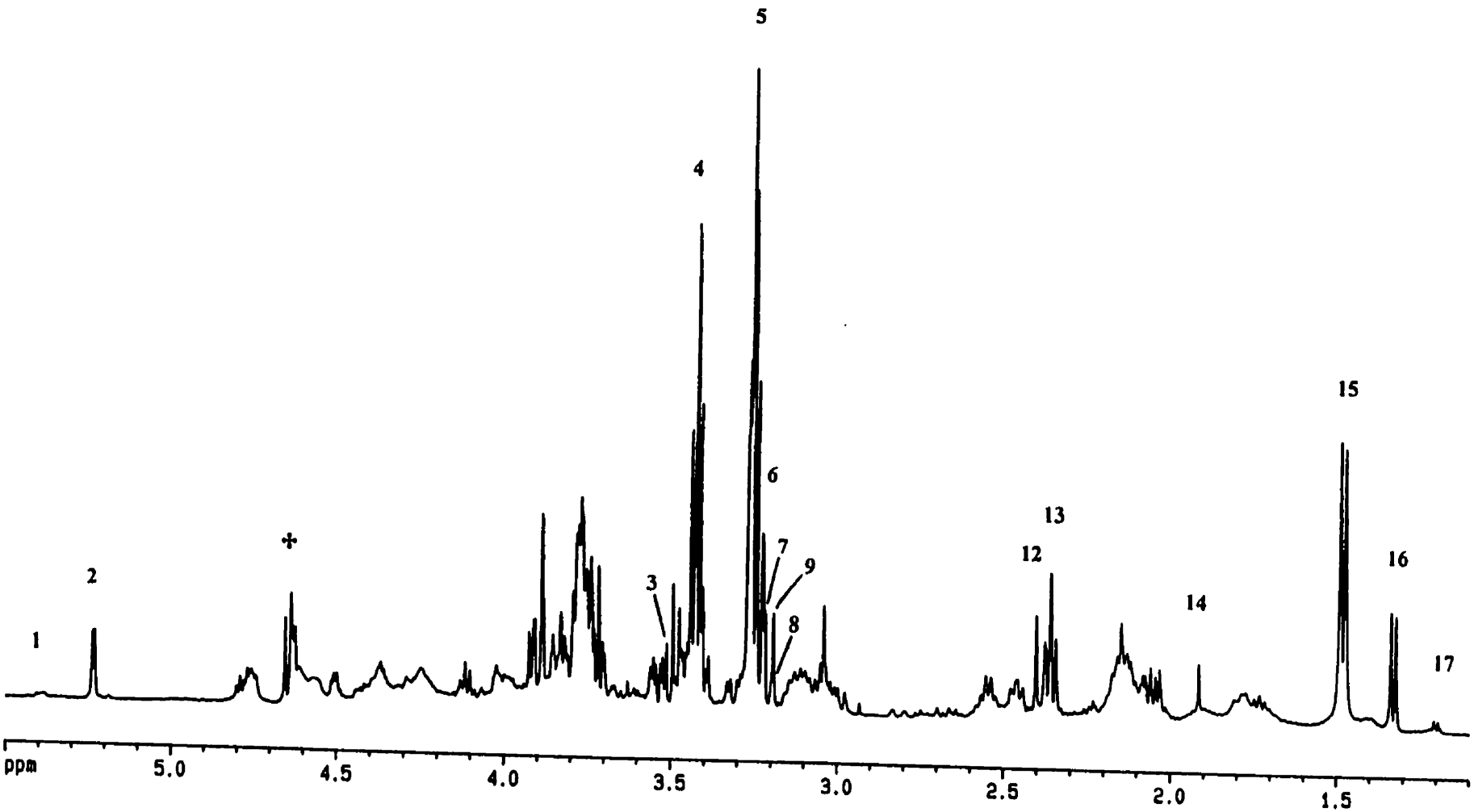

1
2
3
4
5
6
7
8
9
12
13
14
15
16
17
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Fig. 8. A ^{13}C-decoupled ^{1}H MR spectrum of *T. crassiceps* larvae after the introduction of [3-^{13}C]alanine to the host.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

10, CH_2N of lysine

11, CH_2 of citrate

12, CH_2CH_2 of succinate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate

18, CH_2 of FAC

19, CH_3 of FAC

+ Indicates water

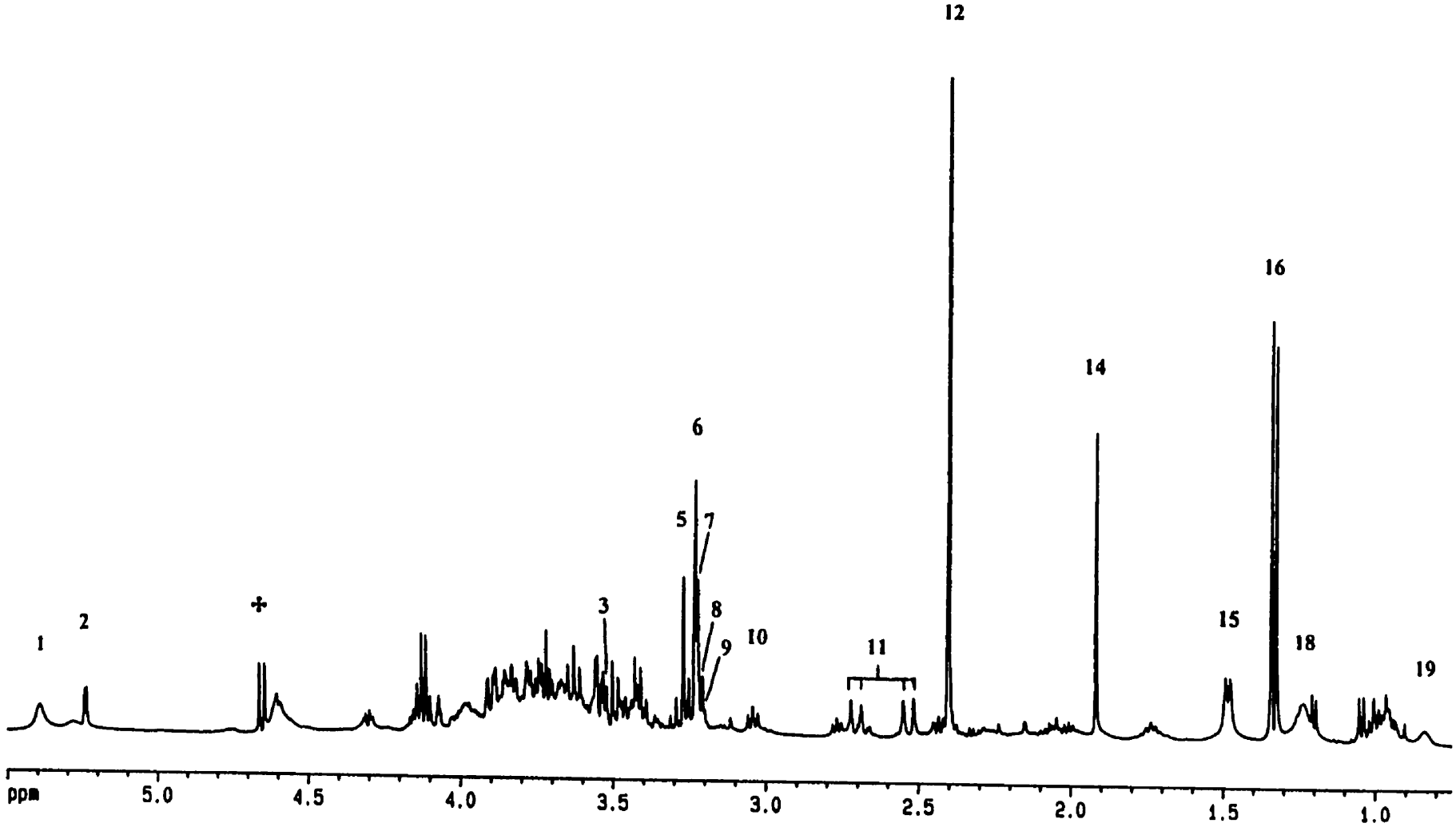

12
16
14
6
5
7
3
8
9
10
11
15
18
19
1
2
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Fig. 9. A ^{13}C-decoupled ^{1}H MR spectrum of liver from an uninfected mouse after the introduction of [3-^{13}C]lactate.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

4, CH_2S of taurine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

12, CH_2CH_2 of succinate

13, γCH_2 of glutamate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate

17, CH_3 of β-hydroxybutyrate

+ Indicates water

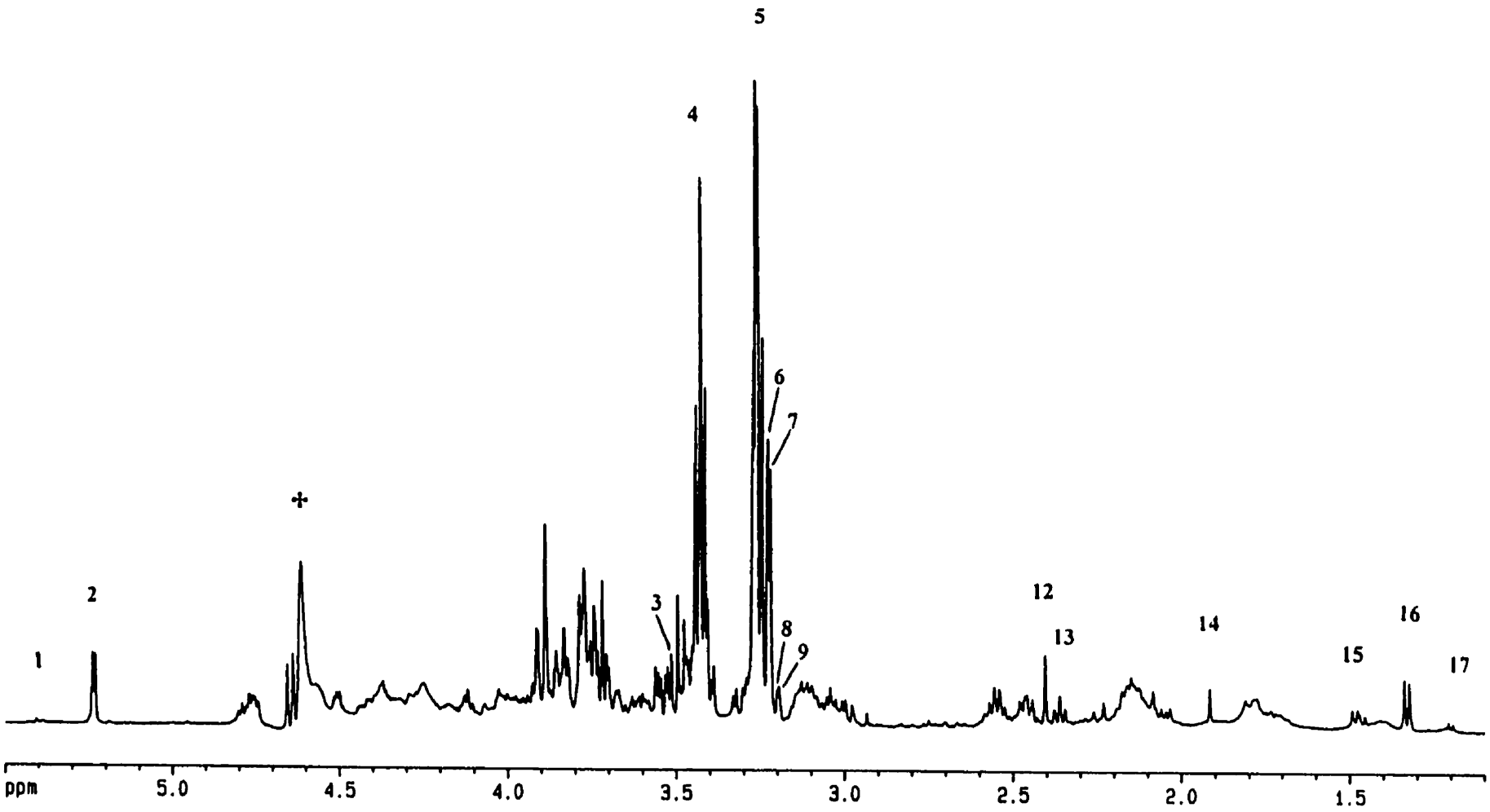

1
2
3
4
5
6
7
8
9
12
13
14
15
16
17
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Fig. 10. A ^{13}C-decoupled ^{1}H MR spectrum of liver from an infected mouse after the introduction of [3-^{13}C]lactate.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

4, CH_2S of taurine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

12, CH_2CH_2 of succinate

13, γCH_2 of glutamate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate

17, CH_3 of β-hydroxybutyrate

+ Indicates water

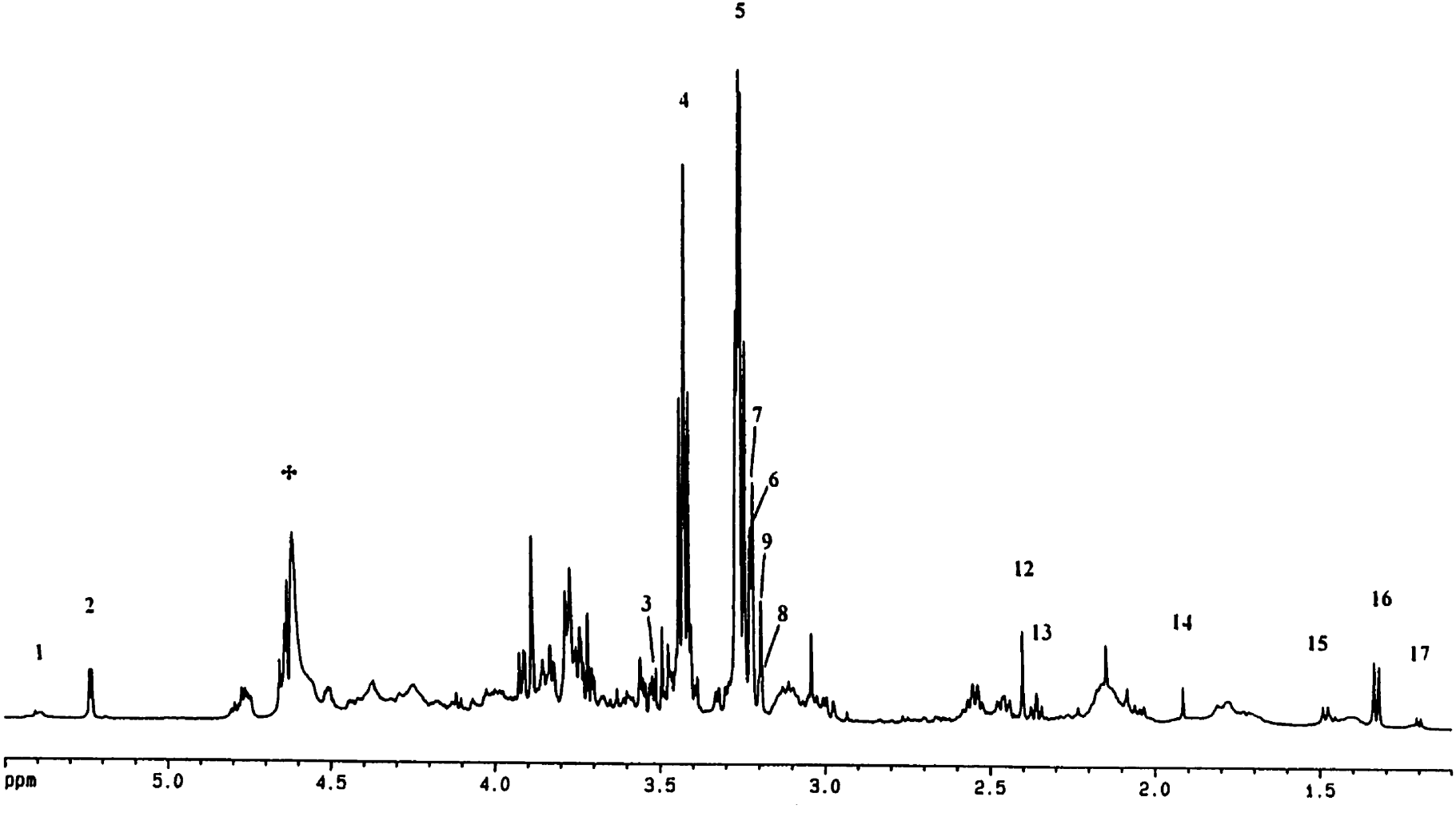

1
2
3
4
5
6
7
8
9
12
13
14
15
16
17
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Fig.11. A ^{13}C-decoupled ^{1}H NMR spectrum of *T. crassiceps* larvae after the introduction of [3-^{13}C]lactate to the host.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

3, CH_2 of glycine

5, $(CH_3)_3N$ of betaine

6, $(CH_3)_3N$ of glycerophosphocholine (GPC)

7, $(CH_3)_3N$ of phosphocholine (PC)

8, $(CH_3)_3N$ of choline

9, $(CH_3)_3N$ of acylcarnitine

10, CH_2N of lysine

11, CH_2 of citrate

12, CH_2CH_2 of succinate

14, CH_3 of acetate

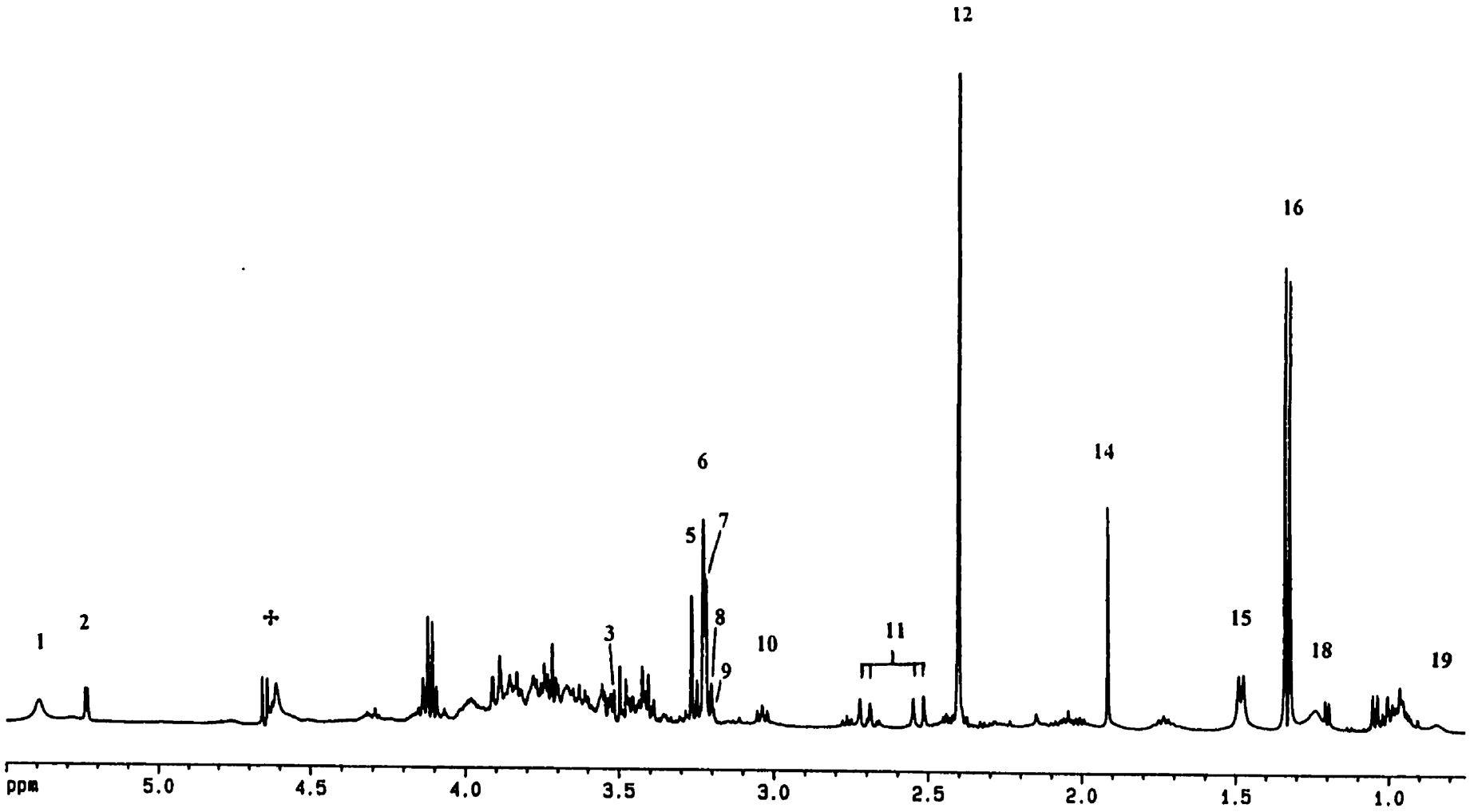
15, CH_3 of alanine

16, CH_3 of lactate

18, CH_2 of FAC

19, CH_3 of FAC

+ Indicates water


12
16
14
6
5
7
8
9
3
10
11
15
18
19
1
2
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

clear resonances of glycogen, glucose, glycine, taurine, betaine, GPC, PC, choline, acylcarnitine, succinate, glutamate, acetate, alanine, lactate and β-hydroxybutyrate (Figs. 6,7,9 and 10). However the resonances of lysine, citrate, and the methylene and methyl of FAC were not measureable. In the spectra of extracts of *T. crassiceps* from mice exposed to either labelled compound, the resonances of taurine, glutamate and β-hydroxybutyrate were not detectable, while those of lysine, citrate, the methylene and methyl of FAC were clearly visible (Figs. 8 and 11).

A representative ^{13}C-decoupled ^{1}H MR spin echo difference spectrum of a PCA liver extract from an uninfected mouse after the introduction of [3-^{13}C]alanine is presented in Fig. 11a. This spectrum is the result of computer subtraction of the [^{12}C -^{13}C] spectrum from the [^{12}C+^{13}C] spectrum, hence only protons attached to ^{13}C nuclei are depicted in this spectrum. Resonances utilized for percent carbon-13 analysis are indicated by peak numbers on the spectrum and correspond to the metabolites listed in Table 3. Resonances not utilized include those from, H-1 of β-glucose at 4.64 ppm, H-2 to H-6 of α, β-glucose and glycogen which produce overlapping resonances extending from 3.90 to 3.24 ppm, methylenes of aspartate at 2.81 and 2.68 ppm, and glutamine, glutathione and β CH_2 of glutamate which give rise to broad resonances at 2.45 to 2.6 ppm and 2.0 to 2.2 ppm. Similar difference spectra result from PCA extracts of livers of infected mice and *T. crassiceps* larvae of the alanine experiment and from liver and larvae extracts of the lactate experiment.

Fig. 11a. A ^{13}C-decoupled ^{1}H MR difference spectrum of liver from an uninfected mouse after the introduction of [3-^{13}C]alanine.

Peak numbers, include:

1, H-1 of glucose units having a 1-4 linkage in glycogen

2, H-1 of α-glucose

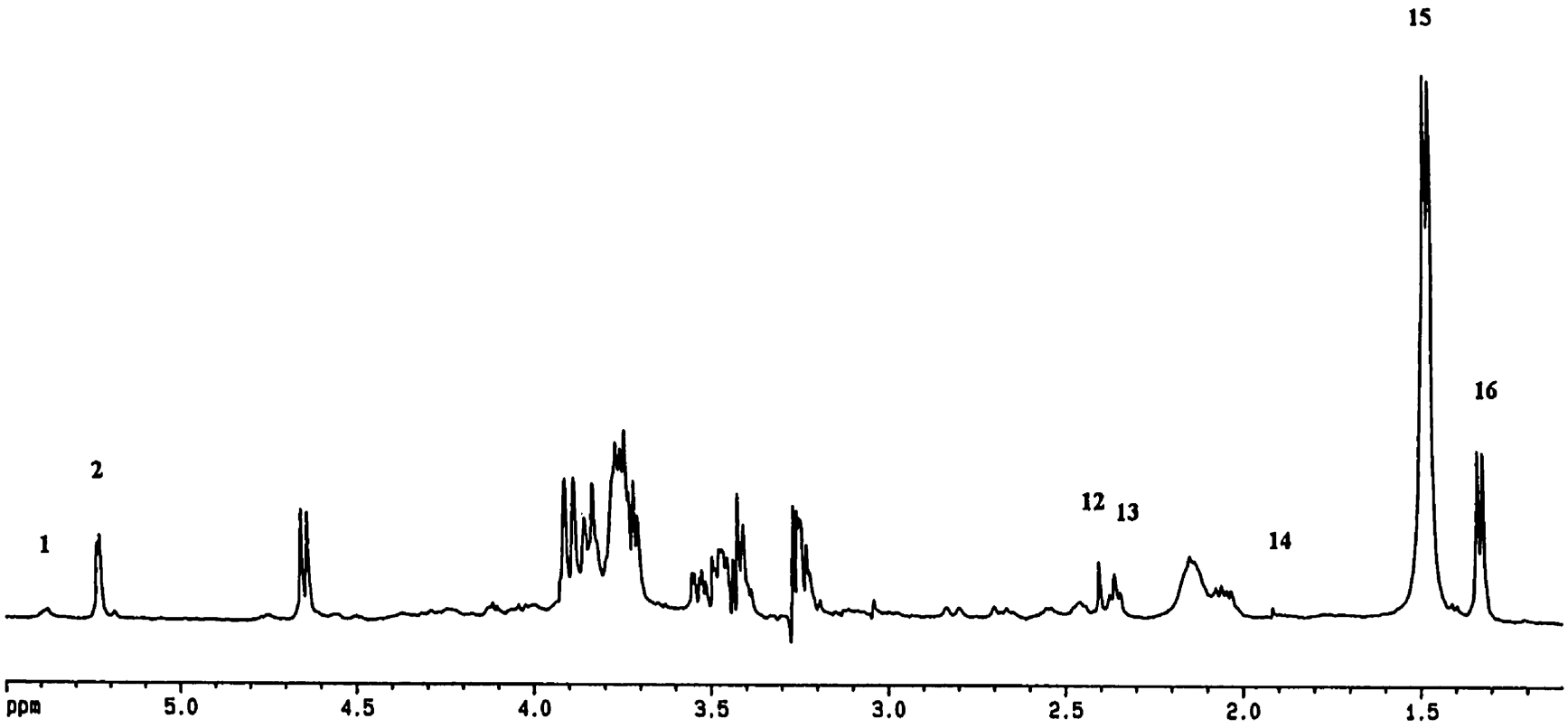
12, CH_2CH_2 of succinate

13, γCH_2 of glutamate

14, CH_3 of acetate

15, CH_3 of alanine

16, CH_3 of lactate


15
16
2
1
12
13
14
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

[3-^{13}C]ALANINE METABOLISM

The total concentrations (unlabelled plus labelled) of hepatic and parasite metabolites are presented in Table 2. The livers of infected mice had less glucose and glutamate, but more betaine, acylcarnitine and β-hydroxybutyrate. When concentrations of *T. crassiceps* metabolites were compared to hepatic metabolites of infected mice, it was found that cysticerci had higher concentrations of glycogen, glycine, GPC, choline, succinate, acetate and lactate but lower concentrations of glucose, betaine, acylcarnitine and alanine.

