GLUCOSE METABOLISM IN *S. MANSONI* INFECTION

BY

KENNETH NSANGYE LEMA, M.D.

University of Dar-es-Salaam

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science (Biochemistry) in the University of Nairobi.
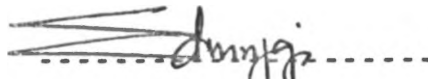
1993

DECLARATION

I, KENNETH NSANGYE LEMA, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.
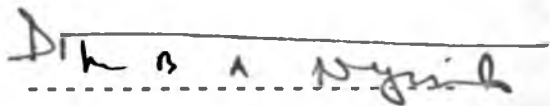
--------‾‾‾‾‾--------
KENNETH NSANGYE LEMA

This thesis has been submitted with our approval as University Supervisors.

--------‾‾‾‾‾--------
DR. E. N. M. NJAGI
1st Supervisor

--------‾‾‾‾‾--------
DR. J. K. MUNYUA
2nd Supervisor

--------‾‾‾‾‾--------
DR. MRAMBA NYINDO
3rd Supervisor

--------‾‾‾‾‾--------
‧PROF. D. W. MAKAWITI
Chairman,
Dept. of Biochemistry

DEDICATION

To Mary and Kaku

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

GLUCOSE METABOLISM IN *S. MANSONI* INFECTION

ABSTRACT

There have been conflicting reports on the effect of *S. mansoni* infection on mammalian host glucose metabolism. Some workers have reported an elevation of host serum glucose concentration, while others have reported normal fasting serum glucose concentration and others have found a lower than normal serum glucose concentration in *S. mansoni* infection.

The present study was conducted to establish the following:

(a)   The effect of *S. mansoni* infection in mice on serum glucose concentration.

(b) The effect of the infection on the production and action of insulin.

(c) The basis of such an effect in terms of the activities of enzymes responsible for glucose metabolism and the end products of glycolysis.

Mice infected with *S. mansoni* showed a statistically significant higher mean serum glucose concentration as compared to normal controls (8.24 mmol/L and 6.31 mmol/L in infected and control mice respectively). *S. mansoni* infection, therefore, appears to cause a mild hyperglycaemia in mice.

To investigate whether the observed mild hyperglycaemia was due to pancreatic malfunction, serum pancreatic lipase concentration was determined. No significant difference was found in serum pancreatic lipase concentration in infected and control mice. This showed that there was no gross pancreatic malfunction, which could have led to the

mild hyperglycaemia observed. There was a significantly greater change in serum glucose concentration following an exogenous dose of insulin in infected mice as compared to normal control animals. From these results, it was suggested that there may be secretion of biologically inactive insulin in *S. mansoni* infected mice such that the insulin sensitive tissues synthesize more insulin receptors. This may lead to the observed greater response to a dose of exogenous insulin, and may have contributed to the mild hyperglycaemia found in *S. mansoni* infection in mice. Serum zinc concentration and the specific activities of the zinc metalloenzymes; hepatic alcohol dehydrogenase and red blood cell catalase were determine to test if there was zinc deficiency in *S. mansoni* infection, leading to poor storage of pro-insulin in this infection. There was no significant change in serum zinc concentration or the activities of hepatic alcohol dehydrogenase and red blood cell catalase in infected and control mice. This suggested that zinc deficiency leading to poor storage of pro-insulin did not contribute to the mild hyperglycaemia observed in this infection. The serum concentration of chromium, which is a constituent of the "glucose tolerance factor" was compared in infected and control mice. No significant change was observed in serum chromium concentration due to *S. mansoni* infection. This suggested that the "glucose tolerance factor" which enhances insulin action *in vitro* is not impaired due to chromium deficiency in *S. mansoni* infection. In order to investigate a possible cause of the observed mild hyperglycaemia, the specific activities of hexokinase, phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase, which are all key enzymes in glucose