Carbon-13 labelled metabolites found in the livers of uninfected and infected mice and in the parasite are presented in Table 3. The livers of both groups of animals contained label in glycogen, glucose, succinate, glutamate, alanine and lactate. Livers from uninfected animals also contained some enrichment in acetate, whereas in those from infected mice the presence of carbon-13 in acetate above natural abundance is dubious. Alanine possessed the highest percent ^{13}C followed in desending order in lactate, glucose, succinate, glutamate, glycogen and acetate. When compared to controls, the livers of infected mice had a lower percent of carbon-13 in alanine and lactate. The percent carbon-13 in other metabolites did not differ statistically between the two liver groups. *T. crassiceps* larvae contained carbon-13 label in glucose, acetate, alanine and lactate. There appears to be no enrichment in glycogen and for succinate it is questionable as to the presence of carbon-13 above the natural abundance level. The majority of label present in the larvae appeared in alanine and lactate followed by that in glucose and acetate. All the labelled metabolites of *T. crassiceps* contained significantly less label than the corresponding hepatic metabolites of the host.

Table 2 . Concentrations (μmol /g wet wt) of metabolites from liver extracts of uninfected and infected mice, and from *Taenia crassiceps* larvae, after introduction of [3-^{13}C]alanine into the host.

Peak No.	Metabolite	LIVER* Uninfected (n=12)	LIVER* Infected (n=12)	*T. crassiceps* larvae (n=12)
1	Glycogen**	2.07± 0.75[a]	1.39± 0.68[a]	3.68± 0.59†
2	Glucose	7.99± 1.17[a]	6.49± 1.48[b]	3.85± 0.42†
3	Glycine	0.23± 0.07[a]	0.22± 012[a]	0.42± 0.14†
4	Taurine	12.57± 1.47[a]	11.97± 2.70[a]	NM
5	Betaine	1.58± 0.25[a]	2.04± 0.64[b]	0.35± 0.07†
6	GPC	0.64± 0.04[a]	0.66± 0.09[a]	0.77± 0.10†
7	PC	0.48± 0.13[a]	0.39± 0.11[a]	0.47± 0.10
8	Choline	0.05± 0.02[a]	0.05± 0.01[a]	0.13± 0.02†
9	Acylcarnitine	0.15± 0.03[a]	0.21± 0.04[b]	0.04± 0.01†
10	Lysine	NM	NM	0.86± 0.18
11	Citrate	NM	NM	1.68± 0.29
12	Succinate	0.30± 0.05[a]	0.38± 0.10[a]	5.60± 0.93†
13	Glutamate	3.47± 0.73[a]	2.47± 0.82[b]	NM
14	Acetate	0.32± 0.07[a]	0.32±0.09[a]	1.75± 0.33†
15	Alanine	10.18± 3.71[a]	7.79± 4.39[a]	3.54± 0.67†
16	Lactate	1.48± 0.30[a]	1.94± 0.55[a]	7.38± 1.03†
17	β-Hydroxybutyrate	0.04± 0.01[a]	0.07± 0.02[b]	NM
18	CH_2 of FAC	NM	NM	3.50± 0.98
19	CH_3 of FAC	NM	NM	0.53± 0.14

* [a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other.
** CH-1 of glucose units having a 1-4 linkage in glycogen.
† Indicates significant difference ($P \leq 0.05$) from corresponding infected mouse metabolite.
NM , Not measureable.

Table 3 . Percent carbon-13 in metabolites from liver extracts of uninfected and infected mice, and from *Taenia crassiceps* larvae, after introduction of [3-^{13}C]alanine into the host.

Peak No.	Metabolite Name	Measured Position	LIVER* Uninfected (n=12)	LIVER* Infected (n=12)	*T.crassiceps* larvae (n=12)
1	Glycogen **	CH-1	5.07± 1.66[a]	4.09± 1.92[a]	1.15± 2.13†
2	α-Glucose	CH-1	11.13± 1.88[a]	10.67± 2.23[a]	2.83± 1.36†
12	Succinate	CH_2CH_2	9.36± 0.82[a]	8.46± 2.08[a]	1.85± 0.78†
13	Glutamate	γCH_2	6.73± 1.88[a]	6.34± 2.62[a]	NM
14	Acetate	CH_3	2.45± 0.71[a]	1.73± 0.95[a]	2.85± 1.41†
15	Alanine	CH_3	48.51± 6.01[a]	37.29± 7.34[b]	6.59± 3.06†
16	Lactate	CH_3	39.65± 5.93[a]	32.20± 7.96[b]	5.22± 1.46†

* [a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other.
** CH-1 of glucose units having a 1-4 linkage in glycogen.
† Indicates significant difference ($P \leq 0.05$) from corresponding infected mouse metabolite.
NM , Not measureable.

[3-^{13}C]LACTATE METABOLISM

The total concentrations (unlabelled plus labelled) of hepatic and parasite metabolites are presented in Table 4. The livers of infected mice contained less glucose and PC but more betaine, choline, acylcarnitine and β-hydroxybutyrate than those of controls. When concentrations of parasite metabolites were compared to those from livers of infected mice, the larvae had more glycogen, glycine, choline, succinate, acetate, alanine and lactate, but less betaine and acylcarnitine.

Carbon-13 labelled metabolites found in the livers of uninfected and infected mice and in the parasite are presented in Table 5. In the livers of uninfected mice carbon-13 label from [3-^{13}C]lactate was found in glycogen, glucose, succinate, glutamate, alanine and lactate. The same hepatic metabolites were also labelled in infected mice except for glycogen, where the presence of carbon-13 above natural abundance is questionable. In both groups of mice lactate and alanine possessed the highest percentage of carbon-13 followed by that in glucose, succinate, glutamate and, in controls, in glycogen. When percent carbon-13 was compared between the two groups, a significantly lower percentage of carbon-13 was observed in hepatic glycogen, succinate, glutamate, and lactate of infected animals. *T. crassiceps* larvae contained carbon-13 label in glucose, alanine and lactate. However, the percent carbon-13 in these three metabolites was lower than that in the corresponding metabolites of the infected hosts.

Table 4. Concentrations (μmol /g wet wt) of metabolites from liver extracts of uninfected and infected mice, and from *Taenia crassiceps* larvae, after introduction of [3-^{13}C]lactate into the host.

Peak No.	Metabolite Name	LIVER* Uninfected (n=12)	LIVER* Infected (n=12)	*T.crassiceps* larvae (n=12)
1	Glycogen **	1.23± 0.81[a]	1.96± 1.53[a]	5.88± 0.91†
2	Glucose	9.85± 1.64[a]	7.62± 1.28[b]	6.70± 1.14
3	Glycine	0.34± 0.06[a]	0.32± 0.05[a]	0.43± 0.15†
4	Taurine	18.95± 3.09[a]	19.00± 2.61[a]	NM
5	Betaine	1.76± 0.47[a]	2.40± 0.73[b]	0.45± 0.12†
6	GPC	0.85± 0.14[a]	0.79± 0.14[a]	0.88± 0.11
7	PC	1.10± 0.46[a]	0.76± 0.14[b]	0.54± 0.08
8	Choline	0.06± 0.02[a]	0.09± 0.03[b]	0.12± 0.01†
9	Acylcarnitine	0.12± 0.03[a]	0.26± 0.08[b]	0.04± 0.01†
10	Lysine	NM	NM	1.02± 0.19
11	Citrate	NM	NM	1.35± 0.33
12	Succinate	0.44± 0.06[a]	0.49± 0.11[a]	4.94± 1.33†
13	Glutamate	0.74± 0.34[a]	0.84± 0.13[a]	NM
14	Acetate	0.30± 0.10[a]	0.33± 0.10[a]	1.59± 0.33†
15	Alanine	0.65± 0.16[a]	0.78± 0.21[a]	3.15± 0.45†
16	Lactate	1.05± 0.19[a]	1.34± 0.41[a]	7.28± 1.16†
17	β-Hydroxybutyrate	0.06± 0.01[a]	0.12± 0.08[b]	NM
18	CH_2 of FAC	NM	NM	3.67± 0.90
19	CH_3 of FAC	NM	NM	0.48± 0.14

* [a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other.

** CH-1 of glucose units having a 1-4 linkage in glycogen.

† Indicates significant difference ($P \leq 0.05$) from corresponding infected mouse metabolite.

NM, Not detectable.

Table 5 . Percent carbon-13 in metabolites from liver extracts of uninfected and infected mice, and from *Taenia crassiceps* larvae, after introduction of [3-^{13}C]lactate into the host.

Peak No.	Metabolite	Measured Position	LIVER* Uninfected (n=12)	LIVER* Infected (n=12)	*T.crassiceps* larvae (n=12)
1	Glycogen	CH-1**	4.74± 2.06[a]	1.37± 0.79[b]	1.50± 1.28
2	Glucose	CH-1	7.80± 1.78[a]	7.40± 1.66[a]	3.00± 1.39†
12	Succinate	CH_2CH_2	7.49± 1.06[a]	5.21± 0.95[b]	1.65± 0.77†
13	Glutamate	γCH_2	6.09± 2.48[a]	3.91± 1.26[b]	NM
14	Acetate	CH_3	1.79± 1.08[a]	0.59± 1.80[b]	1.22± 0.55
15	Alanine	CH_3	20.47± 8.24[a]	18.07± 4.88[a]	2.58± 1.00†
16	Lactate	CH_3	22.95± 7.09[a]	17.36± 5.01[b]	2.75± 0.50†

* [a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other.
** CH-1 of glucose units having a 1-4 linkage in glycogen.
† Indicates significant difference ($P \leq 0.05$) from corresponding infected mouse metabolite.
NM , Not measureable.

LIPID METABOLISM

Mice infected with *T. crassiceps* for approximately 121 days contained on average 14.46 ± 4.35 g of larvae. The livers of these infected mice weighed less than those of uninfected controls, 1.14 ± 0.14 and 1.32 ± 0.13 g, respectively. Representative proton MR spectra of C:M extracts of liver from uninfected and infected mice and *T. crassiceps* larvae are presented in Figs. 12 - 14. Identified peaks are as follows: 1, -C<u>H</u>=C<u>H</u>- of FA chains and sterols; 2, CH of C-2 from the glycerol backbone of TAG, DAG and GPL ; 3, CH of C-1 from the glycerol backbone of GPL; 4, CH attached to C-1/C-3 of glycerol from TAG; 5, $-OC\underline{H}_2CH_2N^+(CH_3)_3$ of phosphatidylcholine (PTC); 6, CH of C-1 from glycerol backbone of TAG, DAG and GPL; 7, $-C\underline{H}_2OPO_2C\underline{H}_2-$ of GPL; 8, -CHOP of the phosphatidylinositol (PTI) ring; 9, $-C\underline{H}_2N^+(CH_3)_3$ of (PTC); 10, $-N^+(C\underline{H}_3)_3$ of choline; 11, $-C\underline{H}_2N^+H_3$ of phosphotidylethanolamine (PTE); 12, $-CH{=}CH(C\underline{H}_2CH{=}CH-)_n$ of FA; 13, -CH=CHC<u>H</u>$_2$CH=CH- of linoleic acid (LA); 14, $-CH{=}CHC\underline{H}_2C\underline{H}_2COO^-$ of docosahexaenoic acid (DHA.); 15, $-CH_2COO^-$ of FA; 16, $-CH_2COOH$ of FFA; 17, $-CH{=}CHC\underline{H}_2CH_2CH_2COO^-$ of arachindonic acid (AA); 18, $-C\underline{H}_2CH{=}CHCH{=}CHC\underline{H}_2-$ of FA; 19, $-C\underline{H}_2CH_2COO^-$ of FA; 20, $-(CH_2)_n-$ of FA and sterols; 21, ω-CH_3 of n-3 FA ; 22, ω-CH_3 of FA and sterols and 23, C-18 methyl of cholesterol . The following Identified peaks : 2, CH of C-2 from the glycerol backbone of TAG, DAG and GPL; 3, CH of C-1 from the glycerol backbone of GPL; 5, $-OC\underline{H}_2CH_2N^+(CH_3)_3$ of PTC; 6, CH of C-1 from glycerol backbone of TAG, DAG and GPL and 10, $-N^+(C\underline{H}_3)_3$ of choline were not quantitatively evaluated since other, better resolved resonances conveyed the same

Fig. 12. A ^{1}H MR spectrum of liver from an uninfected mouse.

Peak numbers, include:

1, -C<u>H</u>=C<u>H</u>- of FA and sterols

2, CH of C-2 from the glycerol backbone of TAG, DAG and GPL

3, CH of C-1 from the glycerol backbone of GPL

4, CH of glycerol backbone C-1/C-3 from TAG

5, -OC<u>H$_2$</u>CH$_2$N$^+$(CH$_3$)$_3$ of PTC

6, CH of C-1 from glycerol backbone of TAG, DAG and GPL

7, -C<u>H$_2$</u>OPO$_2$C<u>H$_2$</u>- of GPL

8, -CHOP of thePTI ring

9, -C<u>H$_2$</u>N$^+$(CH$_3$)$_3$ of PTC

10, -N$^+$(C<u>H$_3$</u>)$_3$ of choline

11, -C<u>H$_2$</u>N$^+$H$_3$ of PTE

12, -CH=CH(C<u>H$_2$</u>CH=CH-)$_n$ of FA

13, -CH=CHC<u>H$_2$</u>CH=CH- of LA

14, -CH=CHC<u>H$_2$</u>C<u>H$_2$</u>COO$^-$ of DHA

15, -CH$_2$COO$^-$ of FA

17, -CH=CHC<u>H$_2$</u>CH$_2$CH$_2$COO$^-$ of AA

18, -C<u>H$_2$</u>CH=CHCHCH=CHC<u>H$_2$</u>- of FA

19, -C<u>H$_2$</u>CH$_2$COO$^-$ of FA

20, -(CH$_2$)$_n$- of FA and sterols

21, ω-C<u>H$_3$</u> of n-3 FA

22, ω-C<u>H$_3$</u> of FA and sterols

23, C-18 methyl of CT

❖ Indicates water ✣ Indicates methanol

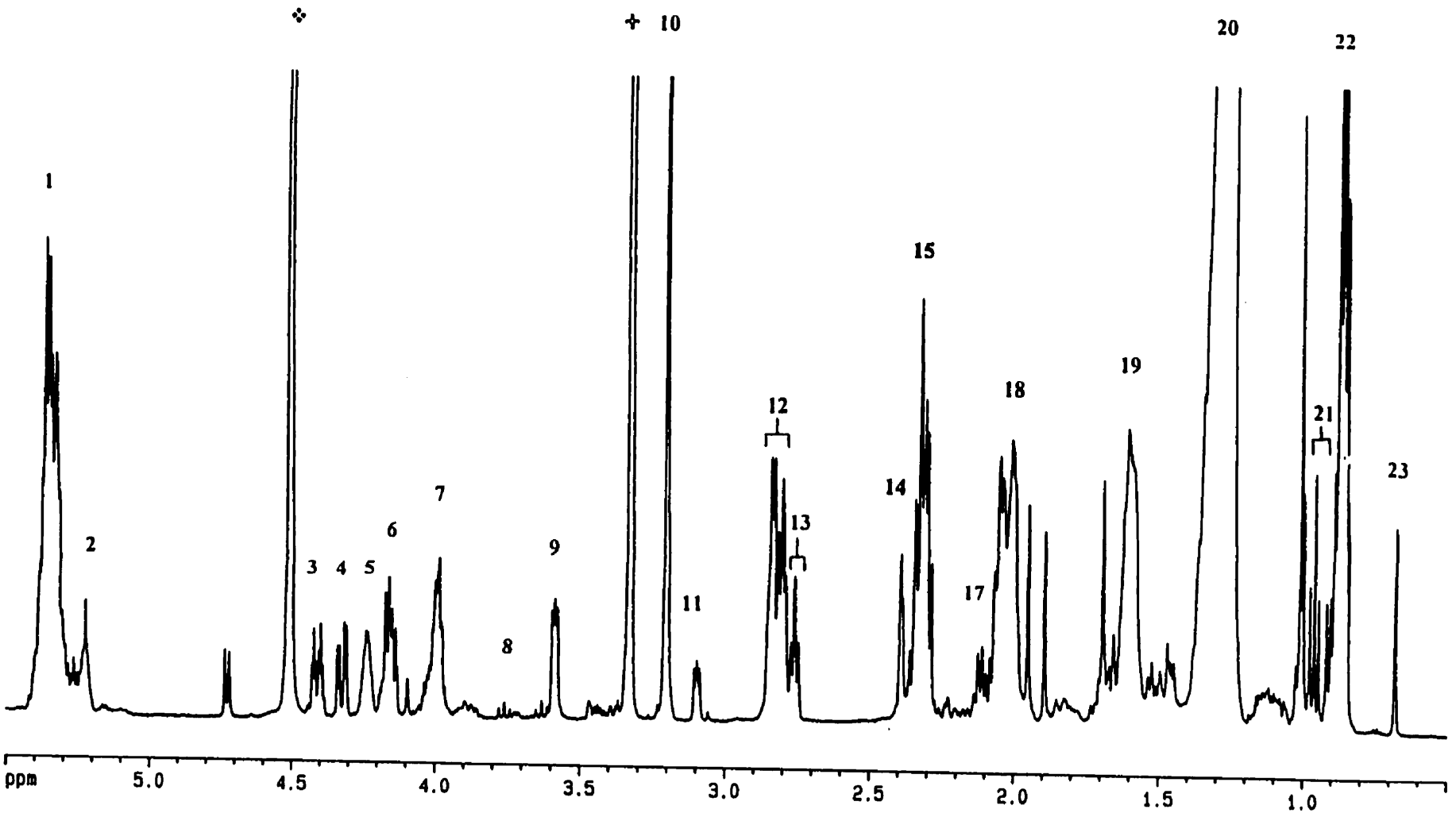

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
17
18
19
20
21
22
23
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Fig. 13. A ^{1}H MR spectrum of liver from an infected mouse.

Peak numbers, include:

1, -C<u>H</u>=C<u>H</u>- of FA and sterols

2, CH of C-2 from the glycerol TAG, DAG and GPL

3, CH of C-1 from the glycerol backbone of GPL

4, CH of glycerol backbone C-1/C-3 from TAG

5, -OC<u>H</u>$_2$CH$_2$N$^+$(CH$_3$)$_3$ of PTC

6, CH of C-1 from glycerol backbone of TAG, DAG and GPL

7, -C<u>H</u>$_2$OPO$_2$C<u>H</u>$_2$- of GPL

8, -CHOP of the PTI ring

9, -C<u>H</u>$_2$N$^+$(CH$_3$)$_3$ of PTC

10, -N$^+$(C<u>H</u>$_3$)$_3$ of choline

11, -C<u>H</u>$_2$N$^+$H$_3$ of PTE

12, -CH=CH(C<u>H</u>$_2$CH=CH-)$_n$ of FA

13, -CH=CHC<u>H</u>$_2$CH=CH- of LA

14, -CH=CHC<u>H</u>$_2$C<u>H</u>$_2$COO$^-$ of DHA

15, -CH$_2$COO$^-$ of FA

17, -CH=CHC<u>H</u>$_2$CH$_2$CH$_2$COO$^-$ of AA

18, -C<u>H</u>$_2$CH=CHCHCH=CHC<u>H</u>$_2$- of FA

19, -C<u>H</u>$_2$CH$_2$COO$^-$ of FA

20, -(CH$_2$)$_n$- of FA and sterols

21, ω-C<u>H</u>$_3$ of n-3 FA

22, ω-C<u>H</u>$_3$ of FA and sterols

23, C-18 methyl CT

❖ Indicates water ✣ Indicates methanol

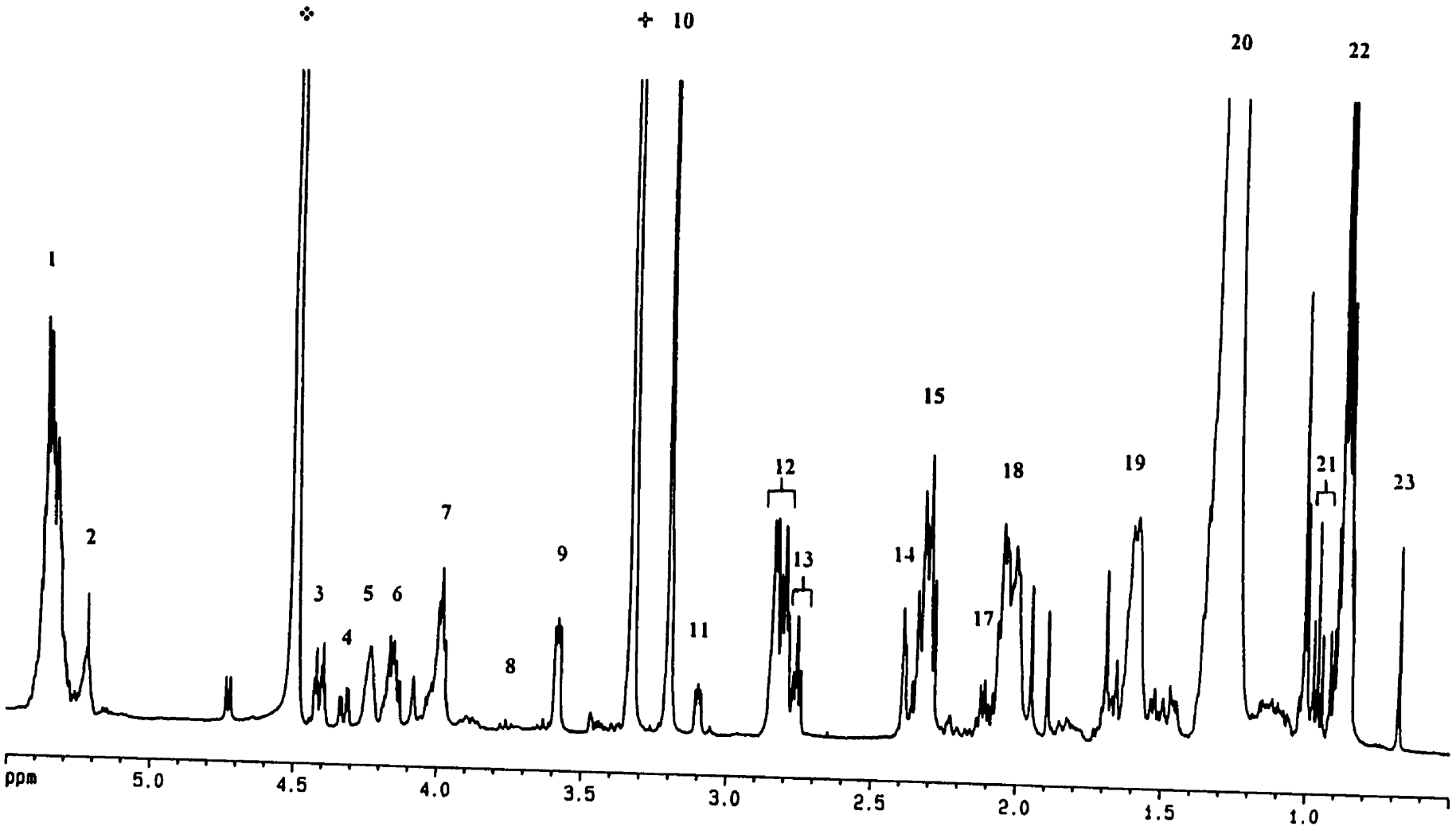

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
17
18
19
20
21
22
23
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Fig. 14. A ^{1}H MR spectrum of *T. crassiceps* larvae.

Peak numbers, include:

1, -C<u>H</u>=C<u>H</u>- of FA and sterols

2, CH of C-2 from the glycerol backbone of TAG, DAG and GPL

3, CH of C-1 from the glycerol backbone of GPL

4, CH of glycerol backbone C-1/C-3 from TAG

5, -OC<u>H</u>$_2$CH$_2$N$^+$(CH$_3$)$_3$ of PTC

6, CH of C-1 from glycerol backbone of TAG, DAG and GPL

7, -C<u>H</u>$_2$OPO$_2$C<u>H</u>$_2$- of GPL

9, -C<u>H</u>$_2$N$^+$(CH$_3$)$_3$ of PTC

10, -N$^+$(C<u>H</u>$_3$)$_3$ of choline

11, -C<u>H</u>$_2$N$^+$H$_3$ of PTE

12, -CH=CH(C<u>H</u>$_2$CH=CH-)$_n$ of FA

13, -CH=CHC<u>H</u>$_2$CH=CH- of LA

14, -CH=CHC<u>H</u>$_2$C<u>H</u>$_2$COO$^-$ of DHA

15, -CH$_2$COO$^-$ of FA

16, -CH$_2$COOH of FFA

18, -C<u>H</u>$_2$CH=CHCHCH=CHC<u>H</u>$_2$- of FA

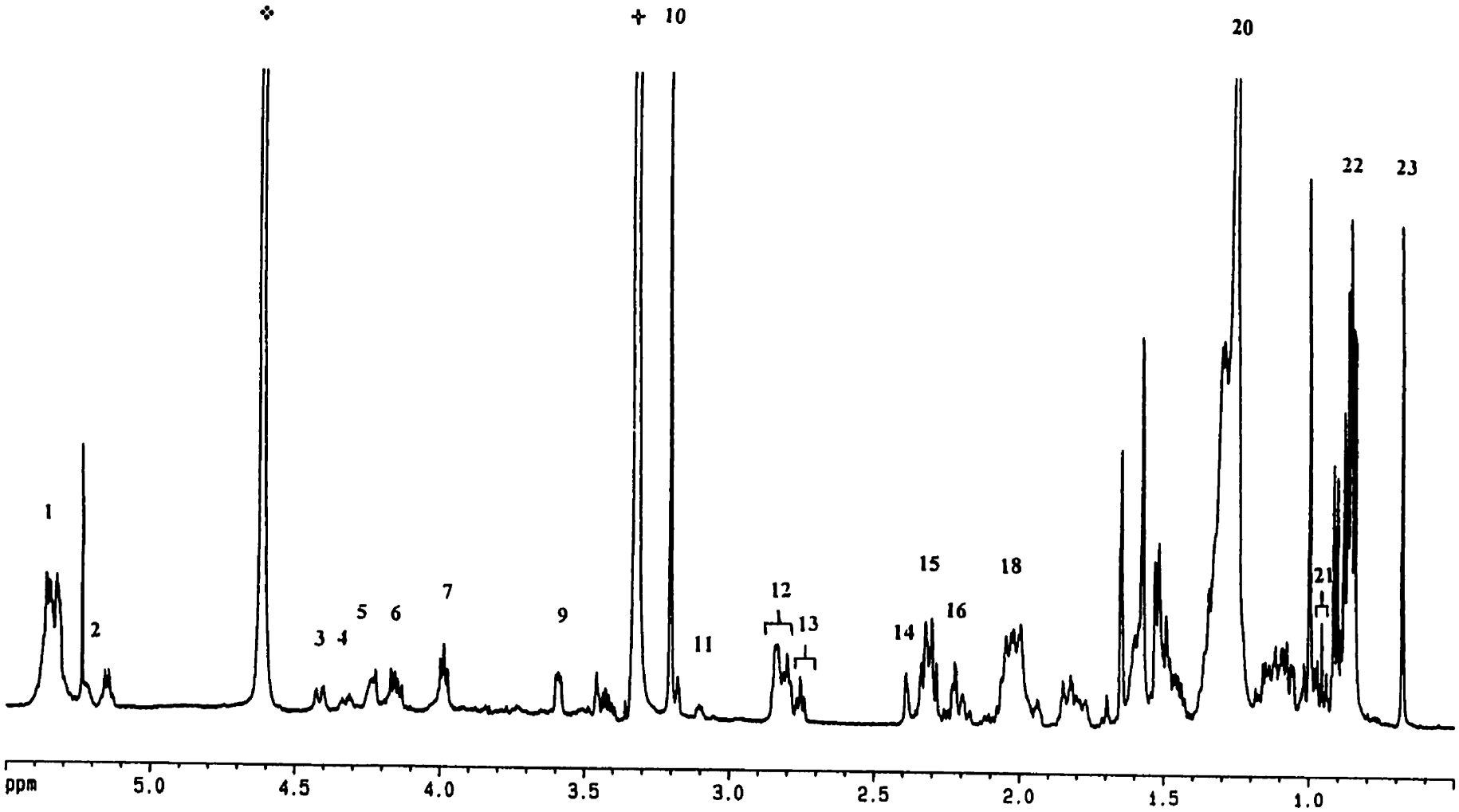
20, -(CH$_2$)$_n$- of FA and sterols

21, ω-CH$_3$ of n-3 FA

22, ω-CH$_3$ of FA and sterols

23, C-18 methyl of CT

❖ Indicates water ✣ Indicates methanol


❖
✢
10
20
22
23
1
2
3
4
5
6
7
9
11
12
13
14
15
16
18
21
ppm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

information. In the liver spectra from uninfected (control) and infected mice (Figs. 12 and 13) the following lipid peaks were evaluated: 1, -CH=CH- ; 4, total TAG; 7, total GPL; 8, PTI; 9, PTC; 11, PTE; 12, -CH=CH(CH_2CH=CH-)$_n$ group; 13, LA; 14, DHA; 15, -CH_2COO^- group; 17, AA; 18, -CH_2CH=CHCH=CHCH_2- group; 19, -$CH_2CH_2COO^-$ group; 20, -$(CH_2)_n$- group; 21, ω-CH_3 of n-3 FAC; 22, ω-CH_3 of FA and sterols and peak 23, cholesterol. The resonance belonging to the FFA moiety of -CH_2COOH (peak 16) could not be detected in the liver spectra. The spectra of *T. crassiceps* larvae (Fig. 14) displayed the same resonances as the liver spectra except that the FFA moiety of -CH_2COOH (peak 16) was clearly visible and the FA moieties of -$CH_2C\underline{H}_2COO^-$ (peak 19), -CH=CH$C\underline{H}_2CH_2CH_2COO^-$ of AA (peak 17) and PTI (peak 8) were absent.

The concentrations of various classes of lipids, total FA and FA components in the liver and the parasite are presented in Table 6. The livers from infected mice contained lower concentrations of PTE, PTC, PTI, total GPL, TAG, total FA and all of the FA components. Only the CT level was similar to that of uninfected controls. Cysticerci contained lower concentrations of every lipid metabolite, with the exception of the FA resonance of -$C\underline{H}_2CH_2COO^-$, the -CH=CH$C\underline{H}_2CH_2CH_2COO^-$ of AA and PTI which could not be detected and the FFA moiety which was not detectable in the host liver.

When the ratios (FA component : total FA) of individual FA components were calculated (Table 7) it was found that the livers from infected animals possessed lower ratios of the saturated methylene moieties of -$(CH_2)_n$- and -CH_2COO^- than those of the uninfected group. Infected mice , however, contained higher ratios of polyunsaturated moieties belonging to the -CH=CH($C\underline{H}_2$CH=CH-)$_n$ group, the -CH=CH$C\underline{H}_2C\underline{H}_2COO^-$

Table 6. Concentrations (μ mol/g wet weight) of lipid metabolites from liver extracts of uninfected and infected mice and *Taenia crassiceps* larvae.

Lipid	uninfected (n=12)	infected (n=12)	larvae (n=12)
Cholesterol	5.51 ± 0.31[a]	5.48 ± 0.30[a]	2.30 ± 0.04†
PTE	8.17 ± 0.77[a]	7.04 ± 1.10[b]	0.37 ± 0.12†
PTC	19.45 ± 1.06[a]	18.06 ± 0.98[b]	1.11 ± 0.20†
PTI	2.08 ± 0.33[a]	1.73 ± 0.30[b]	NM
Total GPL	42.52 ± 3.17[a]	38.21 ± 2.97[b]	3.37 ± 1.39†
TG	11.39 ± 2.94[a]	4.15 ± 1.09[b]	0.62 ± 0.31†
Total FA	123.70 ± 11.97[a]	93.86 ± 6.00[b]	9.86 ± 1.79†
Saturated component			
CH_2 in $-(CH_2)_n-$	1081.49 ± 119.42[a]	777.02 ± 45.71[b]	63.25 ± 10.83†
$-CH_2CH_2COO$	111.42 ± 16.74[a]	80.17 ± 5.62[b]	NM
$-CH_2COO$	106.99 ± 11.92[a]	77.80 ± 4.93[b]	4.88 ± 1.05†
$-CH_2COOH$ (free)	NM	NM	2.30 ± 0.66†
Unsaturated component			
-CH=CH-	172.72 ± 14.02[a]	136.25 ± 15.10[b]	10.52 ± 2.11†
Polyunsaturated component			
$-C\underline{H}_2CH=CHCH=CHC\underline{H}_2-$	66.30 ± 6.67[a]	47.56 ± 5.01[b]	4.17 ± 0.78†
$-CH=CH(C\underline{H}_2CH=CH)n$	87.74 ± 7.88[a]	74.14 ± 11.70[b]	4.78 ± 0.88†
$-CH=CHC\underline{H}_2C\underline{H}_2COO^-$ of DHA	8.26 ± 0.79[a]	7.39 ± 1.22[b]	0.49 ± 0.09†
$-CH=CHC\underline{H}_2CH=CH-$ of LA	18.15 ± 2.57[a]	14.61 ± 1.44[b]	1.11 ± 0.31†
$-CH=CHC\underline{H}_2CH_2CH_2COO^-$ of AA	11.86 ± 1.11[a]	10.35 ± 1.48[b]	N.M

[a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other. † Indicates significant difference ($P \leq 0.05$) from corresponding infected groups. PTE, phosphatidylethanolamine; PTC, phosphatidylcholine; PTI, phosphatidylinositol; GPL, glycerophospholipid; TG, triglyceride; D.H.A., docosahexaenoic acid; L.A., linoleic acid; A.A., arachidonic acid. NM , Not measureable.

Table 7. Fatty acid composition of liver extracts from uninfected and infected mice and of *Taenia crassiceps* larvae.

FA component	Ratio (FA component : total FA) mean ± S.D		
	unifected (n=12)	infected (n=12)	larvae (n=12)
Saturated component			
CH_2 in $-(CH_2)_n-$	8.66 ± 0.18[a]	8.28 ± 0.13[b]	6.46 ± 0.06†
$-CH_2CH_2COO$	0.88 ± 0.04[a]	0.85 ± 0.02[a]	NM
$-CH_2COO$	0.86 ± 0.02[a]	0.83 ± 0.01[b]	0.50 ± 0.04†
$-CH_2COOH$ (free)	NM	NM	0.23 ± 0.42
Unsaturated component			
-CH=CH-	1.40 ± 0.06[a]	1.45 ± 0.12[a]	1.07 ± 0.08†
Polyunsaturated component			
$-CH_2CH=CHCH=CHCH_2-$	0.54 ± 0.02[a]	0.51 ± 0.04[a]	0.45 ± 0.04†
$-CH=CH(CH_2CH=CH)n$	0.71 ± 0.07[a]	0.79 ± 0.11[b]	0.49 ± 0.03†
$-CH=CHCH_2CH_2COO^-$ of DHA	0.07 ± 0.01[a]	0.08 ± 0.01[b]	0.05 ± 0.01†
$-CH=CHCH_2CH=CH-$ of LA	0.14 ± 0.02[a]	0.15 ± 0.01[a]	0.11 ± 0.02†
$-CH=CHCH_2CH_2CH_2COO^-$ of AA	0.09 ± 0.01[a]	0.11 ± 0.02[b]	NM
	Average chain length		
	16.73 ± 0.12[a]	16.50 ± 0.34[a]	12.18 ± 0.62†

[a or b] Means with different letters are significantly different ($P \leq 0.05$) from each other. † Indicates significant difference ($P \leq 0.05$) from corresponding infected group. D.H.A., docosahexaenoic acid; L.A., linoleic acid; A.A., arachidonic acid. NM , Not measureable.

of DHA and the $-CH=CHCH_2CH_2CH_2COO^-$ of AA. The ratios of FA components were also calulated for *T. crassiceps* larvae and compared to those of the infected host (Table 7). *T. crassiceps* contained a shorter average FA chain length and lower ratios of all FA components listed with the exception of $-CH_2CH_2COO^-$ and the $-CH=CHCH_2CH_2CH_2COO^-$ group of AA which could not be detected, and the FFA component $-CH_2COOH$, which was present in the larvae but not detectable in the host liver.

DISCUSSION

[3 -^{13}C]Alanine Metabolism

The high concentration of labelled alanine in the livers of mice from both uninfected and infected groups demonstrates that intraduodenal infusion is an effective method of delivering exogenous agents to the liver. While considerable quantities of this labelled precursor have been metabolized by the liver, as can be seen by the distribution of ^{13}C label among hepatic metabolites, significant amounts remained unmetabolized at the time of dissection. The slow metabolism of labelled alanine in the liver may be related to the transport process involved in moving alanine from the hepatic sinusoids into the hepatocytes. Alanine is transported across the hepatocyte plasma membrane by a neutral amino acid sodium dependent symport called a system A transporter (Haussinger and Gerok, 1986). Although the rate at which alanine is transported across the hepatocyte plasma membrane is regulated by hormones and the energy charge of the cell, the activity of this transporter is generally lower than that of the catalytic enzymes involved in alanine catabolism (Groen *et al.*, 1982). Numerous studies have demonstrated that when large loads of alanine are introduced into the liver, the transport of this amino acid across the plasma membrane is a rate-controlling step for its metabolism (Sips *et al.*, 1980; Fafournoux *et al.*, 1983; Haussinger and Gerok, 1986). Thus the majority of the intrahepatic labelled alanine detected two hours after intraduodenal administration is most likely accumulated [3-^{13}C]alanine located in extracellular compartments.

Although the concentration of alanine in the liver was similar in both groups of mice

the percent label in this substrate was significantly lower in livers of infected mice than in those of controls. This might be, in part, the result of active absorption of [3-^{13}C]alanine by the cysticerci. In this parasitic infection chronic inflammation of the host's peritoneum associated with pronounced vascular changes occurs (Chernin and McLaren, 1983). Circulating fluid, rich in plasma constituents, drains from blood vessels and accumulates in the peritoneal cavity (Davis, 1967; Rhoads and Rhoads, 1976). Thus in infected animals some of the ^{13}C labelled alanine circulating through the vessels of the abdomen presumably leaked into the peritoneal cavity where it became available to the parasite. Indeed a large percentage of labelled alanine was found in the cysticerci. When high intraperitoneal levels of this amino acid are present, significant quantities will enter the cysticerci by diffusion (Pappas, 1983). Under these conditions the entry of [3-^{13}C]alanine into the larvae is the result of osmotic pressure and not metabolic demands made by the parasite. The internalized [3-^{13}C]alanine will be subsequently excreted as the flux of glycolysis favors the excretion of such products (Barrett, 1983). The possibility exists that the reversible enzyme alanine aminotransaminase may convert a small fraction of this labelled substrate to [3-^{13}C]pyruvate. Then, due to the high activity of lactate dehydrogenase in cestodes (Smyth and McManus, 1989), [3-^{13}C]pyruvate would be rapidly converted to [3-^{13}C]lactate which would be either excreted or some of it converted, in a futile cycle, back to labelled alanine.

Alternative metabolism of [3-^{13}C]pyruvate is unlikely as the majority of this short lived intermediate is sequestered by the lactate dehydrogenase reaction. Furthermore, utilization of [3-^{13}C]pyruvate as a gluconeogenic substrate must also be ruled out as cestodes are incapable of gluconeogenesis from pyruvate (Tielens and Bergh, 1993). Therefore [1-

^{13}C]glucose detected in the cysticerci had to be acquired from the host who produced it through gluconeogeneic steps from [3-^{13}C]alanine. As catabolism of glucose by the parasite produces alanine, the labelled alanine found in the larvae had to contain a contribution from this process.

Less label in hepatic alanine also suggests that utilization of this substrate in infected mice could be increased. Although both groups of mice were subjected to the same duration of food deprivation prior to alanine infusion, infected mice presumably experienced, as a result of their long term parasitic infection, a greater degree of starvation. It is known that prolonged starvation causes the mammalian pancreas to produce more glucagon, while down regulating the production of insulin (Boisjoyeux et al., 1986; Hellerstein and Munro, 1994). Circulating glucagon primarily targets the liver, where it binds to glucagon receptors on the outer leaflet of the hepatocyte plasma membrane. This initates a cascade of membrane bound reactions which generates cyclic AMP (cAMP). As a potent metabolic regulator, cAMP promotes the synthesis and liberation of glucose by activating numerous enzymes along the gluconeogenic cascade (Exton *et al.*, 1967; Groen *et al.*, 1986; Pryor *et al.*, 1987; Mathews and VanHolde, 1990). The rate at which alanine is transported into hepatocytes is increased under conditions of starvation or high protein intake (Fafournoux *et al.*, 1983). Therefore a greater influx of [3-^{13}C]alanine should facilitate faster metabolism of this substrate in the infected host. The major pathways of [3-^{13}C]alanine metabolism in the liver are shown in Fig. 15. Once inside the hepatocyte, [3-^{13}C]alanine is converted to pyruvate by the enzyme alanine aminotransaminase (1). The activity of this enzyme is known to increase with prolonged periods of fasting during early starvation (Sharma et al., 1985;

FIGURE : 15 Hepatic metabolism of [3-^{13}C]alanine

Enzymes:

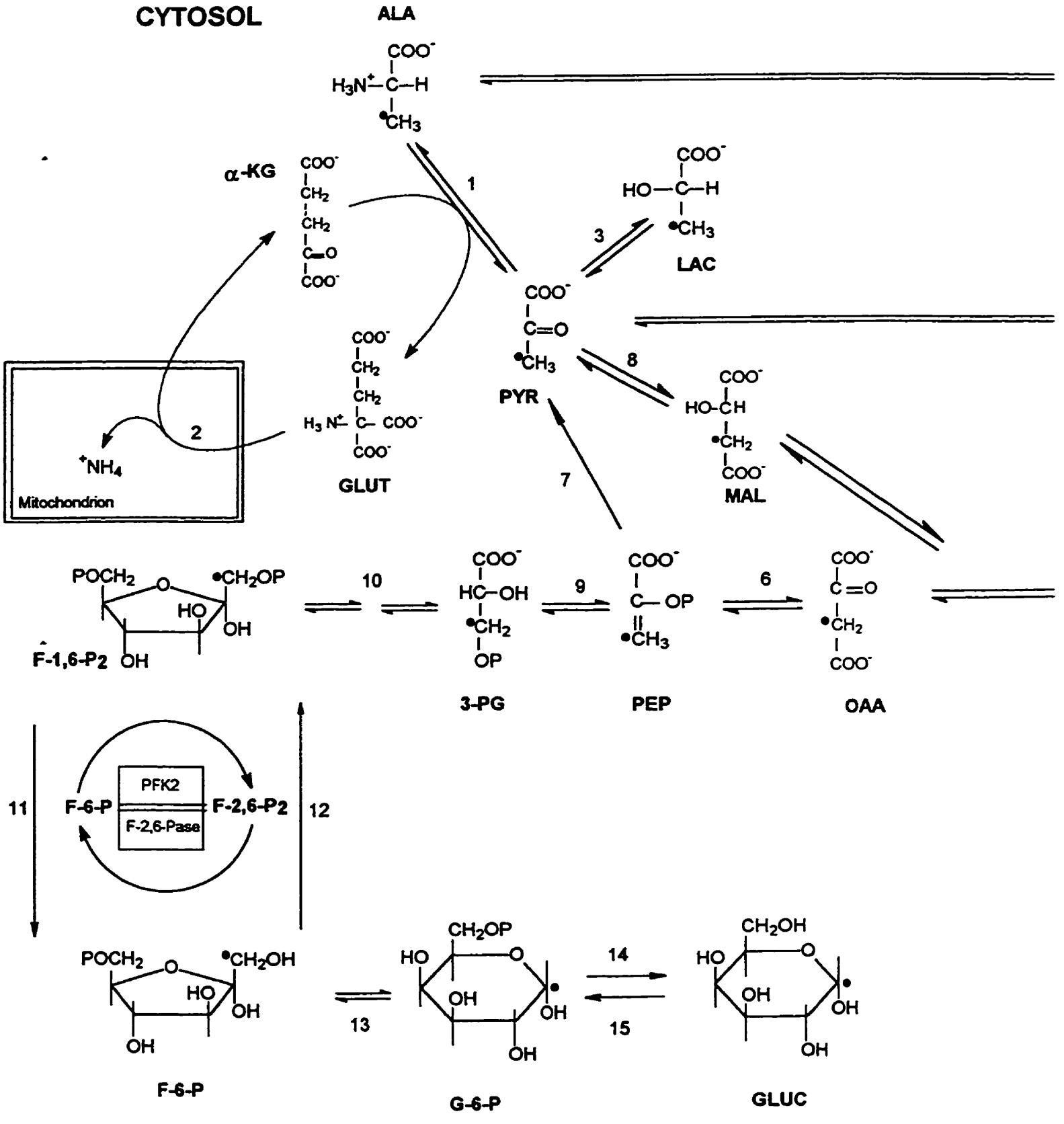
1, alanine aminotransaminase
2, glutamate dehydrogenase
3, lactate dehydrogenase
4, pyruvate dehydrogenase complex (PDC)
5, pyruvate carboxylase
6, phosphoeneolpyruvate carboxykinase
7, pyruvate kinase
8, malic enzyme
9, enolase
10, phosphoglyceromutase, glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase and aldolase
11, fructose-1,6-bisphosphatase
12, phosphofructokinase
13, phosphoglucoisomerase
14, glucose-6-phosphatase
15, glucokinase
16, citrate synthase
17, aconitase and isocitrate dehydrogenase
18, α-ketoglutarate dehydrogenase and succinyl-CoA synthetase
19, succinate dehydrogenase
20, fumerase
21, malate dehydrogenase

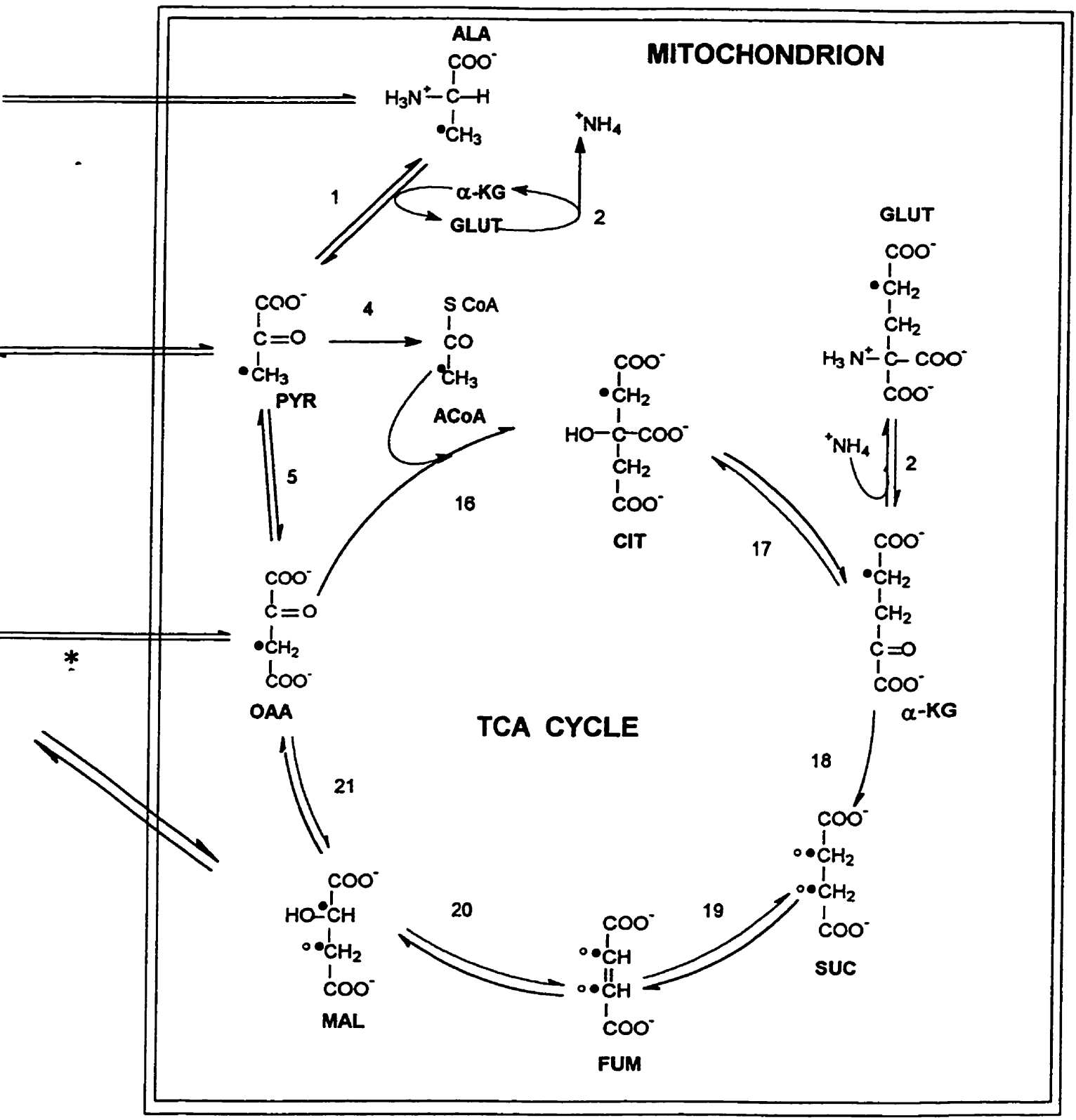
* oxaloacetate enters and leaves the mitochondrion through transamination to aspartate or reduction to malate.