metabolism, were determined in the brain, heart, liver, lungs, spleen, kidneys, testis and skeletal muscle of infected and control mice. The specific activities of pyruvate kinase and hexokinase were found to be significantly elevated in liver of infected mice. This was considered to be due to the presence of the worms and and their eggs, since the adult worms are known to possess a high activity of the key regulatory enzymes of the glycolytic pathway. The activity of phosphofructokinase was not significantly affected by the infection in the liver, suggesting a depression of the host activity of this enzyme in the liver, as the adult worms are known to possess a high activity of phosphofructokinase. These results suggested that glycolysis may be impaired in the liver of *S. mansoni* infected mice. The specific activity of hexokinase was also significantly elevated in the heart of infected mice, but this was not associated with any significant changes in other key glycolytic enzymes in this organ. The specific activities of the Kreb's cycle enzymes; pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were not significantly affected by *S. mansoni* infection in any of the organs studied, showing that *S. mansoni* infection does not affect these Kreb's cycle enzymes.

The serum concentrations of pyruvate and lactate and the specific activity of lactate dehydrogenase in various tissues was determined to establish whether the observed mild hyperglycaemia was due to parasite induced anaerobic respiration in host tissues. Serum concentrations of lactate and pyruvate were not significantly affected by *S. mansoni* infection, and lactate dehydrogenase activity was reduced in lungs of infected mice, but remained unaltered in other tissues. It was concluded that the observed hyperglycaemia was not due to parasite induced

anaerobiosis in the host.

Further work was carried out to investigate whether the observed hyperglycaemia in *S.mansoni* infection in mice was due to increased synthesis of glucose from non-carbohydrate sourc es. This was assesed by dertemining the specific activity of pyruvate carboxylase in infected and control mice. The results showed a significantly increased pyruvate carboxylase activity in liver of infected mice. This increase in pyruvate carboxylase activit y in the liver of infected mice was considered to be due to host enzyme activity since the adult parasites are known to have lower pyruvate carboxylase activity than mammalian liver. These results suggested that gluconeogenesis may be enhanced in liver of *S. mansoni* infected mice. However, the activity of phosphoenolpyruvate carboxykinase, the key regulatory enzyme of gluconeogenesis, was not studied.

In conclusion, the observed mild hyperglycaemia in this study may be due to depressed glycolytic flux, production of biologically inactive insulin and probably increased gluconeogenesis.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1.0.   INTRODUCTION

Schistosomiasis is a parasitic infection caused by trematode worms of the genus schistoscma. There are several species of the genus *schistosoma* which infect man. The most important are; *S. mansoni, S. haematobium, S. japonicum* and *S. intercalatum* (Jordan and Webbe, 1992). Other schistosoma species which also infect man are *S. matheei, S. mekongi* and *S. bovis*.

S. mansoni and *S. haematobium* are found mainly in the African continent and the diseases due to these two are sometimes reffered to as African schistosomiasis. *S. japonicum* is found mainly in the Asian continent (Jordan and Webbe, 1982).

S. mansoni and *S. japonicum* live in mesenteric veins, whereas *S. heamatobium* and *S. intercalatum* live in veins of the urinary bladder.

1.2.0.   LIFE CYCLE OF S. MANSONI

Schistosomes, like other trematode worms, have a complex life cycle and develop by a succession of stages, (Fig 1.), which involve alternating parasitic and free living forms.

ADULT WORMS (1cm long)
Live in mesenteric veins and produce
300 eggs per day.Some of these embolise
in liver and cause fibro-obstructive
granulomatous lesions.
Others (ᴎ 50%) exit body by faeces.

SCHISTOSOMULA
Migrate via lymphatics and
blood vessels through heart and
lungs to hepatic portal system.

EGG (140 x 60 μm)
Hatches in water to
liberate miracidium larva.

CERCARIAE (350 μm long)
Penetrate human skin and
transform into schistosomula.

MIRACIDIUM
Penetrates suitable snail
and proliferates asexually
to form cercaria larvae.

Figure 1.   Life cycle of schistosomes and migration in the host.

In the mammalian host, the adult worms produce a large number of eggs. A single female *S. mansoni* produces 100–300 eggs per day (WHO, 1985) These eggs are passed out in the faeces or become trapped in the host's tissues. The eggs which are passed out through human feaces may hatch in fresh water to release free swimming forms; the miracidia which invade aquatic snails of the genus *Biomphalaria* (Jordan and Webbe, 1982).