Abbreviations

AcoA	acetyl-CoA	G-6-P	glucose-6-phosphate
ALA	alanine	LAC	lactate
CIT	citrate	MAL	malate
FUM	fumerate	OAA	oxaloacetate
F-1,6-P_2	fructose-1,6-bisphosphate	PEP	phosphoenolpyruvate
F-2,6-P_2	fructose-2,6-bisphosphate	PYR	pyruvate
F-6-P	fructose-6-phosphate	SUC	succinate
GLUC	glucose	α-KG	αketoglutarate
GLUT	glutamate	3-PG	3-phosphoglycerate

PFK-2/F-2,6-Pase bifunctional dimer phosphofructokinase-2/fructose-2,6-bisphosphatase


CYTOSOL
ALA
α-KG
LAC
GLUT
PYR
MAL
Mitochondrion
$^{+}NH_4$
F-1,6-P2
3-PG
PEP
OAA
PFK2
F-6-P
F-2,6-P2
F-2,6-Pase
F-6-P
G-6-P
GLUC
1
2
3
6
7
8
9
10
11
12
13
14
15


MITOCHONDRION
ALA
COO^-
H_3N^+-C-H
$\bullet CH_3$
$^+NH_4$
1
α-KG
GLUT
2
GLUT
COO^-
$\bullet CH_2$
CH_2
$H_3N^+-C-COO^-$
COO^-
COO^-
$C=O$
$\bullet CH_3$
PYR
4
S CoA
CO
$\bullet CH_3$
ACoA
COO^-
$\bullet CH_2$
$HO-C-COO^-$
CH_2
COO^-
CIT
$^+NH_4$
2
5
16
17
COO^-
$C=O$
$\bullet CH_2$
COO^-
OAA
COO^-
$\bullet CH_2$
CH_2
$C=O$
COO^-
α-KG
TCA CYCLE
18
21
COO^-
$HO-\bullet CH$
$\circ\bullet CH_2$
COO^-
MAL
20
COO^-
$\circ\bullet CH$
$\circ\bullet CH$
COO^-
FUM
19
COO^-
$\circ\bullet CH_2$
$\circ\bullet CH_2$
COO^-
SUC

Azzoul et al., 1986). Two isoenzymes of alanine aminotransaminase exist in the mammalian liver, one within the cytosol and the other in the mitochondrion (DeRosa and Swick, 1975; Groen *et al.*, 1982; Patel and Olson, 1985). Thus the transamination of alanine may occur in the either compartment. Studies by Patel and Olson (1985) suggest that under conditions of starvation the majority of intracellular alanine is transaminated in the cytosol, and only a fraction of it is transaminated in the mitochondrial matrix. Futile cycling of alanine transport between the mitochondria and cytosol is unlikely to occur as the affinity of the mitochondrial isoenzyme is an order of magnitude greater than that of the cytosolic isoenzyme (DeRosa and Swick, 1975). Therefore, alanine which enters the mitochondria is quickly transaminated to pyruvate. Regardless of which isoenzyme catalyzes the transamination step, the reaction proceeds by cleaving the amino group of alanine and transferring it to α-ketoglutarate, to generate [3-^{13}C]pyruvate and glutamate. The key role α-ketoglutarate plays in accepting ammonia during the transamination of alanine to pyruvate is believed to produce the high concentrations of glutamate (Hall *et al.*, 1988). Although glutamate was clearly measurable in the livers of both uninfected and infected mice, its concentration was significantly lower in infected animals. Upon entering into the mitochondrion through a dicarboxylate transporter, glutamate comes in contact with glutamate dehydrogenase (2), which catalyzes the release of ammonia and regeneration of α-ketoglutarate for further conversion of alanine to pyruvate. Hence with increased alanine aminotransferase activity greater amounts of free ammonia are produced. In order to prevent excess ammonia from reaching toxic levels in the blood the rate of hepatic ureagenesis increases (Haussinger and Gerok, 1986; Ampola, 1994). Thus if in mice infected with *T.*

crassiceps deamination of glutamate was indeed increased, greater quantities of ammonia would be produced, which in turn should be disposed of through the urea cycle. As the rate of ureagenesis was not measured in the present experiment, further work is needed to test this hypothesis.

Further metabolism of the [3-^{13}C]pyruvate generated from [3-^{13}C]alanine (Fig.15) depends on which cellular compartment it is located in, the cytosol or the mitochondrion. A significant fraction of labelled cytosolic pyruvate will encounter the enzyme lactate dehydrogenase (3). This reversible enzyme, found exclusively in the cytosol, catalyzes the reduction of pyruvate to lactate. The equilibrium of the reaction strongly favors the production of lactate. This is evident in the present experiment, as a high percent ^{13}C was detected in hepatic lactate of both uninfected and infected mice. Livers of infected mice, however, contained a lower percentage of carbon-13 in lactate than those of controls. This may, in part, be the result of an increased flux of cytosolic substrates to the mitochondria for gluconeogenesis stimulated by increased levels of glucagon. Cytosolic pyruvate is transported into the mitochondria via a specific carrier in exchange for OH^- or the ketone body, acetoacetate (Kummel, 1987). With an increased duration of starvation, increased ketogenesis is known to occur (Owen et al., 1969; Katz, 1986). The large amounts of acetoacetate produced from mitochondrial reactions create a steep concentration gradient which favors the efflux of acetoacetate, with the concomitant influx of pyruvate. As the activity of this antiport should be accelerated in the infected host, [3-^{13}C]pyruvate would then be shuttled into the mitochondria at a faster rate, which in turn would lead to a decreased level of cytosolic labelled pyruvate.

Increased catabolism of [3-^{13}C]lactate could also be the reason for the lower levels of labelled hepatic lactate found in infected mice. Although the equilibrium of lactate dehydrogenase (3) favors lactate production, increased conversion of lactate to pyruvate occurs under conditions of fasting as a result of increased levels of circulating lactate (Holten *et al.*, 1971; Ferrannini *et al.*, 1993). This would also increase the cytosolic futile cycling of [3-^{13}C]pyruvate → [3^{13}C]lactate → [3^{13}C]pyruvate. With the increased mitochondrial influx of pyruvate, cytosolic [3-^{13}C]lactate becomes a source for pyruvate entering the mitochondria. This could account for lower percentage of [3-^{13}C]lactate seen in infected animals. Further, cytosolic [3-^{13}C]pyruvate may also serve as a substrate for cytosolic malic enzyme (8) which catalyzes the oxidative decarboxylation of pyruvate to malate. The activity of this enzyme functions primarily to provide cytosolic NADPH required for lipogenesis. However, during periods of fasting both lipogenesis and malic enzyme activity are suppressed due to high levels of circulating glucagon (Goodridge *et al.*,1986; Skurat and Dice, 1994; Petersen *et al.*, 1995). Therefore it can be assumed that, under our experimental conditions, the carbon flux through malic enzyme was minimal.

Once inside the mitochondria the fate of [3-^{13}C]pyruvate is governed by the activities of two major enzymes, pyruvate dehydrogenase (4) and pyruvate carboxylase (5). The activities of each of these enzymes is carefully regulated by the metabolic and hormonal status of the cell (Groen *et al.*, 1986; Siess *et al.*, 1988; Sugden *et al.*, 1995). Pyruvate dehydrogenase, more correctly termed the pyruvate dehydrogenase complex (PDC), is a multienzyme complex consisting of three individual enzymes and five distinct coenzymes. The PDC catalyzes the irreversible oxidative decarboxylation of pyruvate to acety-CoA. The

activity of this complex is regulated by the reversible phosphorylation of active sites within the complex (Sugden *et al.*, 1993; Randle *et al.*, 1994; Sugden *et al.*, 1995). The phosphorlylation of these active sites is controlled by two further enzymes, PD kinase and PD phosphatase. The two enzymes operate in a reciprocal fashion, PD kinase inactives the PDC by phosphorylating its active sites, while PD phosphatase activates the PDC by dephosphorylating these sites. PD kinase is activated (i.e. PDC activity is suppressed) by increasing mitochondrial ratios of acetyl-CoA:CoA, ATP:ADP and NADH:NAD (Sugden and Holness, 1994). Conversly the activity of PD phosphatase increases (i.e. PDC is stimulated) by insulin and Ca^{2+} (Sugden and Holness, 1994). Under normal feeding conditions the circulating concentrations of insulin and the intracellular concentrations of Ca^{2+} are sufficient to keep PD phosphatase active. When food deprivation occurs and fasting is continued, insulin levels drop and the mobilization of peripheral fat stores releases increasing amounts of FA which are delivered to the liver. There in the hepatocyte mitochondria they are degraded via β-oxidation. The oxidation of FA generates increasing amounts of acetyl-CoA, NADH, and ATP, which in turn stimulates PD kinase and depresses PDC activity (Randle *et al.*, 1994). Even though PD kinase activity is highly stimulated and that of PD phosphatase activity is during starvation, the flux of pyruvate through the PDC still operates although at a significantly reduced rate (Caterson *et al.*, 1982). The small amount of pyruvate that does pass through the PDC produces acetyl-CoA which is then chanelled towards the TCA cycle and ketogenesis as a result of strict inhibition of lipogenesis.. Labelled [4-^{13}C]glutamate was detected in the livers of both uninfected and infected mice. While the percent ^{13}C at the carbon 4 position of glutamate was equal in both

groups, the concentration of glutamate was lower in mice infected with *T. crassiceps*. This suggests that less [4-^{13}C]glutamate was produced in the livers of infected animals. The labelling pattern of the carbon atoms of glutamate reflects the labelling in the TCA cycle intermediate α-ketoglutarate. Isotopic enrichment of glutamate C4, hence α-ketoglutarate C4, is a direct measure of label from [2-^{13}C]acetyl-CoA produced from the passage of [3-^{13}C]pyruvate through the PDC, as depicted in Fig. 15. Thus the lower levels of hepatic [4-^{13}C]glutamate in infected mice is indicative of a reduced flux of [3-^{13}C]pyruvate through the PDC. These findings suggest the the intramitochondrial environment in the liver of infected mice contained higher ratios of acetyl-CoA:CoA, ATP:ADP and/or NADH:NAD than those in uninfected mice. Mitochondrial [2-^{13}C]acetyl-CoA can also be expected to enter ketogenesis. However, because of the small resonance of β-hydroxybutyrate present in the spectrum, ^{13}C label was not measurable in this metabolite.

Reduced flux of [3-^{13}C]pyruvate through the PDC in hepatocytes of infected animals, would result in more labelled pyruvate being channelled through the pyruvate carboxylase (5) reaction (Fig. 15). This biotin dependent carboxylation of pyruvate to oxaloacetate is the first step to gluconeogenesis. Pyruvate carboxylase and PDC are regulated in a reciprocal fashion, in that high ratios of acetyl-CoA:CoA, ATP:ADP and NADH:NAD stimulate the former (Agius et al., 1985; Siess et al., 1988). Given that these conditions appear to be favored in the livers of infected host, [3-^{13}C]pyruvate would be partitioned more towards carboxylation then decarboxylation. Therefore the fate of [3-^{13}C] pyruvate in infected animals would seem to be more directed to glucose synthesis. However, in order for this to occur a number of subsequent futile cycles and depleting reactions must be overcome.

The [3-^{13}C]oxaloacetate, produced from the pyruvate carboxylase reaction, is the first of such substrates which could be drawn away from glucose production. Because oxaloacetate is a substrate common to both the TCA cycle and gluconeogenesis, dilution of the ^{13}C label occurs. Labelled oxaloacetate molecules randomly enter the TCA cycle in exchange for unlabelled oxaloacetate at both the citrate synthase (16) and equilibrating malate dehydrogenase (21) /fumerase (20) sites. As a result of the rapid equilibration of the malate/fumarate reactions and their reversibility, [3-^{13}C]oxaloacetate may be converted via succinate dehydrogenase (19) to [2-^{13}C]succinate. The same labelling pattern in succinate is also generated from [3-^{13}C]pyruvate's passage through the PDC and clockwise steps of the TCA cycle (Fig.15). Hence labelling in succinate cannot be used to determine the relative activities of the PDC and pyruvate carboxylase reactions. The equivalent percent ^{13}C in hepatic succinate from infected and uninfected animals is not surprising as the majority of ^{13}C label could be derived from the pyruvate carboxylase reaction in the former case, while in the latter the activity of the PDC could be the major contibutor. The reaction that keeps oxaloacetate within the gluconeogenic pathway is its conversion to phosphoenolpyruvate (PEP) in the cytosol, by the enzyme phosphoenolpyruvate carboxykinase (PEPCK) (6). The activity of PEPCK is highly stimulated during conditions of starvation (Boisjoyeux *et al.*, 1986). High levels of circulating cAMP and low levels of insulin activate this enzyme. The former increases transcription of the PEPCK gene and low levels of insulin decreases its suppression of PEPCK mRNA levels (Pilkis *et al.*, 1988). Thus with increased starvation the flux of oxaloacetate through PEPCK becomes more stringent.

Similar to [3-^{13}C]oxaloacetate, the amount of [3-^{13}C]PEP utilized for gluconeogenesis

may also be diminished by competing reactions (Fig.15). In addition to being a substrate for the reversible glycolytic/gluconeogenic enzyme enolase (9), PEP is also acted upon by the irreversible enzyme pyruvate kinase (7). Enolase activity is not significantly altered by the hormonal or metabolic status of the cell, therefore, it is the regulation of pyruvate kinase activity which determines the partition of PEP between enolase and pyruvate kinase (Groen *et al.*, 1983). The activity of pyruvate kinase, like that of PDC, is regulated by a reversible phosphorylation/dephosphorylation state, where phosphorylation of the enzyme by cAMP dependent or Ca^{2+} calmodulin dependent protein kinase suppresses the enzyme's activity and dephosphorylation activates it (Pilkis *et al.*,1988; Seifter and Englard, 1994). Pyruvate kinase is also allosterically activated by high concentrations of fructose-1,6-bisphosphate and inhibited by ATP and certain amino acids, such as alanine. Prolonged starvation, which creates conditions that favor increased phosphorylation of pyruvate kinase and it's allosteric suppression by low levels of fructose-1,6-bisphosphate, have been shown to reduce the flux of hepatic [3-^{13}C]PEP through pyruvate kinase (Cohen, 1987).

Regulation of the fructose-1,6-bisphosphate/fructose-6-phosphate futile cycle is regarded as probably the most important control of the partitioning of carbon flux through gluconeogensis/glycolysis (Pilkis *et al.*, 1988; Mattews and VanHolde, 1990). This regulation is mediated primarily by the concentration of fructose-2,6-bisphosphate (Fig. 15). At high concentration this metabolite allosterically activates phosphofructokinase (PFK-1) (12) / glycolysis and inhibits fructose-1,6-bisphosphatase (F-1,6-P_2ase) (11) /gluconeogenesis, while at low concentration PFK-1 is suppressed and F-1,6-P_2ase is activated. The enzyme responsible for steady state levels of fructose-2,6-bisphosphate is the

bifunctional dimer phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK-2/F-2,6-P_2ase). Upon phosphorylation of this dimer, catalysed by cAMP-dependent protein kinase, PFK-2 is inactivated and F-2,6-P_2ase simultaneously stimulated to hydrolyze fructose-2,6-bisphosphate to fructose-6-phosphate and inorganic phosphate. Conversely when cAMP is low and insulin is high, dephosphorylation promotes PFK-2 activity and suppresses F-2,6-P_2ase, which in turn maintains fructose-2,6-bisphosphate levels and the flux through glycolysis. The bi-directional activity of PFK-2/F-2,6-P_2ase controls not only this futile cycle but also the pyruvate kinase flux by controlling the levels of fructose-1,6-bisphosphate. The influence of fructose-2,6-bisphosphate on the futile cycling of fructose-1,6-bisphosphate/fructose-6-phosphate and pyruvate kinase make this metabolite an important regulator in the partitioning of carbon between glycolysis and gluconeogenesis. It can be assumed that the greater magnitude of starvation experienced by infected animals should create conditions of higher levels of circulating cAMP that, in turn, should decrease fructose-2,6-bisphosphate concentration and therefore favor a greater flux of ^{13}C labelled intermediates towards glucose synthesis.

The last antagonistic pair of irreversible enzymes the gluconeogenic intermediates must face is that of glucose-6-phosphatase (14) and glucokinase (15). Each of these enzymes has a relatively high Michaelis constant (Km) values for their respective substrates, hence regulation of these enzymes is controlled by their substrate concentrations (Mathews and VanHolde, 1990; Seifter and Englard, 1994). When levels of glucose-6-phosphate rise, the activity of glucose-6-phosphatase predominates and free glucose is liberated. Under conditions of starvation when blood glucose levels are low, glucose produced is rapidly

exported from the hepatocyte into the circulation to reestablish blood glucose levels. The glucose transporter, GLUT-2, moves glucose out of the hepatocyte approximately two times faster than glucose-6-phosphatase can hydrolyze glucose-6-phosphate (Seifter and Englard, 1994). Thus when plasma glucose levels are low, glucose produced from gluconeogenesis is rapidly exported from the hepatocyte, thereby reducing the chance of futile cycling through glucokinase.

In the present work, infected animals contained the same percent of ^{13}C in C1 of hepatic glucose, but a lower concentration of this carbohydrate compared to uninfected controls. The results, therefore, suggest that infected mice had less [1-^{13}C]glucose in their liver than uninfected mice. These findings do not contradict the notion that in infected mice the rate of hepatic gluconeogenesis is greater. *De novo* synthesized [1-^{13}C]glucose from [3-^{13}C]alanine would be quickly released into the blood to supply glucose deprived peripheral tissues and as the present data show, a significant portion of this circulating labelled glucose is siphoned off by the parasite. Cysticerci absorb this hexose which is their main source of energy, using an active carrier-mediated system (Craig, 1982). Since cestodes are unable to make glucose *de novo* from pyruvate (Tielens and Bergh, 1993), all labelled glucose found in cysticerci had to come from the host. Within the parasite, absorbed [1-^{13}C]glucose has two fates. It is either polymerized into glycogen or it is catabolized to reduced endproducts. When the host is fasting parasite glycogenesis is known to decrease (Roberts, 1983) and, as in mammalian glycogenesis, glycogen formation in cestodes is inhibited by high levels of cAMP (Smyth and McManus, 1989). In this study glycogen synthesis in *T. crassiceps*, grown in its host for 115 days, was severely impeded as can be seen by the lack of ^{13}C

enrichment in its glycogen. Instead, all of the acquired [1-^{13}C]glucose was used for energy and therefore degraded to reduced end products lactate, alanine, acetate and succinate. Carbon-13 label in lactate and alanine contained major contributions from the degradation of [1-^{13}C]glucose and, as described earlier, possible minor contributions from the direct absorption and futile cycling of [3-^{13}C]alanine. Since the conversion of [3-^{13}C]alanine to [2-^{13}C]acetate or [2-^{13}C]succinate is unlikely due to the irreversible step from pyruvate to PEP (see Fig. 2), these mitochondrial metabolites are strictly products of glucose degradation. The large unlabelled pool of succinate produced from the catabolism of unlabelled glucose in the parasite causes generated [2-^{13}C]succinate to be significantly diluted resulting in the low ^{13}C percent label observed. Regardless of the isotopic nature of the parasite end product, these reduced organic acids are released into surrounding host tissues and are eventually delivered to the liver. At this site parasite end products are then utilized in biochemical pathways of the host.

The altered metabolism of [3- ^{13}C]alanine in infected animals supports the notion that they experienced a greater degree of starvation than uninfected controls. The effect of prolonged starvation is also evident in the loss of liver weight in these infected hosts. Losses of liver mass coinciding with increased starvation have been reported and are mainly attributed to the loss of water content in this organ (Sharma *et al.*, 1985). Although dehydration might be, in part, responsible for the loss of liver weight observed in the present work, other factors such as lipid and protein loss, which occur during prolonged starvation, probably also took place. Evidence for lipid loss is presented in my second experiment.

Elevated concentrations of hepatic acylcarnitine and β-hydroxybutyrate in infected

mice also reflect the effects of parasite induced long term starvation. High levels of the fatty acyl transporter, acylcarnitine, is the result of increased intrahepatic levels of free carnitine and influx of fatty acids to the liver from peripheral adipose tissue (Brass and Hoppel, 1978; Harper *et al.*, 1993). To facilitate more acylcarnitine, the enzyme carnitine palmitoyl transferase I which catalyzes the entry of acylcarnitine into the mitochondria increases in activity (Drynan *et al.*, 1996a and b). The large influx of FA into the mitochondria stimulates the β-oxidation pathway. As the high activity of β-oxidation perpetuates with continued starvation, large amounts of NADH, acetyl-CoA and ATP are generated and accumulate creating the mitochondrial scenario described earlier. Acetyl-CoA produced by β-oxidation is then channelled into the formation of ketone bodies, acetoacetate and β-hydroxybutyrate (Seifter and Englard, 1994). As a result of the highly reduced nature of the mitochondrial matrix, the reduction of acetoacetate by β-hydroxybutyrate dehydrogenase to β-hydroxybutyrate is favored, increasing the β-hydroxybutyrate/acetoacetate ratio. The parallel increase in the levels of both acylcarnitine and β-hydroxybutyrate, as detected in infected animals in the present experiment and also reported by other investigators (Brass and Hoppel, 1978; Hoppel and Genuth, 1980; Yamaguti *et al.*, 1996), is a clear indicator of pronounced starvation. Acylcarnitine was also detected in the cysticerci of *T. crassiceps*. As cestodes are unable to conduct β-oxidation (Barrett, 1983; Frayha and Smyth, 1983; Smyth and McManus, 1989) the role of this metabolite in these organisms is not clear. Whether acylcarnitine is synthesized by the cysticerci to possibly shuttle FA to and from the mitochondria during modifications of absorbed FA, or if it is simply absorbed from the host requires further investigation.

Another metabolite which increased in concentration in livers of infected mice was betaine, a product of choline metabolism. This metabolic alteration was reported previously for this parasitic infection by Corbin *et al.*(1996). Considering that the levels of hepatic choline did not differ between uninfected and infected mice, decreased utilization of betaine and/or increased betaine uptake from the circulation are likely the cause for the observed effect. To date betaine is known to perform two fuctions within the cell. One function is that of an organic osmolyte, which maintains cell volume homeostasis by remaining in or exiting from the cell during altered external osmotic conditions (Petronini et al., 1992). Secondly betaine participates in the hepatic conversion of homocysteine to methionine, following which methionine goes on to produce S-adenosylmethionine (SAM), the the primary methylating agent in the body (Mato *et al.*, 1994). Whether the rise of betaine in infected mice is the result of the need for increased hepatic SAM production (increased uptake of betaine), decreased SAM synthesis (decreased betaine utilization), or osmoregulatory alterations is uncertain. Clearly further studies are required to elucidate the role of betaine under these pathological conditions. In this experiment betaine was also detected in the cysticerci of *T. crassiceps*. Other investigators also reported the presence of betaine in various larval cestodes (Smyth and McManus, 1989; Blackburn *et al.*, 1993). As in mammals, the physiological significance of betaine in larval cestodes is not fully understood. However, the inability of the proliferating larval cestode *Mesocestoides vogae* to convert choline to betaine *in vitro* (unpublished data) suggests that these parasites are unable to synthesize this compound *de novo* and therefore acquire it from the host.

T. crassiceps cysticerci also had detectable amounts of lysine, citrate and the

methylene and methyl groups of lipids in their PCA extracts. These compounds were not measurable by MRS in liver PCA extracts. The high levels of lysine and soluble lipid components are believed to reflect the parasite's ability to actively absorb these compounds from the host and not its synthetic capabilities (Pappas and Read, 1973; Howell, 1976; Hustead and Williams, 1977). Lysine is taken up by the parasite by an active mediated transport system (Pappas and Read, 1973). Such a system allows cysticerci to acquire and retain this amino acid even when the concentration of this metabolite in the surroundings is low (Howell, 1976). The methylene and methyl groups of FAC are not normally expected to be present in aqueous solution, therefore, some substance that aids in their solubilization must be acompaning these lipids in the larvae. Host plasma FA transporter, serum albumin, has been reported as being taken up by cysticerci (Hustead and Williams, 1977). Such a ligand could bind and solubilize FA in an aqueous environment, thereby permitting FA detection by MRS. The transport mechanism whereby this FA transporter is absorbed is uncertain, although diffusion has been suggested (Esch and Kuhn, 1971). Citrate, unlike lysine and lipids, is produced by the parasite. It is formed in the mitochondria from the citrate synthase catalyzed condensation of oxaloacetate and acetyl-CoA. In tapeworm tissues citrate accumulates due to the low aconitase activity and the absence of isocitrate dehydrogenase (Smyth and McManus,1989). While some of the generated citrate is assumed to function in the transport of mitochondrial acetyl-CoA into the cytosol for FAC elongation, the majority of this metabolite is excreted (Corbin, Payette, Blackburn and Novak, unpublished observation).

[3-^{13}C]Lactate Metabolism

In many ways the distribution and fate of the ^{13}C label among hepatic metabolites from [3-^{13}C]lactate was similar to that in the [3-^{13}C]alanine experiment. However, a number of distinct and underling differences surfaced between the two experiments. Considering that equimolar amounts of [3-^{13}C]alanine and [3-^{13}C]lactate were introduced intraduodenally to mice in their respective experiments, the most striking difference was that noticeably less [3-^{13}C]lactate was found in the liver in the lactate experiment than [3-^{13}C]alanine in the alanine experiment. Hepatic concentrations of total lactate (labelled and unlabelled isotopomers) in the lactate experiment were an order of magnitude lower than the concentration of total alanine in the alanine experiment. There are two possible explanations for these results. Either the animals metabolized the labelled lactate at a much faster rate than the labelled alanine or lower quantities of [3-^{13}C]lactate arrived at the liver in the lactate experiment than of [3-^{13}C]alanine in the alanine experiment. The former suggestion is unlikely as there is no evidence of any highly enriched product indicative of such activity in the lactate experiment. Therefore the latter suggestion must be considered.

Superficially the rate of absorption of a sodium [3-^{13}C]lactate solution should be similar to that of an equimolar solution of [3-^{13}C] alanine. Both substrates are three carbon gluconeogenic precursors whose unlabelled isotopomer counterparts are highly distributed throughout the body. However, the entry of the lactate anion into the intestinal enterocyte occurs via a mediated monocarboxylate transport system and during high extracellular

concentrations by passive diffusion (Hildmann *et al.*, 1980; Tiruppathi *et al.*, 1988). Regardless of which form of transport is utilized, a co-transport exists where with the entry of each lactate anion into the cell, a hydrogen ion (H^+) or Na^+ must also enter the cell (Hildmann *et al.*, 1980; Poole and Halestrap, 1993; Takanaga *et al.*, 1995). Although lactate ions can enter the cell with Na^+, studies have shown that it is the co-transport with H^+ that is of major importance to cellular uptake of lactate (Tiruppathi *et al.*, 1988; Poole and Halestrap, 1993). In the intestine the pH in the close vicinity of the brush border membrane is acidic (pH 5.5-6.0) (Tiruppathi *et al.*, 1988; Takanaga *et al.*, 1995). However, the intracellular pH of intestinal epithelial cells is approximately 7.0 (Kurtin and Charney, 1984). The resultant proton gradient which favours the inward flow of H^+ into the enterocyte, has been suggested as providing the driving force for the absorption of lactate from the intestine whether it be by diffusion or mediated transport (Tiruppathi *et al.*, 1988; Takanaga *et al.*, 1995). Obviously in the present experiment, upon administration of sodium [3-^{13}C] lactate physiological conditions favouring the absorption of lactate became disturbed. Labelled sodium lactate, the product of [3-^{13}C]lactic acid titrated with concentrated sodium hydroxide, is present as an alkaline solution whose introduction into the intestinal lumen changes not only the luminal pH but also the microenvironmental pH proximal to the brush border membrane. Therefore it can be assumed that when the alkaline substrate solution was introduced into the duodenum the excess H^+ ions, proximal to the brush border membrane, were quickly neutralized by the large influx of hydroxide ions (OH^-), significantly diminishing the proton gradient and thus the absorption of labelled lactate. Studies performed on brush border membrane vesicles by Tiruppathi *et al.* (1988) reported

similar findings of reduced lactate uptake with high extravesicular pH. Although the co-transport of lactate and H^+ was restrained by changes in luminal pH, the abundance of Na^+ in the administered solution in this experiment had to facilitate the uptake of some [3-^{13}C]lactate as this labelled substrate was detectable in the livers of both uninfected and infected mice.

The absorption of [3-^{13}C]lactate by the parasite is probably a minor process, as lactate which is generated in large amounts in cysticerci is continually expelled from their cells. Therefore any exogenous lactate which is absorbed by the parasite remains in their tissues for a very short period of time before it is excreted. However, during the brief time labelled lactate is internalized by the cysticerci, a fraction of the substrate may be converted to [3-^{13}C]pyruvate which can be transaminated to [3-^{13}C]alanine. But again, once [3-^{13}C]alanine is formed by this futile cycle its tendency is, like lactate, to be eliminated from the parasites tissues. Thus most [3-^{13}C]lactate and [3-^{13}C]alanine detected in the cysticerci had to be derived from the catabolism of acquired [1-^{13}C]glucose and not from the direct absorption of labelled lactate or alanine.

Assuming that the parasite removed very little [3-^{13}C]lactate from the host, increased hepatic utilization of this substrate in infected mice must be considered. Upon arrival at the liver lactate is transported into the hepatocyte by both a pH dependent monocarboxylate mediated carrier and by passive diffusion (Fafournoux *et al.*, 1985; Poole and Halestrap, 1993). Unlike the hepatocyte A system amino acid transporter, conclusive evidence for the hepatocyte lactate carrier as a possible regulatory site for lactate metabolism has not been established. In addition, recent reviews suggest that it is unlikely that the

transport of lactate across the hepatic plasma membrane is a rate-determining step for its overall utilization and production of glucose (Poole and Halestrap, 1993). Once [3-^{13}C]lactate traverses the hepatocyte plasma membrane and enters the cytosolic compartment, it is converted to [3-^{13}C]pyruvate by the enzyme lactate dehydrogenase (Fig.15). Unlike alanine aminotransaminase, there is also no evidence in the literature of direct hormonal regulation of lactate dehydrogenase. Instead the activity of this enzyme is controlled by the availability of substrates and coenzymes (Holten *et al.*, 1971; Buchalter *et al.*, 1989). During the oxidation of lactate to pyruvate, the enzyme requires that the coenzyme nicotinamide-adenine dinucleotide (NAD^+) receives the released hydride ion of lactate, reducing it to NADH. If the NADH produced from the lactate dehydrogenase reaction is allowed to accumulate, the cytosol would become highly reduced, which in turn would inhibit the consumption of lactate and favour its production. Therefore, generated NADH must then be converted back to NAD^+ to allow continued production of [3-^{13}C]pyruvate. NAD^+ may be regenerated through the cytoplasmic coupled reaction associated with gluconeogenesis, in which 1,3-bisphophoglycerate is converted to glyceraldehyde-3-phosphate with the concomitant oxadation of NAD^+ to NADH (Williamson *et al.*, 1967). However, recent studies have demonstrated that the mitochondrial oxidation of NADH is the primary means whereby NAD^+ is regenerated for cytoplasmic oxidation of lactate (Berry *et al.*, 1992). Since the inner mitochondrial membrane is impermeable to both NAD^+ and NADH, shuttle systems exist which transport these reducing equivalents between cytosolic and mitochondrial compartments. A transport system particularly active in the hepatocyte is the malate/aspartate shuttle (Fig. 16). The NADH generated from the lactate

FIGURE : 16 Malate-Aspartate Shuttle

Enzymes:

1, lactate dehydrogenase

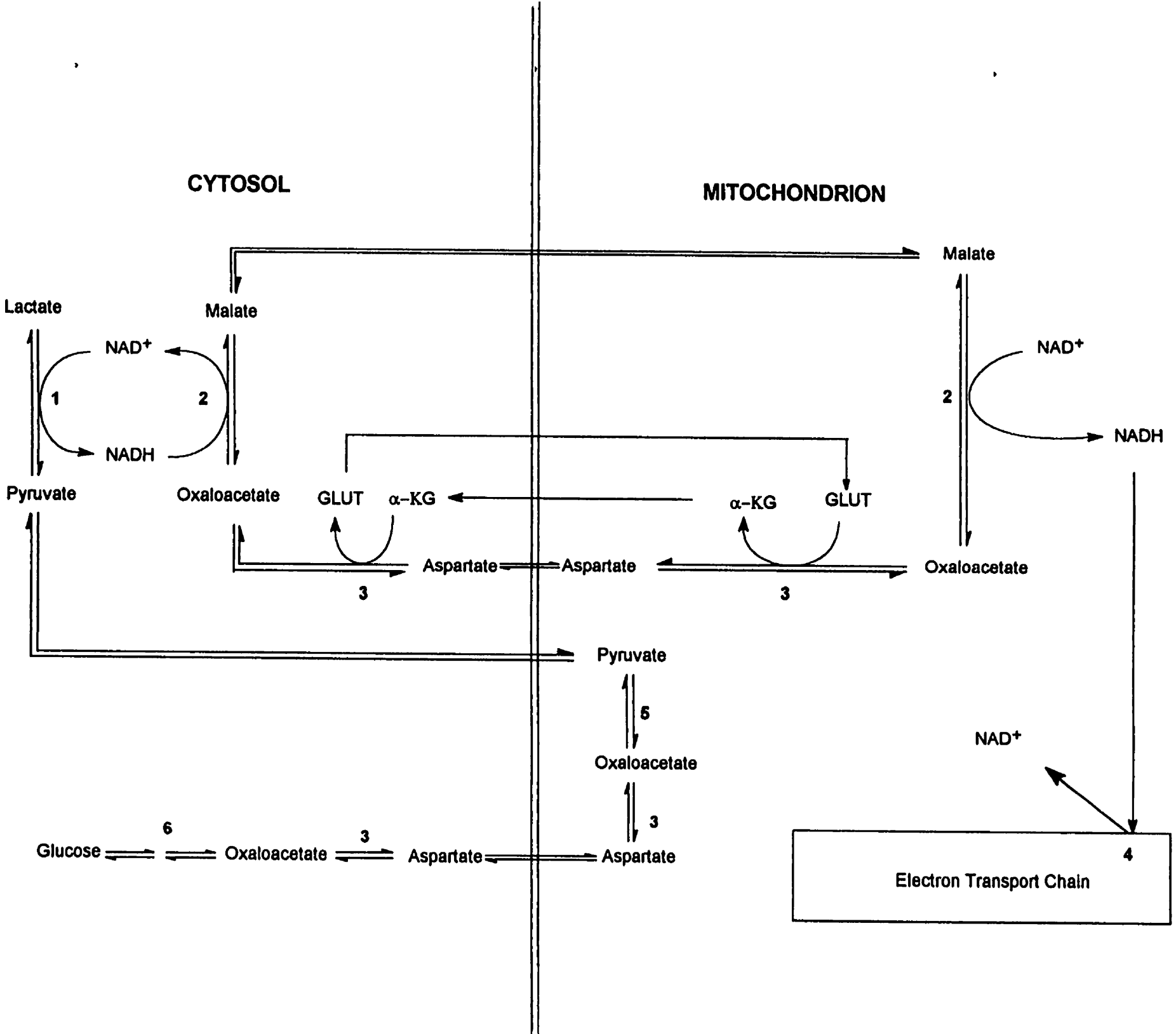
2, malate dehydrogenase

3, aspartate aminotransaminase

4, NADH dehydrogenase

5, pyruvate carboxylase

6, enzymes involved in the conversion of oxaloacetate to glucose (see Fig.15).


CYTOSOL
MITOCHONDRION
Lactate
Malate
NAD+
NADH
1
2
Pyruvate
Oxaloacetate
GLUT
α-KG
Aspartate
3
Malate
NAD+
NADH
2
Oxaloacetate
Pyruvate
5
Oxaloacetate
3
Glucose
6
Oxaloacetate
3
Aspartate
Aspartate
NAD+
4
Electron Transport Chain

dehydrogenase (1) reaction is utilized in the cytosolic catalyzed conversion of oxaloacetate to malate by malate dehydrogenase(2). Malate then traverses the inner mitochondrial membrane by a dicarboxylate transporter. Once inside the mitochondrial matrix malate is oxidized to oxaloacetate by the mitochondrial isoenzyme malate dehydrogenase(2) with the concomitant generation of NADH. NADH then goes on into the electron transport chain where the enzyme NADH dehydrogenase (4) converts NADH back NAD^+. Oxaloacetate formed from malate is transaminated to aspartate by mitochondrial aspartate aminotransaminase (3), which then exits the mitochondrion. Upon arriving in the cytosol aspartate is reconverted back to oxaloacetate by cytosolic aspartate aminotransaminase, (3) completing the cycle. As a result of this shuttle's dependence on aspartate aminotransaminase, α-ketoglutarate must be continuously exported from the mitochondria while glutamate is imported.

With continued activity of the malate-aspartate shuttle system, NAD^+ is delivered to the cytosolic compartment to facilitate further conversion of [3-^{13}C]lactate to [3-^{13}C]pyruvate. Labelled pyruvate then enters the mitochondrion via a monocarboxylate transporter where it serves as a substrate for gluconeogenic or energy generating pathways. The rate with which pyruvate enters the mitochondrion is regulated by the pyruvate/acetoacetate monocarboxylate antiport, where for every molecule of pyruvate that enters the mitochondrion a molecule of acetoacetate is exported. When the rate of ketogenesis increases, as in starvation, the greater efflux of acetoacetate facilitates a greater influx of pyruvate (Kummel, 1987; Metcalfe *et al.*, 1992). Therefore it can be assumed that due to the greater degree of starvation experienced by mice infected with *T. crassiceps* , the

rate with which pyruvate enters hepatic mitochondria should be accelerated. In both the cytosol and mitochondrion [3-^{13}C]pyruvate may be transaminated to [3- ^{13}C]alanine by the cytosolic or mitochondrial isoenzyme of alanine aminotransaminase. The high activity of of this enzyme is reflected by the substantial content of ^{13}C found in hepatic alanine in both uninfected and infected mice. However, the formation of labelled alanine is again a futile reaction, which subsequently leads only to alanine's reconversion back to pyruvate. The cross over of carbon skeletons from amino acids such as alanine into the lactate/pyruvate pool and vice versa, as described in the futile cycle above, is known to occur at substantial rates within hepatocytes (Kaloyianni and Freedland, 1990).

Hepatic [3-^{13}C]pyruvate, as previously discussed, seems to be partitioned to a greater extent towards pyruvate carboxylation in infected mice compared to that in uninfected controls. Intramitochondrial conditions of high NADH:NAD$^+$, ATP:ADP and acetyl-CoA:CoA ratios brought about by increased β-oxidation activity which reduces the flux through the PDC is further perpetuated by the oxidation of lactate. As greater quantities of labelled lactate are oxidized in the livers of infected mice, due to the greater influx of pyruvate into the mitochondria, the activity of the malate-aspartate shuttle must also operate at an accelerated rate. The high activity of this shuttle drains the mitochondrial compartment of NAD$^+$, while simultaneously introducing increasing quantities of NADH to it. This additional increase of the NADH:NAD$^+$ ratio inhibits not only the activity of of the PDC, but also that of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase, all of which catalyze oxidation reactions of the TCA cycle that are NAD$^+$ dependent. In addition, the pool of TCA cycle intermediates is also diminished, as many of

these molecules are utilized in the transport of reducing equivalents in the malate-aspartate shuttle. The cumulative effects of suppressed TCA cycle activity is reflected in the lower percent ^{13}C in glutamate and succinate of infected mice. The labelling pattern of glutamate C-4 as previously described reflects the labelling of α-ketoglutarate at the C-4 position, which is a direct measure of the flux of [3-^{13}C]pyruvate through the PDC. The lower percentage of hepatic [4-^{13}C]glutamate in infected mice leads us to believe that the flux of [3-^{13}C]pyruvate through the PDC was indeed inhibited in these animals. Although [2-^{13}C]succinate is not a direct measure of the flux of [3-^{13}C]pyruvate through the PDC, the lower percentage of [2-^{13}C]succinate in the liver of infected mice suggests a reduction of both the entry of [3-^{13}C]pyruvate into the PDC and the entry of [2-^{13}C]oxaloacetate into the TCA cycle.

With decreased decarboxylation of [3-^{13}C]pyruvate through the PDC, an increased carboxylation of pyruvate through pyruvate carboxylase is expected. Mitochondrial [2-^{13}C]oxaloacetate, produced from the pyruvate carboxylase reaction, is driven to the cytosol due to the significantly suppressed activity of the TCA. In order to cross the inner mitochondrial membrane, oxaloacetate must first be converted to malate or aspartate which are able to traverse this membrane. When lactate is the gluconeogenic precursor, oxaloacetate is preferentially transaminated to aspartate rather than being reduced to malate, as this prevents further perturbation of the reducing equivalents pool (Williamson *et al.*, 1967; Berry *et al.*, 1992). Upon the entry of aspartate into the cytosol it is converted back to oxaloacetate which can enter the gluconeogenic pathway (Fig.16). Along this pathway it will encounter a number of competing irreversible reactions. These rate determining reaction

sites, which include PEP/pyruvate and fructose-6-phosphate/fructose-1,6-bisphosphate sites, are, however, under strict hormonal regulation. Therefore higher levels of circulating glucagon in infected animals, induced by their prolonged state of starvation, should suppress the activity of reactions competing with gluconeogenesis, thereby promoting the flux of labelled intermediates towards glucose synthesis. Despite the fact that the rate of gluconeogenesis in infected mice should be increased, lower concentrations of hepatic [1-^{13}C]glucose were detected in these animals. As discussed in the alanine experiment, synthesized [1-^{13}C]glucose is rapidly released by hepatocytes into the systemic circulation. Some of this glucose leaks into the peritoneal cavity where it is acquired by the parasite. Since cestodes are unable to make this carbohydrate from pyruvate (Tielens and Bergh, 1993), the presence of [1-^{13}C]glucose in the cysticerci indicates that they did indeed take up labelled glucose from the host. Absorbed labelled glucose was then catabolized by the parasite to reduced end products, most notably cytosolic lactate and alanine.

The greater degree of starvation experienced by mice infected with *T. crassiceps* was also reflected in their elevated concentrations of hepatic acylcarnitine and β-hydroxybutyrate. The high levels of these metabolites indicate increased mobilization and delivery of FA to the liver and an increased rate of ketogenesis, both of which are known to occur during the process of starvation (Brass and Hoppel, 1978; Hoppel and Genuth, 1980; Yamaguti *et al.*, 1996). In addition, higher concentrations of hepatic betaine were detected in infected animals. Although this is not believed to be a result of starvation, nevertheless, it was a consistent feature among infected mice in both experiments and requires further investigation.

Infected mice in this experiment, unlike those in the alanine experiment, experienced a decrease in the concentration of hepatic PC with a concomitant increase in the concentration of choline. These soluble lipid metabolites are closely related in that choline is directly converted to PC via a substrate level phosphorylation catalyzed by the enzyme choline kinase (Hill and Lands, 1970; Mathews and van Holde, 1990). Thus alterations detected in the concentrations of these membrane metabolites may be explained by perturbed activity of choline kinase in the infected host. This cytosolic enzyme displays maximum activity at pH 8-9 (Ansell and Spanner, 1982; Kusel *et al.*, 1990). Studies performed by Kuesel et al.(1990) demonstrated that upon lowering the pH in the medium of cultured cells, lower levels of PC were detected in the cells as a result of suppressed activity of choline kinase. The possibility exists that in infected mice the cytosolic compartment of hepatocytes experienced a lower pH than that of controls, as with a higher influx of [3-^{13}C]lactate the monocarboxylate transporter must also increase the co-transport of H^+ into the cells. The acidification of the cytosol would then retard the activity of choline kinase and therefore the synthesis of PC, resulting in the accumulation of choline.

Lipid Metabolism

The present experiment revealed that the concentration of many lipid metabolites and the FA composition in mouse liver became significantly altered upon infection with *T. crassiceps*. Among the numerous changes detected, the most pronounced was the depletion of TAG. Given that TAG are composed of three FAC esterified to a glycerol backbone, it is not surprising that the concentration of total FA and each FA moiety investigated also decreased in the liver of infected mice. TAG are a class of neutral lipids whose primary function in the mammalian cell is to serve as a main source of energy (Mathews and van Holde, 1990; Marinetti, 1990). The high content of TAG normally found in the liver is likely due to the highly active and mobile intracellular pool of TAG. They are synthesized in hepatocytes on the cytosolic face of the endoplasmic reticulum (Chao et al., 1986) from FA transported to or synthesized by the liver. Once produced, TAG are mobilized in a convoluted manner which involves their migration to the cytoplasm where they are temporarily stored in lipid droplets. When the need arises, TAG stores are hydrolyzed and liberated FA are either reesterified in the cisternae of the endoplasmic reticulum for lipoprotein secretion or oxidized in the mitochondria for energy production or ketogenesis. Thus intracellular levels of TAG are dependent on the coordinated activities of substrate supply, intracellular events and their subsequent mobilization. A disturbance in any one of these activities may result in perturbed concentrations of hepatic TAG. Hence the alterations detected in the concentrations of hepatic TAG, total FA and each FA component in infected mice may be explained by one or a combination of the following: i) decreased delivery of

dietary lipids to the liver, (ii) decreased lipogenesis and/or (iii) altered hepatic utilization of lipids.

The supply of dietary fat is known to greatly influence the content of lipid in the liver (Stubbs and Smith, 1984; Herzberg, 1991). Upon ingestion of dietary fat, which is composed mainly of TAG, various intestinal lipases hydrolyze TAG predominately to 2-MAG and FFA (Shiau, 1987; Marinetti, 1990; Tso and Fujimoto, 1991). Subsequent interactions with bile salts secreted into the intestinal lumen, solubilize MAG and FFA into micelles which are then able to enter into the intestinal enterocytes. Once internalized in the enterocyte these hydrolyzed products migrate to the endoplasmic reticulum where the biosynthesis of complex lipids takes place. MAG and FFA are utilized in the construction of TAG either by the MAG pathway where MAG are reacylated to TAG, or by the α-glycerophosphate pathway, in which glycerol-3-phosphate is converted to phosphatidic acid which then is hydrolyzed to DAG followed by acylation to TAG. During normal lipid absorption the MAG pathway predominates as the reacylation of MAG to TAG is an extremely efficient process (Shiau, 1987; Tso and Fujimoto, 1991). The resynthesized TAG goes on to be packaged along with cholesterol esters and phospholipids in a coating of apoproteins to form a class of lipoprotein called chylomicrons, whose main function is to transport dietary TAG from the intestine to peripheral tissues. Unlike most products of digestion which enter the portal system , chylomicrons secreted by intestinal cells are picked up by the lymphatic system. From the lymphatic circulation these lipoproteins enter, via the thoracic duct, the blood vascular system and eventually end up in the liver. Thus with respect to dietary lipids the liver does not exhibit first pass extraction of this nutrient. Within

the circulation, chylomicrons acquire specific apoproteins from other circulating lipoproteins [high density lipoproteins (HDL)], which serve as receptors for lipoprotein lipases (LPL). These enzymes, closely associated with capillary endothelial cells, bind to circulating chylomicrons and hydrolyze the TAG rich core of the lipoprotein liberating FFA and MAG which are taken up by underlying cells. Most cells in the mammals, especially those in the heart and skeletal muscle, utilize FFA liberated from chylomicron TAG as a main energy source (Cahill, 1986; Mathews and van Holde, 1990). Adipose tissue also recovers a large fraction of MAG and FA released by LPL. In the adipocyte these products are either catabolized for energy or reesterified into TAG and stored as a future fuel source for peripheral tissues. As TAG are hydrolyzed and released from chylomicrons the lipoprotein particle becomes smaller until it is reduced to a CT-enriched packet called a chylomicron remnant. The chylomicron remnant then reenters the circulation and is eventually deposited in the liver where the remaining CT, TAG and GPL are utilized in intrahepatic processes while the apoprotein covering is recycled for later lipoprotein synthesis.

In mice infected with *T. crassiceps* a number of metabolic alterations occur which can impede the delivery of chylomicron TAG to the liver. During the course of the infection cysticerci must acquire lipids from the host since they are incapable of *de novo* FA and sterol synthesis (Barrett, 1983; Smyth and McManus, 1989). To date there is no indication in the literature of *T. crassiceps* cysticerci being able to absorb lipoproteins. Therefore their acquisition of lipids most likely occurs through the absorption of water soluble components, such as choline, glycerol and short chain FA, and micelles of long chain FA and MAG released from hydrolyzed chylomicrons. In the present study, evidence for the uptake of

FFA by cysticerci is illustrated by the high concentration of the unesterified FA moiety, -CH_2COOH. Although intracellular hydrolysis of endogenous TAG and GPL from the cysticerci tissues could contribute to levels of FFA detected, the inability of cestodes to catabolize FA seems to suggest that the hydrolysis of these complex lipids would be a minor process. Indeed this appears to be the case, as Mills *et al.*(1981) report very low levels of MAG, DAG and lysophosphatidylcholine/ethanolamine (deacylated phosphatidylcholine/ethanolamine) in the tissues of *T. crassiceps* larvae. As the FA from the TAG core in chylomicron are consumed by peripheral tissues and the parasite, the resulting chylomicron remnants arrive at the liver depleted in TAG. Thus it is possible that in infected animals the reduction in TAG took place prior to its arrival at the liver and this may account for the lowered hepatic concentrations of TAG and FA.

In addition to the removal of FA (depletion of TAG), the cysticerci also acquire substantial amounts of glucose from the host (previous experiment). As a result of this hypoglycemic condition, the liver attempts to correct this metabolic disturbance by mobilizing glycogen stores (Corbin *et al.*, 1996) and accelerating the gluconeogenic process. Because both these glucose-generating events are stimulated by high glucagon/low insulin levels (Jungermann and Katz, 1986; Seifter and Englard, 1994), hormonal conditions in the infected host seem to be such that the utilization of glucose in secondary pathways, such as FA synthesis, is inhibited (Katz, 1986). Normally *de novo* synthesis of FA is controlled by a number of regulatory enzymes within its complex pathway. During periods of low glucose availability the activity of key lipogenic enzymes, PDC, acetyl-CoA carboxylase, malic enzyme and FA synthase, are suppressed both by reduced enzyme synthesis and allosteric

effectors (Goodridge *et al.*, 1986; Sugden *et al.*, 1993). Thus with decreased activity of hepatic FA synthesis, FA esterification and TAG synthesis are also expected to be markedly diminished.

With reduced availability of circulating glucose for FA synthesis and diminished dietary TAG arriving at the liver, the organ must rely on other extrahepatic fuel reserves to supply it with necessary FA substrates. Under normal conditions lipolysis of TAG stores in adipose tissue provides the liver and peripheral tissues with a large supply of FA and glycerol (Seifter and Englard, 1994). During the hydrolysis of these TAG reserves, FA are released into the plasma where they bind to albumin. By forming these FA-protein complexes, FA are solubilized within plasma, thereby permitting its circulation to peripheral tissues. Upon entering the circulation these albumin bound FA may also become accessible to the parasite. Studies performed by Hustead and Williams (1977) demonstrate that *T. crassiceps* are indeed capable of internalizing albumin. Therefore it is likely that albumin bound FA derived from adipose tissue is also captured by the cysticerci. The transport mechanism whereby this FA transporter is taken up by the cysticerci is uncertain, although diffusion has been suggested (Esch and Kuhn, 1971). As the competition continues between the cysticerci and host tissues for FFA released from adipose tissue, not only is the liver likely to receive reduced quantities of FFA, but other tissues too, notably skeletal and cardiac muscle. In response to the continuous demand for energy substrates by peripheral tissues, further hydrolysis of adipose TAG stores are initiated. The perpetual mobilization of adipose deposits coupled with the reduced potential for adipocytes to synthesize FA and TAG, due to the decreased availability of circulating glucose and uptake of dietary FA, in this infection,

may contribute to the significant loss of host body fat previously reported by Crompton *et al.* (1985). In this experiment a loss in liver mass was also found in infected mice. Although the depletion in hepatic TAG and FA cannot account for the total loss of liver mass, it is likely that their reduction contributed to this process.

When the amount of hepatic FA is low and the capacity to synthesize them is compromised, the available FA and TAG are quickly channelled into energy producing pathways. The FA that enter the liver have three possible fates: (1) esterification into TAG and subsequent exportation from the liver as very low density lipoproteins (VLDL); (2) oxidation to ketone bodies; or (3) complete oxidation to CO_2 and H_2O for energy generation. The partitioning of incoming FA into these alternative pathways is primarily regulated by the concentration of malonyl-CoA (Katz, 1986; Hellerstein and Munro, 1994). The concentration of this regulatory metabolite increases when the supply of glucose exceeds that needed for energy generation. As the levels of malonyl-CoA accumulate the formation of acylcarnitine is inhibited, thereby blocking the mitochondrial processes of ketogenesis and β-oxidation while concomitantly promoting the microsomal activities of TAG and VLDL synthesis and secretion. Because the availability of circulating glucose is low in infected mice, the concentration of malonyl-CoA will also be low. Therefore FA entering the liver in infected mice are expected to be partitioned to a greater extent towards the mitochondrial processes of ketogenesis and complete oxidative degradation rather than TAG and VLDL synthesis. Although serum VLDL levels have not been measured in rodents infected with *T. crassiceps*, significantly lower levels of circulating FA and VLDL were found in sera from gerbils infected with the metacestode *Echinococcus multilocularis* (Novak et al.,1989).

These findings, along with the fact that liver RNA and protein levels rapidly decrease early in starvation (Goodman and Ruderman, 1980), suggest that hepatic VLDL synthesis and secretion could be compromised in mice infected with *T. crassiceps*.

FA which enter the mitochondrion, whether for ketogenesis or complete oxidation are first degraded along the β-oxidation sequence to acetyl-CoA. Once acetyl-CoA is generated it either enters the β-ketothiolase and HMG-CoA synthase reaction which incorporates it into ketogenesis or the citrate synthase reaction for TCA cycle energy production (see Fig. 4). Ketogenesis is stimulated when an increased supply of exogenous FA enter the liver (Robinson and Williams, 1980; Zammit, 1981). Thus this pathway predominately serves to accept surplus FA when the supply of FA presented to the liver occurs faster than it can be oxidized to CO_2 and H_2O (Marinetti, 1990). With the reduced amount of FA found in the liver of infected animals, it can be assumed that FA are no longer available in excess to be channelled into ketogenic processes. Indeed the findings of decreased concentrations of hepatic β-hydroxybutyrate in mice infected with *T. crassiceps* Corbin *et al.*(1996) support this hypothesis and suggests that infected animals do run ketogenesis at a reduced rate. Alternatively, the diminished levels of β-hydroxybutyrate could reflect high exportation rates of this substance. However this is unlikely as the hepatic supply of FA does not appear to be sufficient to support this process. It seems then that the FA available in the liver were used for energy generation. Continued complete oxidation of FA is essential for the liver as it derives the majority of its energy from this process (Seifter and Englard, 1994). This energy, in turn, is used to fuel the many biosynthetic, degradative and secretory activities necessary to maintain both the integrity and vitality of this organ.

Although the concentration of each FA component in the liver of infected mice was significantly lower than that of controls, the ratio of certain FA components relative to total FA differed. Saturated FA methylene components of $-(CH_2)_n-$ and $-CH_2COO^-$ groups were reduced, while the ratios of unsaturated FA components belonging to AA, DHA and $-CH=CH(CH_2CH=CH)_n$ were higher. Lower ratios of the hepatic FA methylene components of $-(CH_2)_n-$ and $-CH_2COO^-$ in infected mice indicate that there was less of these components per FAC than that in uninfected mice. Likewise higher ratios of unsaturated FA belonging to AA, DHA and $-CH=CH(CH_2CH=CH)_n$ means that there was more of these moieties per FAC in infected animals compared to uninfected controls. Thus the change in the ratio of certain saturated and unsaturated moieties both contributed to the alteration in the composition of hepatic FA in infected mice. Changes in the amount of $-CH_2COO^-$ and $-CH=CH(CH_2CH=CH)_n$ per FAC are difficult to interpret and provide little information when studied independently. These moieties must be viewed in conjunction with $-(CH_2)_n-$ and AA/DHA respectively. The methylene detected in the $-CH_2COO^-$ resonance in the proton MR spectrum represents a methylene alpha to a carbonyl group with no other neighbouring functional groups within three bonds, with the exception of repeating $-CH_2-$ groups. Therefore, the $-CH_2COO^-$ is likely to be frequently associated with the $-(CH_2)_n-$ group and hence may also be a measure of saturated moieties in FA. Likewise the $-CH=CH(CH_2CH=CH)_n$ group is associated with AA and DHA. As depicted below, the $-CH=CH(CH_2CH=CH)_n$ moiety is actually a major constituent of both AA and DHA. It repeats three times in the AA chain and five times in the DHA chain. Thus the increased ratio of the $-CH=CH(CH_2CH=CH)_n$ group both complements and reflects the corresponding

elevated ratios of AA and DHA.

AA

$HOOCCH_2CH_2CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_2CH_2CH_2CH_3$

DHA

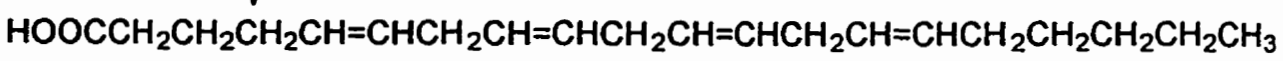
$HOOCCH_2CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH\text{-}CH_2CH_3$

Higher quantities of moieties belonging to AA and DHA could be explained by increased synthesis/uptake or decreased utilization of these FA. Intrahepatic AA and DHA can be derived from two potential sources. Either they are synthesized within hepatocytes or sinusoidal cells of the liver or they are taken up from the circulation bound to chylomicrons or albumin. Synthesis of AA and DHA in the liver requires the respective precursors LA and linolenic acid (LnA). Both LA and LnA must be acquired from the diet since mammalian cells neither possess the $\Delta^{12\text{-}}$ nor $\Delta^{15\text{-}}$ FA desaturase enzymes necessary to synthesize them from oleic acid (Marinetti, 1990; Mathews and Van Holde, 1990). In the liver microsomes of the endoplasmic reticulum, a fraction of these 18 carbon FA precursors, are enzymatically converted into AA and DHA via successive desaturation and elongation reactions. Thus if increased hepatic biosynthesis of AA and DHA were to occur in infected animals a change in the proportions of LA and LnA would also have to reflect this activity. The fact that the hepatic concentration of LA was depleted to the same degree as the concentration of total FA suggests that neither excess LA was available for increased

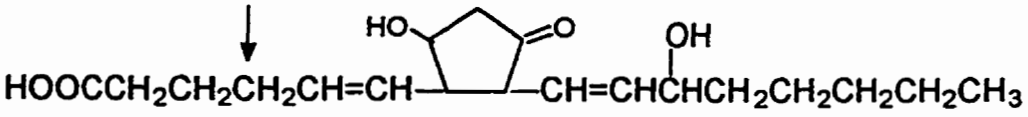
conversion to AA, nor was LA further depleted which would indicate enhanced AA biosynthesis. In addition, under conditions of low circulating levels of insulin the activity of liver microsomal desaturase is known to be depressed (De Gomez Dumm et al., 1976; Brenner, 1977; Poisson and Cunnane, 1991). The presumed low levels of insulin in infected mice would inhibit the activity of FA desaturation, which would infer that enhanced biosynthesis of AA and DHA are unlikely in these animals.

The identification of AA and DHA in this experiment were based on the unique resonances produced by the C_4 methylene alpha to olephenic group for AA and the methylenes at positions 2 and 3 alpha/beta to an olephenic group for DHA. It was found that while the orientation of these methylene groups are not exclusive to the acyl chains of AA and DHA, the other FA which also possess these particular orientations of methylene groups are present in animal tissues at only minute levels. Therefore it has been the convention in proton MRS studies of animals lipids to regard all the resonances generated by these methylene groups as measures of AA and DHA (Casu *et al.*, 1991; Choi *et al.*, 1993; Adosraku *et al.*, 1994). However, there is one group of fatty acyl derivatives which may interfere with the interpretation of the C_4 methylene resonance as a measure of AA. These are the eicosanoids, namely prostaglandin (P) GI_2, PGE_2 and thromboxane A_2 (TXA_2). They all possess a C_4 methylene alpha to an olephenic group (Fig. 17). Eicosanoid synthesis is initiated in mammalian cells when hormonal or inflammatory stimuli activate phospholipase A_2 and C which hydrolyze AA from the carbon-2 position of membrane PTC/PTE and PTI respectively (Mathews and van Holde, 1989; Smith et al., 1991).

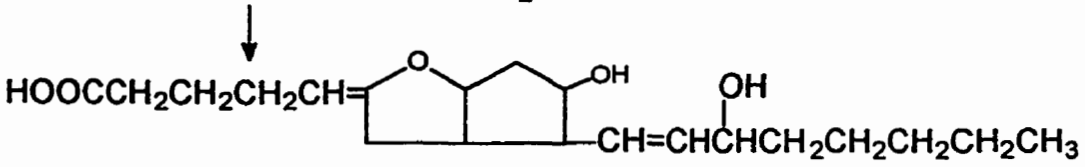
AA



PGE_2



PGI_2



TXA_2

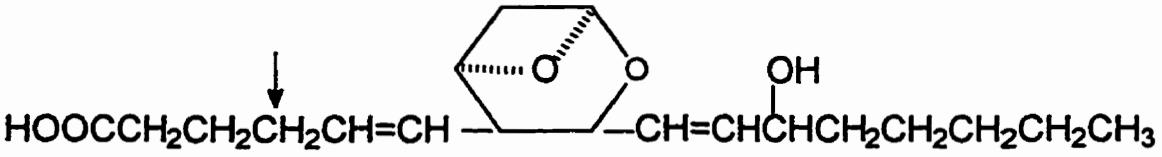


FIGURE : 17 The chemical structures of arachidonic acid, AA; prostaglandin E_2, PGE_2; prostaglandin I_2, PGI_2 and thromboxane A_2, TXA_2. The arrow indicates the C_4 methylene alpha to an olephenic group in each lipid molecule.

Released AA then enters a cascade of microsomal oxygenation reactions catalyzed by prostaglandin G/H synthase to produce the prostaglandins and thromboxanes or the lipoxygenase pathway to generate leukotrienes or related hydroxy acids. Once synthesized eicosanoids are released from the cell and act locally by binding to nearby target cells where they modulate numerous physiological processes as vasodilation, platelet aggregation, immune cell proliferation and chemotaxis (Mathews and van Holde, 1989; Smith *et al.*, 1991; Keppler *et al.*, 1994). Recent studies have documented that many parasitic helminths are also capable of converting AA, through a series of oxygenating reactions, into various eicosanoids (Fusco *et al.*, 1985; Liu *et al.*, 1992; Fukushima *et al.*, 1993). In the case of cestodes, the cysticerci of *T. taeniaeformis* are able to synthesize and secrete the eicosanoids PGE_2, PGI_2 and TXA_2 (Leid and McConnell, 1983a; Leid and Mc Connell, 1983b). Once these potent lipid mediators are released they are believed to be taken up into surrounding host tissues where they induce immunosuppressive effects by inhibiting macrophage and T- and B-cell functions, and altering haemostasis in surrounding vessels (Leid and McConnell, 1983a; Leid and Mc Connell, 1983b; Belley and Chadee, 1995). The cysticerci of *T. crassiceps* are closely related to those of *T. taeniaeformis* in that both share numerous morphological, physiological and biochemical similarities (Hustead and Williams, 1977; Mills *et al.*, 1981; Novak and Dowset, 1983; Ito *et al.*, 1996). As well they both elicit similar immune responses in their intermediate hosts (Freeman, 1964; Siebert *et al.*, 1980; Williams *et al.*, 1982). Hence it is highly likely that the cysticerci of *T. crassiceps* may also synthesize and release eicosanoids as a means of modulating the host's inflammatory and immune response. In mammals, dietary LA is the necessary precursor from which AA and,

in turn, eicosanoids are formed (Marinetti, 1990; Lands, 1991). However, while abundant LA was detected by MRS in *T. crassiceps* cysticerci, the inability of cestodes to desaturate FA (Barrett, 1983; Smyth and McManus, 1989) renders them incapable of synthesizing AA from this FA. Therefore any AA in the parasite must be acquired from the host as a solubilized FFA. Although in this experiment levels of AA in the cysticerci were not measurable, chromatographic experiments performed by Mills et al.(1981) detected AA in this parasite, albeit at low concentrations. The low concentration of AA could be due to high rates of eicosanoid synthesis and secretions by the parasite which would account for the marked immunomodulation (down regulation of Th1 cells /upregulation of the Th2 cells) of the host (Sciutto et al., 1995; Villa et al., 1995). While it is likely that *T. crassiceps* larvae are capable of secreting various eicosanoids, it is also highly possible that some eicosanoids are generated by the host. The inflammation of the peritoneum and marked leucocytosis which accompanies this infection (Freeman, 1962; 1964) also extends to the host liver. Histological studies by Chernin and McLaren (1983) reported fibrosis and an accumulation of inflammatory cells in this organ. Within the liver eicosanoids are mainly synthesized by the Kupffer, Ito and sinusoidal endothelial cells while hepatocytes are the major sites of eicosanoid uptake (Keppler *et al.*, 1994). Once these lipid mediators bind to hepatocytes, their target cells, they are rapidly inactivated via degradative β - and ω-oxidative pathways (Mathews and van Holde, 1989; Smith et al., 1991; Keppler, 1994). Further studies, however, are needed in this area to determine whether eicosanoids generated by *T. crassiceps* cysticerci or host tissues contribute to the higher AA ratio observed in this experiment.

The nutritional state of mammals is known to have a direct effect on the FA composition of their liver (Sugano et al., 1975; Crane and Masters, 1981; Cunnane, 1996). When food is deprived and sources of lipids and lipid precursors are not available, the liver must adapt to this challenge with specific metabolic alterations. Many of these alterations involve preferential retention and utilization of specific FA which result in a change in the hepatic FA composition. It is well established that during fasting some hepatic PUFA, such as AA and DHA, are preserved while acyl chains of palmitic (16:0), palmitoleic [16:1(n-7)] and oleic [18:1 (n-9)] are readily utilized (Sugano et al., 1975; Cunnane, 1988; Chen and Cunnane, 1992; Andriamampandry et al., 1996). The observed alterations in the FA composition in the liver of mice infected with *T. crassiceps* are similar to descriptions of changes in hepatic FA composition of fasting animals (Sugano et al., 1975; Cunnane, 1988; Chen and Cunnane, 1992; Andriamampandry et al., 1996). With the reduced quantity of hepatic FA in infected mice it seems likely that these animals, similar to fasting animals, would also engage in the preferential retention/utilization of specific hepatic FA. In spite of the pronounced depletion of total hepatic TAG, AA and DHA were preserved to a greater extent than other FA. In fasting rodents these modifications are believed to be a result of the retention of adipose tissue derived, preformed AA and DHA (Cunnane et al., 1986; Cunnane, 1988; Chen and Cunnane, 1992). After adipose tissue TAG stores are hydrolyzed, liberated AA and DHA which subsequently bind to albumin and are later deposited and reacylated in the liver manage to evade oxidative degradation by tissues. Gavino and Gavino (1991) demonstrated that the reactivity of mitochondrial palmitoyltransferase to AA and DHA was significantly lower than to other FA, which may explain how the liver selectively

retains these PUFA during lipid deprivation. In the liver various reacylation steps result in the retention of AA in TAG (Cunnane, 1990; Chen and Cunnane, 1992) while DHA is retained in both TAG and GPL (Chen and Cunnane, 1992; Andriamampandry et al., 1996). It is possible that similar mechanisms for AA and DHA preservation might be also operating in infected animals. However, because the parasite is able to steal adipose- liberated FFA from the circulation, the increased amount of AA and DHA per FAC was not as pronounced as the amounts reported previously for fasted animals (Cunnane, 1988; Chen and Cunnane, 1992). The ability of the cysticerci to take up FFA is depicted in the detectable levels of DHA and LA in their tissues. Both LA and DHA have been previously described as major components in FA composition of *T. crassiceps* larvae (Mills *et al.*, 1981). High concentrations of LA have been also reported for numerous cestodes (Barrett, 1983), but only rarely has DHA been identified. In mammals, DHA is found in high quantities in neural tissues where it serves as an essential membrane constituent for normal functional development of the retina and brain (Simopoulos, 1991; Pawlosky *et al.*, 1996). The role of DHA in nonneural mammalian tissues, however, is uncertain. Likewise the functional significance of DHA and LA in *T. crassiceps* cysticerci is not known, but most investigators seem to agree that the accumulation of specific FA in cestodes is a reflection of the FA composition of the host's diet and not the result of intended efforts of the parasite (Barrett, 1983; Smyth and Mc Manus, 1989). *T. crassiceps* cysticerci contain considerable amounts of long chain FA ranging from 16 to 22 carbons in length, as well as very long chain FA in the order of 26 to 30 carbons (Mills *et al.*, 1981; Dennis *et al.*, 1992). Since cestodes cannot synthesize FA *de novo* these lipids are obtained by absorbing preformed long chain FFA in

micelles or bound to albumin. Cysticerci probably do not acquire very long chain FA in this manner as they are not prominent in mammalian plasma. Instead these FA are synthesized through the elongation of acquired shorter chain FA (Barrett, 1983; Frayha and Smyth, 1983; Smyth and Mc Manus, 1989). During this process acetyl-CoA units are sequentially added to the growing FAC, however, double bonds are not introduced to the acyl chain since cestodes are incapable of FA destaturation. This may explain why in this study *T. crassiceps* cysticerci possessed less double bonds per FAC than host liver, and why others have reported high levels of 26:0, 28:0 and 30:0 FA in this parasite (Dennis et al., 1992). Short to medium chain FA ranging from 5 to 12 carbons are readily soluble in blood without the aid of solubilizing plasma proteins (Shiau, 1987). Thus as these FA are easily absorbed and retained among longer FA within the parasite's small pool of FA, the value for the average FAC length is easily skewed, producing a shorter average FAC length of 12.18 carbons per FAC for the cysticerci, compared to 16.50 carbons per FAC for host liver.

While AA and DHA are selectively preserved in lipid-deprived animals, palmitic, palmitoleic and oleic acids are depleted at a faster rate to continue generating energy via oxidative pathways (Cunnane, 1988; Cunnane, 1990; Chen and Cunnane, 1992). Studies have shown that the mitochondrial acyl transporter enzyme carnitine palmitoyltransferase has a relatively high affinity for palmitic acid (Gavino and Gavino, 1991). This is consistent with the notion that the marked depletion of hepatic TAG, seen during fasting, is due to oxidative degradation of FA such as palmitic acid (Cunnane, 1990). Although palmitic acid could not be directly measured in this experiment, the pronounced depletion of $-(CH_2)_n-$ and $-CH_2COO^-$ moieties in the liver of infected mice likely reflects the enhanced oxidative

degradation of palmitic acid. The decreased levels of hepatic palmitoleic and oleic acid reported in fasting animals may also be due to increased β-oxidation of these FA. However, decreased synthesis of these FA from palmitic and stearic acid must also be considered as the activity of desaturase is known to be reduced during fasting (De Gomez Dum et al., 1976; Brenner, 1977; Poisson and Cunnane, 1991). Although palmitoleic and oleic acids could not be directly measured in the present experiment, these FA are suspected of having been metabolized by the liver of infected mice in a manner similar to those in fasting animals. The decreased ratio of saturated moieties in hepatic FA from infected mice could also be a result of the selective absorption of saturated FA by the cysticerci. Such mechanisms of saturated long chain FA absorption have not previously been observed for cestodes and warrant further investigation.

The concentrations of PTC, PTE, PTI and total GPL were all lower in liver of infected mice than in uninfected controls. Although PTC, PTE and PTI are common lipid constituents of both the plasma and organelle membranes of cells, it is unlikely that their reduced concentration is solely the result of membrane breakdown. Such activity would compromise the integrity of the cell which would ultimately lead to cell death. Studies on hepatic lipid content and turnover during starvation revealed that GPL were preserved to a greater extent than TAG due to low rates of membrane GPL degradation (Groener and Van Golde, 1977; Moir and Zammit, 1992). Mechanisms of membrane GPL preservation, via suppressed membrane degradation, are also believed to be operating in the liver of infected animals as data of Corbin et al.(1996) showed that in mice infected with *T. crassiceps,* levels of hepatic GPC (a product of PTC membrane degradation) were not elevated. Therefore, the

reduced concentration of hepatic GPL in infected mice must have resulted from a disturbance in the mobile pool of GPL in the liver. A substantial fraction of the mobile pool of hepatic GPL, which include PTC, PTE and PTI, is derived from dietary sources of either preformed GPL or water soluble GPL precursors. Intestinal chylomicrons are packaged with an outer covering composed mainly of apoproteins, choline and ethanolamine containing GPL. As chylomicrons are released into the circulation to deliver FA to peripheral tissues, the GPL components are transferred to other circulating lipoproteins, primarily the HDL (Redgrave and Small, 1979). HDL in turn transports the majority of intestinal preformed PTC and PTE to peripheral tissues and the liver (Ansell and Spanner, 1982; Engelmann *et al.*, 1996). While cestodes probably could obtain their supply of membrane lipids by absorbing micelles of preformed GPL liberated from HDL, there is no evidence for this process. Instead cestodes are likely to acquire their membrane constituents through the absorption of water soluble precursors such as PC, choline, PE, ethanolamine, inositol and glycerol (Barrett, 1983; Fryha and Smyth, 1983; Smyth and McManus, 1989). Within the parasite these precursors are constructed into membrane GPL by the cytidine 5'-diphosphate (CDP) linked cytidyltransferase catalyzed condensation of glycerol derived DAG with choline, ethanolamine or inositol. Phosphatidylserine and cardiolipin are also constructed by CDP pathways in cestodes. Mills et al. (1981) showed that PTC is the most prominent GPL in *T. crassiceps* cysticerci, followed by PTE, PTS, PTI and finally cardiolipin. A similar trend was also found in this study. As in mammals, cestode GPL function primarily as structural membrane constituents. Given the high rates of membrane turnover and asexual reproduction, cysticerci must acquire considerable amounts of GPL precursors to maintain

continued membrane biosynthesis. The host in turn is robbed of these precursors which may compromise the capability of the liver to synthesize GPL. While the parasite's active uptake of membrane precursors may be contributing to this reduction, the low levels of FA in the liver appear to be a greater deterrent to the synthesis of hepatic GPL. In the study by Corbin et al. (1996) mice infected with *T. crassiceps* cysticerci had increased concentrations of hepatic choline and PC. With less FA in the liver of the infected host, the availability of DAG, a key participant in GPL synthesis, becomes limited (see Fig. 18). When DAG is not available to participate in the CDP-choline 1,2-DAG PC transferase (9) reaction, PTC synthesis is impeded and choline and PC start to accumulate in the cell. PTE and PTI may also be synthesized by the condensation of CDP-ethanolamine and myo-inositol with DAG and CDP-DAG respectively. Therefore the limited supply of DAG in the liver would also contribute to the reduced concentration of PTE and PTI in infected mice. Since individual GPL are no longer in excess, the rates of head group exchange and interconversion may be reduced which could also contribute to lower concentrations of other GPL observed. For instance low levels of PTE may contribute to the decrease in PTC concentration as excess PTE is no longer available to participate in the PTE-N-methyltransferase (6) synthesis of PTC. Although hepatic content of phosphatidylserine, phosphatidylglycerol and cardiolipins were not detectable by MRS in this study, their concentrations are also suspected of being reduced in infected animals due to the limited availability of DAG, and the suspected reduction in GPL head group exchange and interconversion. All of this would lead to the decreased concentration of total GPL, which was found in this experiment.

Surprisingly the concentration of CT remained unchanged in the liver of infected

FIGURE : 18 Effects of low hepatic FA content on GPL synthesis

Enzymes

1, Ethanolamine kinase

2, CTP-phosphoethanolamine cytidylyltransferase

3, CDP-ethanolamine 1,2-diacylglycerol phosphoethanolamine transferase

4, Phosphatidylserine decarboxylase

5, Phosphatidylethanolamine serinetransferase (head group exchange enzyme)

6, Phosphatidylethanolamine-N-methyltransferase

7, Choline kinase

8, CTP-phosphocholine cytidylyltransferase

9, CDP-choline 1,2-diacylglycerol phosphocholine transferase

10, Glycerol-3-phosphate acyltransferase

11, Glycerol-3-phosphate acyltransferase and phophatidate cytidylyltransferase

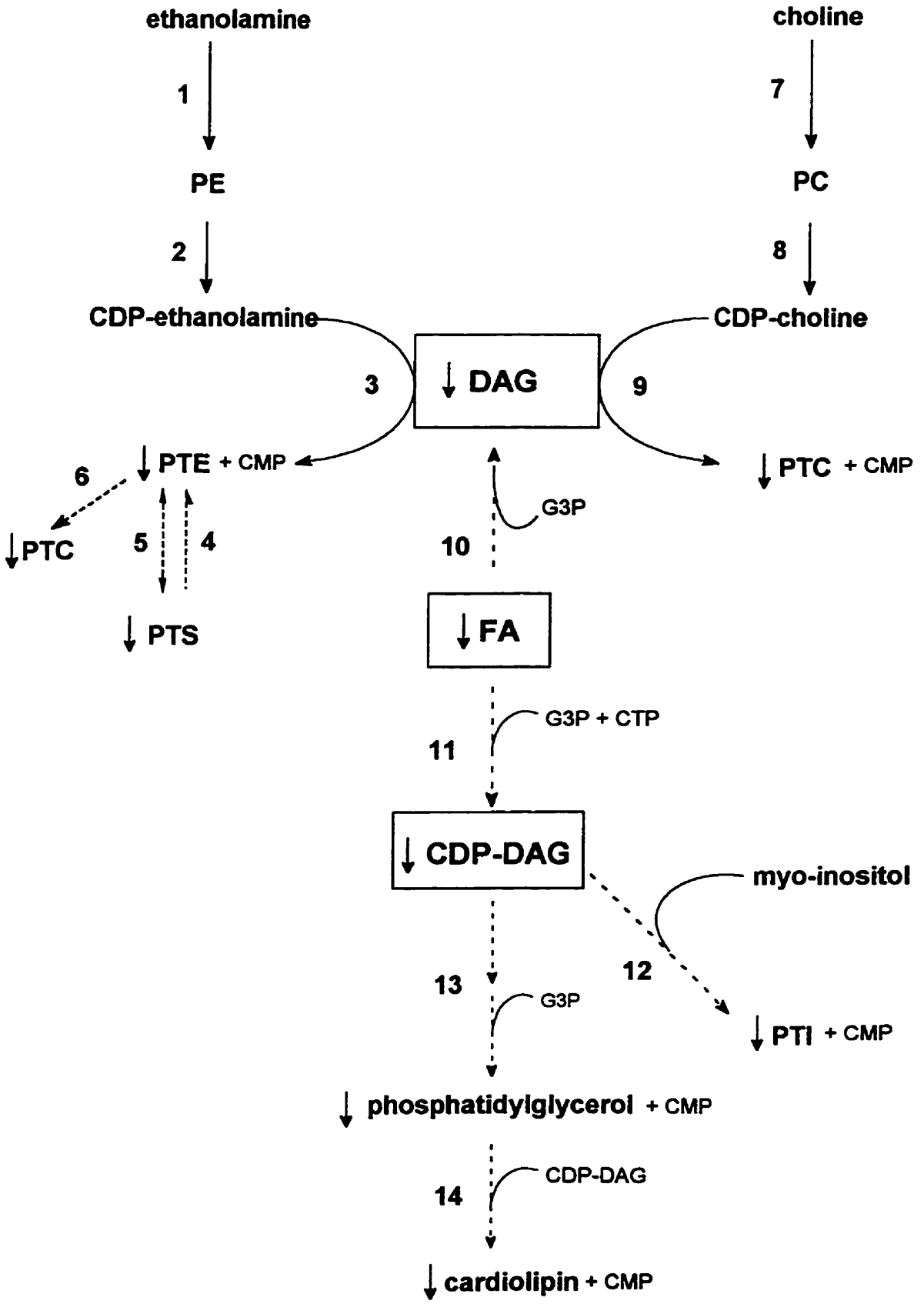
12, Phosphatidylinositol synthase

13, Glycerophosphate phosphatidyltransferase

14, Cardiolipin synthase

Abbreviations

CDP	cytidine 5'-diphosphate	PE	phosphoethanolamine
CMP	cytidine 5'-monophosphate	PTC	phosphatidylcholine
DAG	diacylglycerol	PTE	phosphatidylethanolamine
FA	fatty acid	PTI	phosphatidylinositol
G3P	glycerol-3-phosphate	PTS	phosphatidylserine
PC	phosphocholine		


ethanolamine
choline
1
7
PE
PC
2
8
CDP-ethanolamine
CDP-choline
3
DAG
9
PTE + CMP
PTC + CMP
6
PTC
5
4
PTS
10
G3P
FA
G3P + CTP
11
CDP-DAG
myo-inositol
12
13
G3P
PTI + CMP
phosphatidylglycerol + CMP
14
CDP-DAG
cardiolipin + CMP

mice. Yet high levels of CT found in cysticerci indicate active absorption from the host as cestodes are unable to synthesize sterols *de novo* (Barrett, 1983; Smyth and McManus, 1989). In spite of removal of this lipid by the parasite the host was still able to maintain its normal concentration of CT.

It can be concluded that indeed *T. crassiceps* dramatically alters hepatic carbohydrate and lipid metabolism and that magnetic resonance spectroscopy is an appropriate technique for analyzing such complex systems.

FUTURE INVESTIGATIONS

Several new ideas and important questions worth investigating in future studies arose from the data generated from the experiments in this study. Quantitative ^{13}C spectroscopic analysis of tissue extracts from [3-^{13}C]alanine and [3-^{13}C]lactate studies should be one of the first sets of experiments conducted. The spin echo spectroscopy, which was used in the present study, is able to detect the ^{13}C label at only one carbon position in most metabolites analyzed, due to the complex overlap of resonances in the proton MR spectrum. More information could be obtained by applying ^{13}C spectroscopy, as ^{13}C enrichment could be measured at several different carbon positions in each metabolite. Carbon-13 enrichment in different isotopomers of glutamate and glucose would be especially informative as they permit a much more direct and accurate measure of the partitioning of the ^{13}C label into gluconeogenic and TCA biochemical pathways. In addition, metabolites such as glutamine and aspartate, which are normally hidden in the proton spectrum by overlapping resonances, could be clearly detected and quantified via ^{13}C spectroscopy.

A consistent finding in all three experiments in this study was the fact that mice infected with *T. crassiceps* displayed a different hepatic metabolic response to both exogenous and endogenous supplies of carbohydrate and lipid substrates than uninfected mice. Many theories were proposed, based on the change in concentration and ratio of various liver metabolites, to account for the difference seen in the metabolic responses between the two groups of animals, but to truly explain these differences in hepatic metabolism the biological agents which regulate the processes of fuel metabolism, insulin,

glucagon and the catecholamines epinephrine and norepinephrine, should be directly measured. In a simple experiment, blood samples could be collected from a group of infected and uninfected mice and the levels of these hormones quantitated by standard techniques such as radioimmunoassays or gas chromatography. Differences in the concentration and ratio of these hormones would provide a definite explanation for the alterations seen in the utilization and partitioning of fuel substrates in infected animals.

The interactions numerous extrahepatic tissues have with the liver may also greatly influence many intrahepatic metabolic processes. Peripheral organs which are primarily involved in the integrated exchange of fuel substrates and end products with the liver include adipose tissue and skeletal muscle. By measuring the content of selected substrates such as glucose and FA and end products like lactate in these tissues, a better understanding of the role peripheral distribution and supply of fuel substrates have on hepatic metabolism in infected mice. MRS would be an appropriate technique to use in such studies as it would allow the simultaneous detection of several classes of compounds. In addition, the administration of ^{13}C labelled substrates ([1-^{13}C]glucose and ^{13}C labelled FA) would allow the investigator to trace the substrate from the point of administration to the tissue under analysis and its subsequent entry into various biochemical pathways at this site. Experiments of a similar kind could also be expanded to include the brain, where metabolites such as glucose and β-hydroxybutyrate could be followed to determine if the metabolism of distant organs is altered in this infection.

Lipid metabolism could also be more effectively monitored utilizing labelled substrates and MRS. By introducing an intravenous bolus of chylomicrons containing ^{13}C

labelled TAG, CT or ^{31}P labelled GPL into infected animals, the distribution of labelled lipid metabolites in the liver, adipose tissue, skeletal muscle, plasma lipoproteins and the parasite could be examined. Data generated from a study such as this would not only support or dispute the explanations proposed for the alterations seen in the hepatic lipid profile in infected mice in this study, but it would also provide new information and insight into the role dietary lipids play in the metabolic interactions between the parasite and the host. In particular selectively tracing ^{13}C labelled CT and its by-products in these tissues could also help determine which mechanisms were involved in the homeostasis of hepatic CT during this infection. In the studies involving ^{13}C labelled TAG or CT it is recommended that ^{13}C spectroscopy be utilized as it would offer more specific and detailed information on the metabolism of individual carbons of interest in these lipid molecules than SEDS. Likewise ^{31}P spectroscopy should be used in the ^{31}P labelled GPL study.

Future experiments should also address whether *T. crassiceps* cysticerci do indeed synthesize eicosanoids from acquired AA. By culturing this parasite *in vitro* with an exogenous source of AA, the production eicosanoids PGE_2, PGI_2 and TXA_2 could be determined in the parasites tissue and the media by radioimmunoassays. Determining whether *T. crassiceps* cysticerci are able to synthesize eicosanoids would not only help clarify the results obtained in the present experiment, but it would also provide a better understanding of the mechanisms involved in the complex immunological interactions between the parasite and the host in this infection.

REFERENCES

Ambrosio, J., Landa, A., Merchant, M.T. and Laclette, J.P. 1994. Protein uptake by cysticerci of *Taenia crassiceps. Arch. Med. Res.* 25: 325-330.

Adosraku, R.K., Anderson, M.M., Anderson, G.J., Choi, C., Yardely, V., Phillipson, J.D.,Gibbons, W.A. 1993. Proton NMR lipid profile of *Leishmania donovani* promastigotes. *Mol. Biochem. Parasitol.* 62: 251-262.

Adosraku, R.K., Choi, G.T.Y., Constantinou-Kokotos, V., Anderson, M.M., and Gibons, W.A. 1994. NMR lipid profiles of cells, tissues, and body fluids: Proton NMR analysis of human erythrocyte lipids. *J. Lipid Res.* 35: 1925-1931.

Agius, L. and Alberti, G.M.M. 1985. Regulation of flux through pyruvate dehydrogenase and pyruvate carboxylase in rat hepatocytes: Effects of fatty acids and glucagon. *Eur. J. Biochem.* 152: 699-707.

Ampola, M.G. 1994. The urea cycle: enzymes and defects. In *Liver Biology and Pathobiology.* 3rd ed. Raven Press, Ltd. New York. pp. 365-377.

Andriamampandry, M.D., Bnouham, M., Michard, D., Gutbier, G., Le Maho, Y. and Leray, C. 1996. Food deprivation modifies fatty acid partitioning and β-oxidation capacity in rat liver. *J. Nutr.* 126: 2020-2027.

Ansell, G.B. and Spanner, S. 1982. Phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine. In *Phospholipids.* Elsevier Biomedical Press. New York. pp. 1-49.

Azzout, B., Boisjoyeux, B., Chanez, M. and Peret, J. 1986. Development of gluconeogenesis from various precursors in isolated rat hepatocytes during starvation or after feeding a high protein, carbohydrate-free diet. *J. Nutr.* 117:164-169.

Barrett, J. 1983. Lipid Metabolism. In *Biology of Eucestoda.* Vol. 2. Academic Press Inc. London. pp.391-420.

Barrett, J. 1984. The anaerobic end-products of helminths. *Parasitology* 88: 179-198.

Belley, A. And Chadee, K. 1995. Eicosanoid production by parasites: from pathogenesis to immunomodulation? *Parasitology Today* 11: 327- 334.

Berners-Price, S.J., Sant, M.E., Christopherson, R.I., and Kuchel, P.W. 1991. ^{1}H and ^{31}P NMR and HPLC studies of mouse L121 leukemia cell extracts: the effect of Au (I) and Cu (I) diphosphine complexes on cell metabolism. *Magn. Reson. Med.* 18:142-158.

Berry, M.N., Phillips, J.W., Gregory, R.B., Grivell, A.R. and Wallace, P.G. 1992. Operation and energy dependence of the reducing-equivalent shuttles during lactate metabolism by isolated hepatocytes. *Biochim. Biophys. Acta* 1136: 223-230.

Blackburn, B.J., Hudspeth, C. and Novak,M. 1993. Proton magnetic resonance analysis of liver metabolites from mice infected with *Mesocestoides vogae. Int. J. Parasit.* 23: 953-957.

Boisjoyeux, B., Chanez, M., Azzout, B. and Peret, J. 1986. Comparison between starvation and consumption of a high protein diet: Plasma insulin and glucagon and hepatic enzymes of gluconeogenic enzymes during the first 24 hours. *Diabete et Metabolisme* 12: 21-27.

Brass, E.P. and Hoppel, C.L. 1978. Carnitine metabolism in the fasting rat. *J. Biol. Chem.* 253: 2688-2693.

Brenner, R.R. 1977. Regulatory function of Δ6 destaturase- key enzyme of polyunsaturated fatty acid synthesis. *Adv. Exp. Med. Biol.* 83: 85-101.

Buchalter, S.E., Crain, M.R. and Kreisberg, R. 1989. Regulation of lactate metabolism in vivo. *Diabetes/ Metabolism Reviews* 5: 379-391.

Cahill, Jr. G.F. 1986. Physiology of gluconeogenesis. In *Hormonal Control of Gluconeogenesis*. Vol. 1. CRC Press, Inc. pp. 3-14.

Canedo, L., Garrido, F. and Gonzalez, M. 1976. Host-parasite relationship of cyticerciasis in man. In *Biochemistry of Parasites and Host-Parasite Relationships*. North- Holland Publishing Company. Amsterdam. pp. 385-392.

Casu, M., Anderson, G.J., Choi, G., and Gibbons, W.A. 1991. NMR lipid profiles of cells, tissues and body fluids- 1D and 2D proton NMR of lipids from rat liver. *Magnet. Reson. Chem.* 29:594-602.

Caterson, I.D., Fuller, S.J. and Randle, P.J. 1982. Effect of the fatty acid inhibitor 2-tetradecyglycidic acid on pyruvate dehydrogenase activity in starved and alloxan-diabetic rats. *Biochem. J.* 208: 53-60.

Chao, F.F., Stiers, D.L. and Ontko, J.A. 1986. Hepatocellular triglyceride synthesis and transfer to lipid droplets and nascent very low density lipoproteins. *J. Lipid Res.* 27: 1174 -1181.

Chen, Z.Y. and Cunnane, S.C. 1992. Preferential retention of linoleic acid-enriched triacylglycerols in liver and serum during fasting. *Am. J. Physiol.* 263: R233-R239.

Chernin, J. and McLaren, D. 1983. The pathology induced in laboratory rats by metacestodes of *Taenia crassiceps and Mesocestoides corti* (Cestoda). *Parasitology* 87: 279-287.

Cherrington, A.D. and Vranic, M. 1986. Hormonal control of gluconeogenesis in vivo. In *Hormonal Control of Gluconeogenesis*. Vol. 1. CRC Press, Inc. pp. 15-38.

Choi, G.T.Y., Casu, M. and Gibbons, W.A. 1993. NMR lipid profiles of cells, tissues and body fluids: neutral, non-acidic and acidic phospholipid analysis of Bond Elut chromatographic fractions. *Biochem. J.* 290: 717-721.

Christie, W.W. 1982. *Lipid Analysis: Isolation, Seperation, Identification and Structural Analysis of Lipids.* 2nd ed. Pergamon Press. Oxford, England.

Cohen, S.M., Rognstad, R., Shulman, R.G. and Katz, J. 1981. A comparison of ^{13}C nuclear magnetic resonance and ^{14}C tracer studies of hepatic metabolism. *J. Biol. Chem.*26: 3428-3432.

Cohen, S.M. 1983. Application of nuclear magnetic resonance to the study of liver physiology and disease. *Hepatology* 3: 738-749.

Cohen, S.M. 1987. Effects of insulin on perfused liver from streptozotocin-diabetic and untreated rats: ^{13}C NMR assay of pyruvate kinase flux. *Biochemistry* 26: 573-580.

Corbin, I., Blackburn, B.J., Wolowiec, T. and Novak. M. 1996. Metabolite profile of the liver of mice infected with cysticerci of *Taenia crassiceps*. *Paraisitol. Res.* 82: 273-275.

Craig, P.S., Rogan, M.T. and Allan, J.C. 1996. Detection, screening and community epidemiology of Taeniid cestode zoonoses: cystic echinococcsis, alveolar echinococcsis and neurocysticercosis. *Adv. Parasitol.* 38:170-250.

Craig, F.C.M. 1982. Mechanisms and specificity of monosaccharide transport in Taenia crassiceps larvae. In *Proceedings of the British Society for Parasitology.* Portsmouth, England. pp. 63.

Crane, D. And Masters, C. 1981. On the turnover of tissue lipids in the mouse during early staravation. *Int. J. Biochem.* 13: 117-120.

Crompton, D.W.T., Arnold, S.E., Walters, D.E. and Whitefield, P.J. 1985. Food intake and body weight changes in mice infected with metacestodes of *Taenia crassiceps. Parasitology*. 90: 449-456.

Cunnane, S.C., Huang, Y.S. and Manku, M. 1986. Triacylglycerol content of arachidonic acid varies inversely with total triacylglycerol in liver and plasma. *Biochim. Biophys. Acta* 876: 183-186.

Cunnane, S.C. 1988. Differential utilization of long chain fatty acid during triacylglycerol depletion. II. Rat liver after starvation. *Lipids:* 23: 372-374.

Cunnane, S.C. 1990. Differential utilization of long chain fatty acids during fasting-induced triacylglycerol depletion. III. Comparison of n-3 and n-6 fatty acid in rat plasma and liver. *Biochim. Biophys. Acta* 1036: 64-70.

Davis, J.H. 1967. Current concepts of peritonitis. *Am. Surg.* 33: 673-681.

De Gomez Dumm, I.N.T., De Alaniz, M.J.T. and Brenner, R.R. 1976. Comparative effects of glucogon, dibutyryl cyclic AMP, and epinephrine on the destaturation and elongation of linoleic acid by rat microsomes. *Lipids* 11: 833-836.

Dennis, R.D., Baumeister, S., Geyer, R., Peter-Katalinic, J., Rudolf, H., Egge, H., Geyer, E. and Weigandt, H. 1992. Glycosphingolipids in cestodes: Chemical structures of ceramide monosaccharide, disaccharide, trisaccharide from metacestodes of fox tapeworm, *Taenia crassiceps* (Cestoda:*Cyclophyllidea*). *Eur. J. Biochem.*207: 1053-1062.

DeRosa, G. and Swicks, R.W. 1975. Metabolic implications of the distribution of the alanine aminotransferase isoenzymes. *J. Biol. Chem.* 250: 7961-7967.

Desmoulin, F., Coufort-Gouny, F., Masson, S., Bernard, M., Doddrell, D.M., and Cozzne, P.J. 1990. Application of reverse-DEPT polarization transfer pulse sequence to study organs by ^{1}H- NMR spectroscopy. *Magn. Reson. Med.* 15:456-461

Drynan, L., Quant, P.A. and Zammit, V.A. 1996 (a). Flux control exerted by mitochondrial outer membrane carnitine palmitoyltransferase over β-oxidation, ketogenesis and tricarboxylic acid cycle in hepatocytes isolated from rats in differnt metabolic states. *Biochem. J.* 317: 791-795.

Drynan, L., Quant, P.A. and Zammit, V.A. 1996 (b). The role of changes in the sensitivity of hepatic mitochondrial overt carnitine palmitoyltransferase in determining the onset of the ketosis of starvation in the rat. *Biochem. J.* 318: 767-770.

Dumas, M., Grunitzky, E., Deniau, M., Dabis, F., Bouteille, B., Belo, M., Pestre, M., Catanzano, G., Darda, M.L.D. and Almeida, M. 1989. Epidemiology study of neurocysticercosis in northern Togo (West Africa). *Acta Leidensia*. 57: 191-196.

Engelmann, B., Kogl, C., Kulschar, R. And Schaipp, B. 1996. Transfer of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin from low- and high-density lipoprotein to human platelets. *Biochem. J.* 315: 781-789.

Esch, G.W. and Kuhn, R.E. 1971. The uptake of ^{14}C- chlorella protein by larval Taenia crassiceps (Cestoda). *Parasitology* 62: 27-29.

Evanochko, W.T., Sakai, T.T., Ng, T.C., Krishna, N.R., Kim, H.D., Zeidler, R.B., Ghanta, V.K., Brockman, R.W., Schifer, L.M., Braunscheiger, P.G., and Glickson, J.D. 1984. NMR study of in vivo RIF-1 tumors. Analysis of perchloric acid extracts and identification of ^{1}H-, ^{31}P- and ^{13}C- resonances. *Biochim. Biophys. Acta*, 805: 104-116.

Exton, J.H. and Park, C.R. 1967. Control of gluconeogenesis in liver: General features of gluconeogenesis in the perfused livers of rats. *J. Biol. Chem.* 242: 2622-2636.

Fafournoux, P., Remesy, C. and Demigne, C. 1983. Control of alanine metabolism in rat liver by transport processes or cellular metabolism. *Biochem. J.* 210: 645-652.

Fafournoux, P., Demigne, C. and Remesy, C. 1985. Carrier-mediated uptake of lactate in rat hepatocytes: Effects of pH and possible mechanisms for L-lactate transport. *J.Biol. Chem.* 260: 292-299.

Felig, P., Pozefsky, T., Marliss, E. and Cahill, G.F. 1970. Alanine: Key role in gluconeogenesis. *Science* 167: 1003.

Felig, P. 1973. Progress in endocrinology and metabolism: The glucose-alanine cycle. *Metabolism* 22:179-207.

Ferrannini, E., Natali, A., Brandi, L.S., Bonadonna, R., De Kreutzemberg, S.A., Del Prato, S. and Santoro, D. 1993. Metabolic and thermogenic effects of lactate infusion in humans. *Am. J. Physiol.* 265: E504-E512.

Frayha, G.J. and Smyth, J.D. 1983. Lipid metabolism in parasitic helminths. *Adv.Parasitol.* 22: 309- 387.

Freeman, R.S. 1962. Studies of the biology of *Taenia crassiceps*. *Can. J. Zool.* 40: 969-990.

Freeman, R.S. 1964. Studies on responses of intermediate host to infection with *Taenia crassiceps*. *Can. J. Zool.* 42: 367-385.

Fukushima, T., Isobe, A., Hojo, N., Shiwaku, K., Yamane, Y. and Torii, M. 1993. The metabolism of arachidonic acid to prostaglandin E_2 in plerocercoids of *Spirometra erinacei*. *Parasitol. Res.* 79: 634-638.

Fusco, A.C., Salafsky, B. and Kevin, M.B. 1985. *Schistosoma mansoni*: Eicosanoid production by cercariae. *Exp. Parasitol.* 59: 44-50.

Gavino, G.R. and Gavino, V.C. 1991. Rat outer mitochondrial carnitine palmitoyltransferase activity towards long chain polyunsaturated fatty acids and their CoA esters. *Lipids* 26: 266-270.

Gemmell, M.A., Matyas, Z., Pawlowiski, Z., Soulsby, E.J.L., Larralde, C., Nelson, G.S. and Rosicky, B. 1983. In *Guildelines for Surveillance Prevention and Control of Taeniasis/ Cysticercosis*. VPH/83.49. Genuva, Switzerland. World Health Organization.

Gillies, R.J. 1992. Nuclear magnetic resonance and its application to physiological problems. *Annu. Rev. Physiol.* 54: 733-748.

Gilroy, F.V., Edwards, M.R., Norton, R.S., and Sullivan, W.J.C. 1988. Metabolic studies of the protozoan parasite, *Crithidia Luciliae*, using proton nuclear magnetic resonance spectroscopy. *Mol. Biochem. Parasitol.* 20:83-86.

Glickman, R.M. and Sabesin, S.M. 1994. Lipoprotein metabolism. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 391-414.

Good, A.H. and Miller,K.L. 1976. Depression of the immune response to sheep erythrocytes in mice infected with *Taenia crassiceps* larvae. *Infect. and Immun.* 14: 449-456.

Goodman, M.N. and Ruderman, N.B. 1980. Starvation in the rat. I. Effects of age and obesity on organ weights, RNA, DNA, and protein. *Am. J. Physiol.* 239: E269- E276.

Goodridge, A.G., Donald, W.B., Wilson, S.B. and Goldman, M.T. 1986. Regulation of genes for enzymes involved in fatty acid synthesis. *Ann. N.Y. Acad. Sci.* 478: 46-62.

Groen, A.K., Sipis, H.J., Vervoorn, R.C. and Tager, J.M. 1982. Intracellular compartmentation and control of alanine metabolism in rat liver parenchymal cell. *Eur. J. Biochem.* 122: 87-93.

Groen, A.K., Vervoorn, R.C., Van der Meer, R. and Tager, J.M. 1983. Control of gluconeogenesis in rat liver cells I. Kinetics of the individual enzymes and the effect of glucagon. *J. Biol. Chem.* 258: 14346-14353.

Groen, A.K., van Roermund, C.W.T., Vervoorn, R.C. and Tager, J.M. 1986. Control of gluconeogenesis in rat liver cells: Flux control coefficients of the enzymes in gluconeogenic pathway in the absence and presence of glucagon. *Biochem. J.* 237: 379-389.

Groener, J.E.M. and Van Golde, L.M.G. 1977. Effects of fasting and feeding a high-sucrose, fat-free diet on the synthesis of hepatic glycerolipids in vivo and in isolated hepatocytes. *Biochim. Biophys. Acta* 487: 105-114.

Grove, D.I. 1990. *A History of Human Helminology*. Wallingford: CAB International.

Hall, J.E., Mackenzie, N.E., Mansfield, J.M., McCloskey, D.E. and Scott, A.I. 1988. ^{13}C-NMR analysis of alanine metabolism by isolated perfused livers from C3HeB/FeJ mice infected with African trypanosomes. *Comp. Biochem. Physiol.* 89B: 679-685.

Harper, P., Wadstrom, C. and Cederblad, G. 1993. Carnitine measurements in liver, muscle tissue, and blood in normal subjects. *Clin. Chem.* 39: 592-599.

Harrison, L.J.S. and Swell, M.M.H. 1991. The zoonotic taeniae of Africa. In *Parasitic Helminths and Zoonoses in Africa*. London: Unwin Hyman. pp. 54-82.

Haussinger, D. and Gerok, W. 1986. Metabolism of amino acids and ammonia. In *Regulation of Hepatic Metabolism: Intra- and intercellular compartmentation.* Plenum Press. New York. pp. 253-291.

Hellerstein, M.K. and Munro, H.N. 1994. Interactions of liver, muscle and adipose tissue in the regulation of metabolism in response to nutritional and other factors. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 1169-1191.

Herzberg, G.R. 1991. Dietary regulation of fatty acid triglyceride metabolism. *J. Physiol. Pharmacol.* 69: 1637-1647.

Hildmann, B., Storelli, C., Haase, W., Barac-Nieto, M. and Murer. 1980. Sodium ion/Lactate co-transport in rabbit small intestinal brush-border-membrane vesicles. *Biochem. J.* 186: 169-176.

Hill, E.E. and Lands, W.E.M. 1970. Phospholipid metabolism. In *Lipid Metabolism.* Acedemic Press, Inc. New York. pp. 185-279.

Holten, C.H. Muller, A. and Rehbinder, D. 1971. *Lactic Acid: Properties and Chemistry of Lactic Acid and Derivatives*. Verlag Chemie. Copenhagen, Denmark.

Hoppel, C.L. and Genuth, S.M. 1980. Carnitine metabolism in normal-weight and obese human subjects during fasting. *AM. J. Physiol.* 238: E4109-E415.

Howell, M.J. 1976. The peritoneal cavity of vertebrates. In *Ecological Aspects of Parasitology*. North- Holland Publishing Company. Amsterdam. pp. 243-268.

Hustead, S.T. and Williams, J.F. 1977. Permeability studies on Taeniid metacestodes: Uptake of protein by larval stages of *Taenia taeniaeformis, Taenia crassiceps and Echinococcus granulosus. J. Parasitol.* 63: 314-321.

International Task Force for Disease Eradication. 1990. *Morb. Mortal. Weekly Rep.* 39: 209-217.

Ito, A., Takami, T. and Itoh, M. 1991. Vaccine effect of intact metacestodes of *Taenia crassiceps* against *T. taeniaeformis* infection in rats. *Am. J. Trop. Med. Hyg.* 44: 696-701.

Jungermann, K. and Katz, N. 1986. Metabolism of carbohydrates. *In Regulation of Hepatic Metabolism: Intra- and intercellular compartmentation.* Plenum Press. New York. pp.211-236.

Kalinna, B. and Mc Manus, D.P. 1993. An IgG (Fcγ)-binding protein of *Taenia crassiceps* (Cestoda) exhibits sequence homology and antigenic similarity with schistosome paramyosin. *Parasitology*. 106: 289-296.

Kaloyianni, M. and Freedland, R.A. 1990. Contribution of several amino acids and lactate to gluconeogenesis in hepatocytes isolated from rats fed various diets. *J. Nutr.* 120: 116-122.

Katz, M. 1986. Metabolism of lipids. *In Regulation of Hepatic Metabolism: Intra- and intercellular compartmentation.* Plenum Press. New York. pp. 237-252.

Keppler, D. 1994. Leukotrienes and other eicosanoids in liver pathology. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 1015-1029.

Koretsky, A.P. and Williams, D.S. 1992. Application of localized in vivo NMR to whole organ physiology in the animal. *Annu. Rev. Physiol.* 54: 799-826.

Kuesel, A.C., Graschew, G., Hull, W.E., Lorenz, W. and Thielmann, H.W. 1990. ^{31}P NMR of cultured human tumor cells. Influence of pH on phospholipid metabolite levels and the detection of cytidine 5'-diphosphate choline. *NMR Biomed.* 3: 78-79.

Kummel, L. 1987. Mitochondrial pyruvate carrier- a possible link between gluconeogenesis and ketogenesis in the liver. *Biosci. Rep.* 7: 593-597.

Kunz, J., Baumeister, S., Dennis, R.D., Kuytz, B., Weigandt, H. and Geyer, E. 1991. Immunological recognition of larval *Taenia crassiceps* glycolipids by sera from parasite-infected mice. *Parasitol. Res.* 77: 443-447.

Kurtin, P. and Charney, A.N. 1984. Intestinal ion transport and intracellular pH during acute respiratory alkalosis and acidosis. *Am. J. Physiol.* 247: G24-G31.

Larralde, C., Sotelo, J., Montoya, M., Palencia, G., Padilla, A., Govezensky, T., Diaz, M.L., and Sciutto, E. 1990. Immunodiagnosis of human cysticercosis in cerebrospinal fluid: Antigens fom murine *Taenia crassiceps* cysticerci effectively substitute those from porcine *Taenia solium*. *Arch. Pathol. Lab. Med.* 114:926- 928.

Larralde, C., Morales, J., Terrazas, I., Govezensky, T. and Romano, M.C. 1995. Sex hormone changes induced by the parasite lead to feminization of the male host in murine *Taenia crassiceps* cysticercosis. *J. Steroid Biochem. Molec. Biol.* 52: 575-580.

Lazarow, P.B. 1994. Peroxisomes. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 293-308.

Lehninger, A.L., Nelson, D.L. and Cox, M.M. 1993. *Priciples of Biochemistry*. Worth Publishers. New York.

Leid, R.W. and Mc Connell, L.A. 1983a. PGE_2 generation and release by the larval stage of the cestode, *Taenia Taeniaeformis*. *Prostaglandins Leukot. Med.* 11: 317-323.

Leid, R.W. and Mc Connell, L.A. 1983b. Thromboxane A_2 generation by the larval cestode, *Taenia taeniaeformis*. *Clin. Immuno. Immunopathol.* 28: 67-76.

Lowry, O.H. and Passonneau, J.V. 1972. *A Flexible System of Enzymatic Analysis*. Acedemic Press. New York.

Lui, L.X., Buhlmann, J.E. and Weller, P.F. 1992. Release of prostaglandin E_2 by microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. *Am. J. Trop. Med. Hyg.* 46: 520-523.

Mathews, C.K. and Van Holde, K.E. 1990. *Biochemistry*. The Benjamin/Cummings Publishing Company, Inc. California.

Mato, J.M., Alvarez, L., Corrales, F.J. and Pajares, M.A. 1994. S-adenosylmethionine and the liver. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 461-470.

Marinetti, G.V. 1990. *Disorders of Lipid Metabolism*. Plenum Press. New York.

Mc Manus, D.P. and Lamsam, S. 1990. *Taenia crassiceps* surface immunoglobulins: parasite- or host-derivied? *Parasitology*. 101: 127-137.

Meneses, P. And Glonek.T. 1988. High resolution ^{31}P NMR of extracted phospholipids. *J. Lipid Res.* 29: 679-689.

Metcalfe, H.K., Monson, J.P., deAllie, F. and Cohen, R.D. 1992. Effects of D-3-hydroxybutyrate and acetoacetate on lactate removal in isolated perfused livers from straved and fed rats. *Metabolism*. 41: 435- 440.

Mills, G.L., Taylor, D.C. and Williams, J.F. 1981. The lipid composition of the helminth parasite *Taenia crassiceps. Comp.Biochem. Physiol.* 69B: 553-557.

Moir, A.M.B. and Zammit, V.A. 1992. Selective labelling of hepatic fatty acids in vivo: Studies on the synthesis and secretion of glycerolipids in the rat. *Biochem. J.* 283: 145-149.

Muniz, R., Burguillo, L. and del Castillo, J.R. 1993. Effects of starvation on neutral amino acid transport in isolated small-intestinal cells from guinea pigs. *Pflugers Arch.* 423: 59-66.

Novak, M. and Dowsett, J.A. 1983. Scanning electron microscopy of the metacestode of *Taenia crassiceps. Int. J. Parasit.* 13: 383- 388.

Novak, M., Marat, K., Johnson, L. and Blackburn, B.J. 1989. ^{1}H and ^{13}C NMR studies of sera from normal and *Echinococcus multilocularis* infected jirds. *Int. J. Parasit.* 19: 395-400.

Novak, M., Modha, A., Lee, J., Buist, R. and Blackburn, B. 1995. Metabolism of D-[1-^{13}C]glucose in livers of *Meriones unguiculatus* infected with *Echinococcus multilocularis. Can. J. Zoo.* 73: 58-66.

Owen, O.E., Felig, P., Morgan, A.P., Wahren, J. and Cahill, G.F. 1969. Liver and kidney during prolonged starvation. *J. Clin. Invest.* 48: 574-594.

Pappas, P.W., Uglem, G.L. and Read, C.P. 1973. *Taenia crassiceps*:absorption of hexoses and partial characterization of Na^{+}- dependent glucose absorption by larvae. *Expl. parasit.* 33: 127-137.

Pappas, P.W. and Read, C.P. 1973. Permeability and membrane transport in the larva of *Taenia crassiceps. Parasitology.* 66:33-42.

Pappas, P.W. 1983. Host-parasite interface. In *Biology of Eucestoda*. Vol. 2. Academic Press Inc. London. pp. 297-334.

Patel, T.B. and Olson, M. 1985. A reexamination of the role of the cytosolic alanine aminotransferase in hepatic gluconeogenesis. *Arch. Biochem. Biophys.* 240: 705-711.

Pawlosky, R.J., Ward, G. and Salem, N. 1996. Essential fatty acid uptake and metabolism in the developing rodent brain. *Lipids* 31: S103- S107.

Petersen, K.F., Blair, J.B. and Shulman, G.I. 1995. Triiodothyronine treatment increases substrate cycling between pyruvate carboxylase and malic enzyme in perfused rat liver. *Metabolism*. 44: 1380- 1383.

Petronini,P.G., DeAnglelis, E.M., Burghetti, P. and Bourghetti, A.F. 1992. Modulation of betaine cellular responses to osmotic stress. *Biochem. J.* 282: 69-73.
Phinney, S.D., Fisler, J.S., Tang, A.B. and Warden, C.H. 1994. Liver fatty acid composition correlates with body fat and sex in a multigenic mouse model of obesity. *Am. J. Clin. Nutr.* 60: 61- 67.

Pilkis, S.J., Claus, T.H. and El-Maghrabi M.R. 1988. The role of cyclic AMP in rapid and long-term regulation of gluconeogenesis and glycolysis. *Adv. Second Messenger Phosphoprotein Res.* 22:175- 191.

Poisson, J.P. and Cunnane, S.C. 1991. Long chain fatty acid metabolism in fasting and diabetes: Relation between altered destaturase activity and fatty acid composition. *J. Nutr. Biochem.* 2: 60-70.

Pollesello, P., Masutti, F., Croce, L.S., Toffanin, R., Eriksson, O., Paoletti, S., Hockerstedt, K. and Tiribelli. 1993. ^{1}H NMR spectroscopic studies of lipid extracts from human fatty liver. *Biochem. Biophys. Res. Comm.* 192: 1217-1222.

Poole, R.C. and Halestrap, A.P. 1993. Transport of lactate and other monocarboxylates across mammalian plasma membranes. *Am. J. Physiol.* 264: C761- C782.

Pryor, H.J., Smyth, J.E., Quinlan, P.T. and Halestrap, A.P. 1987. Evidence that the flux control coefficient of the respiratory chain is high during gluconeogenesis from lactate in hepatocytes from starved rats. *Biochem. J.* 247: 449-457.

Rafter, J.E.M., Bates, T.E., Bell, J.D. and Iles, R.A. 1991. Metabolites in the developing rat liver-a proton nuclear magnetic resonance spectroscopy study. *Biochim. biophys. Acta.* 1074: 263-269.

Randle, P.J., Priestman, D.A., Mistry, S.C. and Halsall, A. 1994. Glucose fatty acid interactions and the regulation of glucose disposal. *J. Cell. Biochem.* 55S: 1-11.

Reddy, M.C., Koneru, B., Soni, S. and Patel, D. 1996. ^{31}P nuclear magnetic resonance study of phospholipids in ischemia/ reperfusion injury in a rat fatty acid model. *Transplantation.* 61: 1151- 1155.

Redgrave, T.G. and Small, D.M. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* 64: 162-171.

Rhoads, J.E. and Rhoads, E.R. 1976. The peritoneum. In *Gastroenterology.* W.B. Saunders Company. Philadelphia. Pp.33- 47.

Roberts, L.S. 1983. Carbohydrate metabolism. In *Biology of Eucestoda.* Vol. 2. Acedemic Press Inc. London. pp. 343-390.

Roberts, T., Murrell, K.D. and Marks, S. 1994. Economic losses caused by food borne diseases. *Parasitology Today.* 11: 419-423.

Robinson, A.M. and Williamson, D.H. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* 60: 143-187.

Schmidt, G.D. and Roberts, L.S. 1989. Class Cestoidea: Form, function, and classification of the tapeworms. In *Foundations of Parasitology.* 4th ed. Mosby Company. St. Louis, Missouri. pp. 313- 3445.

Sciutto, E., Fragoso, G., Trueba, L., Lemus, D., Montoya, R.M., Diaz, M.L., Govezensky, T., Lomeli, C., Tapia, G. and Larralde, C. 1990. Cysticercosis vaccine: cross protecting immunity with *T. solium* antigens against experimental murine *T. crassiceps* cysticercosis. *Parasite Immunol.* 12: 687-696.

Sciutto, E., Fragoso, G., Baca, M., De La Cruz, V., Lemus, L. and Lamoyi, E. 1995. Depressed T-cell proliferation associated with suspeptibility to experimental *Taenia crassiceps* infection. *Infect. Immun.* 63: 2277-2281.

Seifter, S. and Englard, S. 1994. Engery metabolism. In *Liver Biology and Pathobiology.* 3rd ed. Raven Press, Ltd. New York. pp. 323-364.

Shaka, A.J., Barker, P.B., and Freeman, R. 1985. Computer-optimized decoupling scheme for wideband applications and low level operation. *J. Magn. Reson.* 64, 547-552.

Sharma, M., Lal, H. and Saini, A.S. 1985. Effect of prelonged starvation and refeeding on fuel metabolism in rats. *Ind. J. Physiol. Pharac.* 29: 10^{7}-11^{0}.

Shea, M., Maberley, A.L., Walters, J., and Freeman, R. 1972. Tapeworms turn up in a new place, the eye. *JAMA*. 222: 891-1192.

Shiau, Y.F. 1987. Lipid digestion and absorption. In *Physiology of the Gastrointestinal Tract*. 2nd ed. Raven Press. New York. pp. 1527-1557.

Shulman, G.I., Rossetti, L., Rothman, D.L., Blair, J.B. and Smith,D. 1987. Quantitative analysis of gylcogen repletion by nuclear magnetic resonance spectroscopy. *J. Clin. Invest.* 76: 1229-1236.

Siebert, A.E., Good, A.H. and Simmons, J.E. 1978. Ultrastructure aspects of early immune damage to *Taenia crassiceps* metacestodes. *Int. J. Parasit.* 8: 45-53.

Siebert, A.E. and Good, A.H. 1980. *Taenia crassiceps*: Immunity to metacestodes in BALB/c and BDF1 mice. *Expt. Parasit.* 50: 437- 446.

Siebert, A.E., Blitz, R.R., Morita, C.T. and Good, A.H. 1981. *Taenia crassiceps*: Serum and surface immunoglobulins in metacestode infections of mice. *Expt. Parasit.* 51:418-430.

Siess, E.A., Banik, E. and Neugebauer, S. 1988. Control of pyruvate carboxylase activity by the pyridine-nucleotide redox state in mitochondria from rat liver. *Eur. J. Biochem.* 173: 369-374.

Silverstein, R.M., Bassler, G.C. and Morrill, T.C. 1991. ^{13}C NMR spectrometry. In *Spectrometric Identification of Organic Compounds*. 5th ed. John Wiley and Sons, INC. New York. pp.227-266.

Simopoulos, A.P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54: 438-463.

Sips, H.J., Groen, A.K. and Tager, J.M. 1980. Plasma-membrane transport of alanine is rate limiting for its metabolism in rat-liver parenchymal cells. *FEBS Letts.* 119: 271-274.

Skurat, R.M. and Dice, J.F. 1994. Degradation of cytoplasmic proteins. In *Liver Biology and Pathobiology*. 3rd ed. Raven Press, Ltd. New York. pp. 229-238.

Smith, W.L., Borgeat, P. and Fitzpatrick, F.A. 1991. The eicosanoids: lipoxygenase and epoxygenase pathways. In *Biochemistry of Lipids, Lipoproteins and Membranes*. Elsevier. Amsterdam. pp. 297- 326.

Smyth, J.D. and McManus, D.P. 1989. *The Physiology and Biochemistry of Cestodes*. Cambridge University Press. Cambridge.

Sparling , M.L., Zidovetzki, R., Muller, L., and Sunney, I.C. 1989. Analysis of membrane lipids by 500 Mhz ^{1}H NMR. *Anal. Biochem.*178: 67-76.

Stubbs, C.D. and Smith, A.D. 1984. The modification of mammalian polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta.* 779:89-137.

Sugano,M., Imaizumi, K., and Kamo, F. 1975. Nutritional regulation of lipid metabolism in rats. VII. Effects of overnight fasting on liver lipids of rats fed different levels of essential fatty acids. *Nutr. Metabol.* 19: 65-72.

Sugden, M.C., Howard, R.M., Munday, M.R. and Holness, M.J. 1993. Mechanisms involved in the coordinate regulation of strategic enzymes of glucose metabolism. *Advan. Enzyme. Regul.* 33: 71-95.

Sugden, M.C. and Holness, M.J. 1994. Interactive regulation of pyruvate dehydrogenase complex and the carnitine palmitoyltransferase system. *FASEB J.* 8: 54-61.

Sugden, M.C., Orfali, K.A. and Holness, M.J. 1995. The pyruvate dehydrogenase complex: Nutrient control and the pathogenesis of insulin resistance. *J. Nutr.* 125: 1746S-1752S.

Swergold, B. 1992. NMR spectroscopy of cells. *Annu. Rev. Physiol.* 54: 775-798.

Sze, D.Y. and Jardetzky, O. 1990a. Determination of metabolite and neucleotide concentrations in proliferating lymphocytes by ^{1}H-NMR of acid extracts. *Biochim. Biophys. Acta.* 1054: 181-197.

Sze, D.Y and Jardetzky, O. 1990 b. Characterization of lipid composition in stimulated human lymphocytes by proton NMR. *Biochim. Biophys. Acta.* 1054: 198-206.

Takanga, H., Tamai, I., Inaba, S., Sai, Y., Higashida, H., Yamamoto, H. and Tsuji, A. 1995. cDNA cloning and functional characterization of rat intestinal monocarboxylate transporter. *Biochem. Biophys. Res. Comm.* 217: 370-377.

Terrazas, L.I., Bojalil, R., Govezensky, T. and Larralde. 1994. A role for 17-β-estradiol in immunoendocrine regulation of murine cysticercosis (*Taenia crassiceps*). *J. Parasitol.* 80: 563-568.

Tielens, A.G.M. and Bergh, S.G. van den. 1993. Aerobic and anerobic energy metabolism in the life cycle of parasitic helminths. In *Surviving Hypoxia: Mechanisms of Control and Adaptation.* CRC Press, Inc. pp. 19-40.

Tiruppathi, C., Balkovetz, C.D.F., Ganapathy, V., Mujamato, Y. and Leibach, F.H. 1988. A proton gradient, not a sodium gradient, is the driving force for active transport of lactate in rabbit intestinal brush border membrane vesicles. *Biochem. J.* 256: 219-223.

Thompson, S.N., Platzer, E.G. and Lee, R.W.K. 1987. In vivo ^{31}P NMR spectrum of *Hymenolepis diminuta* and its change on short-term exposure to mebendazole. *Mol. Biochem. Parasitol.* 22: 45-54.

Tso, P. and Fujimoto, K. 1991. The absorption and transport of lipids by the small intestine. *Brain Res. Bill.* 27: 477- 482.

Villa, O.F. and Kuhn, R.E. 1996. Mice infected with larvae of *Taenia crassiceps* exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. *Parasitology.* 112: 561- 570.

Vlahcevic, Z.R., Hylemon, P.B. and Chaing, J.Y. 1994. Hepatic cholesterol metabolism. In *Liver Biology and Pathobiology.* 3rd ed. Raven Press, Ltd. New York. pp. 379-389.

Wakil, S.J. 1970. Fatty acid metabolism. In *Lipid Metabolism.* Acedemic Press, Inc. NewYork. pp 1- 48.

White, C. 1997. Neurocysticercosis: A major cause of neurological disease worldwide. *Clin. Infect. Dis.* 24: 101-115.

Williams, J.F., Engelkirk, P.G. and Lindsay, M.A. 1982. Mechanisms of immunity in rodent cysticercosis. In *Cyticercosis: Present State of Knowledge and Perspectives.* Acedemic Press Inc. New York. pp. 621- 632.

Williamson, J.R., Browning, E.T. and Olson, M.S. 1967. Interrelations between fatty acid oxidation and the control of gluconeogenesis in perfused rat liver. *Adv. Enzyme Regul.* 6: 67- 100.

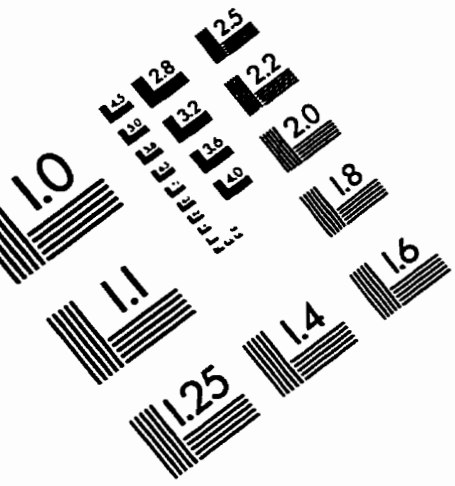
Yacoe, M.E., Sommer, G., and Reehl, D. 1991. *In vitro* proton spectroscopy of normal and abnormal prostate. *Magn. Reson. Med.* 19:429-438.

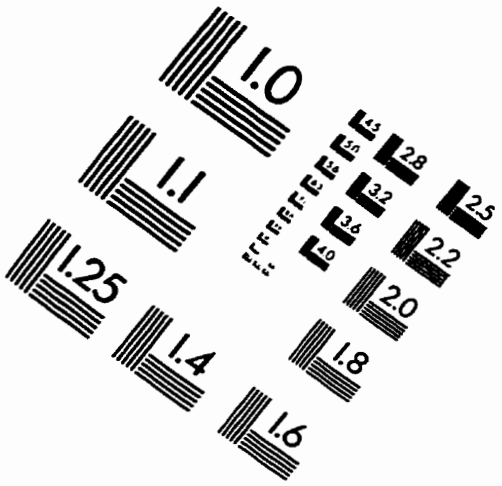
Yamaguti, K., Kurastsune, H., Wantanabe, Y., Takahanshi, M., Nakamoto, I., Machii, T., Jacobsson, G., Onoe, H., Matsumura, K., Valind, S., Langstrom, B. and Kitani, T. 1996. Acylcarnitine metabolism during fasting and after refeeding. *Biochem. Biophys. Res. Comm.* 225: 740-746.

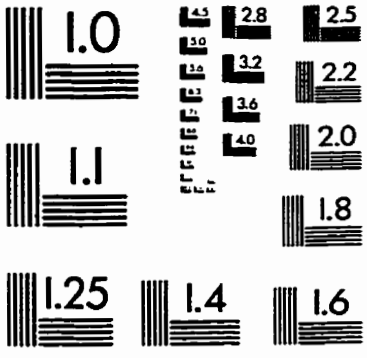
Zammit, V.A. 1981. Intrahepatic regulation of ketogenesis. *TIBS*. 6: 46- 49.

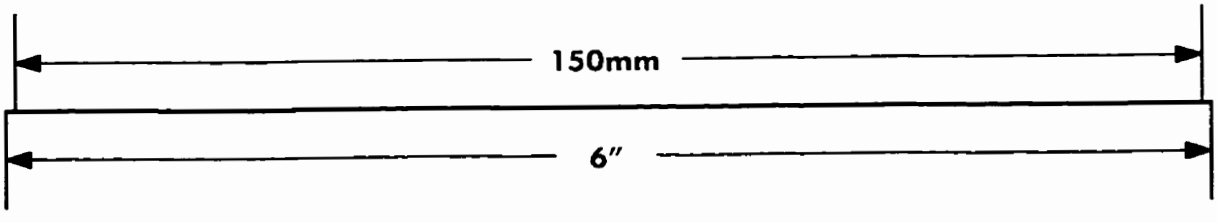
Zang, L., Rothman, D.L. and Shulman, R.G. 1990. ^{1}H NMR visibility of mammalian glycogen in solution. *Proc. natn. Acad. Sci. U.S.A.* 87: 1678-1680.

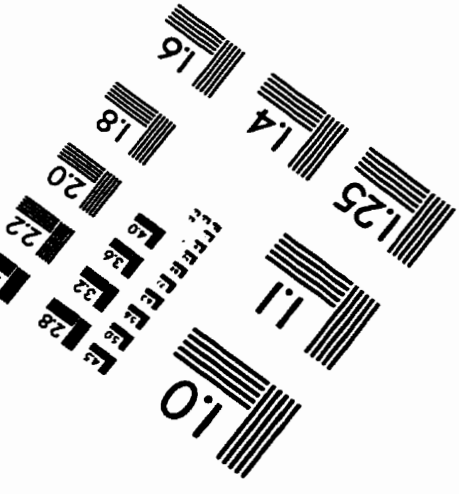
IMAGE EVALUATION
TEST TARGET (QA-3)

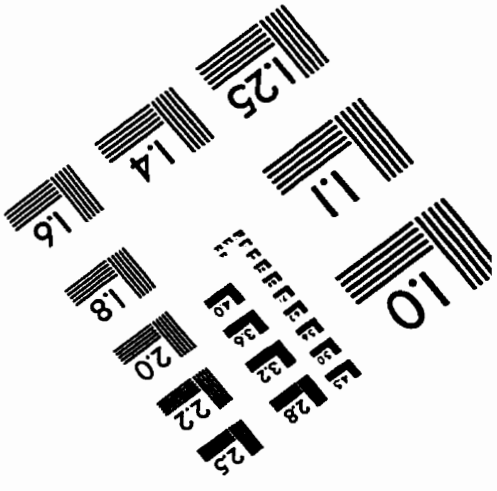












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989