In the snail, miracidia transform into sporocysts which undergo asexual multiplication, and are released as cercariae into the water in large numbers. This stage infects man after skin penetration. It rapidly transforms to a schistosomulum, which migrates via lymphatics and blood vessels through the heart and lungs to the hepatic portal system. The route of migration of *S. mansoni* schistosomula from the lungs to liver· is entirely intravascular with probably several passages round the pulmonary and systemic circulations, before chance entry into arteries leading to the hepatic portal system (Miller and Wilson, 1980). Maturation to adulthood and mating take place in the liver, and the mated *S. mansoni* pairs remain in the liver or migrate to the messenteric veins. The mature worms remain paired at all times throughout their life, except during oviposition when the female detaches itself from the gynaecophoric canal of the male and migrates into the small blood vessels where she deposits eggs, one at a time.

The eggs retain their position after the body of the parent worm has been withdrawn. The embryo, protected by the egg shell,

develops into a ciliated organism within six days and begins to secrete proteolytic enzymes which are released through ultramicroscopic pores in the egg shell. The proteolytic enzymes digest the surrounding host tissues, and enable the egg to pass out of the venules, and into the intestines. *S. mansoni* eggs are characterized by a lateral spine and escape with faeces (Cowper, 1971).

Although adult schistosome worms may live for 30 years or more, the average life span is estimated to be about five years (Baron, 1939; Fairley, 1931; Harris *et al*, 1984; Market *et al* 1978).

1.3.0.   GEOGRAPHICAL DISTRIBUTION OF SCHISTOSOMIASIS

An estimated 200 million people in 76 countries have schistosomiasis in close association with water resources development, and the periurban areas of Africa (World Health Statistics Annual, 1991). The geographical distribution of the parasites is dependent on the distribution of the snail, which is the intermediate host (Kinoti, 1971a,b). In areas where both *Bulinus* and *Biomphalaria* species of the snails co-exist, also both *S. haematobium* and *S. mansoni* infections occur. Table 1. shows the geographical distribution of schistosomiasis by species.

S. mansoni is endemic in most African countries, parts of Arabia, in Northern and Eastern parts of South America and in some Carribean Islands (WHO, 1985).

S. japonicum occurs in the Phillipines, Japan, China and some parts of Thailand and Indonesia (WHO, 1985).

Table 1 Geographical distribution of schistosomiasis by species, by WHO region.

Country or area	S. mansoni	S. haematobium	S. intercalatum
African Region			
Algeria		+	
Angola	+	+	
Benin	+	+	
Botswana	+	+	
Burkina Faso	+	+	
Burundi	+		
Cameroon	+	+	+
Central African Republic	+	+	+
Chad	+	+	+
Congo	+	+	+
Ethiopia	+	+	
Gabon	+	+	+
Gambia	+	+	
Ghana	+	+	
Guinea-Bissau	+	+	
Guinea	+	+	
Ivory Coast	+	+	
Kenya	+	+	
Liberia	+	+	
Madagascar	+	+	
Malawi	+	+	
Mali	+	+	
Mauritania		+	
Mauritius		+	
Mozambique	+	+	
Namibia	+	+	
Niger	+	+	
Nigeria	+	+	
Rwanda	+		
Sao Tome and Principe		+	
Senegal	+	+	
Sierra Leone	+	+	
South Africa	+	+	
Swaziland	+	+	
Togo	+	+	
Uganda	+	+	
United Republic of Tanzania	+	+	
Zaire	+	+	+
Zambia	+	+	
Zimbabwe	+	+	
Region of the Americas			
Antigua	+		
Brazil	+		
Dominican Republic	+		
Guadeloupe	+		
Martinique	+		
Montserrat	+		
Puerto Rico	+		
Saint Lucia	+		
Suriname	+		
Venezuela	+		
Eastern Mediterranean Region			
Democratic Yemen	+	+	
Egypt	+	+	
Iran, Islamic Republic of		+	
Iraq		+	
Lebanon		+	
Libyan Arab Jamahiriya	+	+	
Oman	+		
Saudi Arabia	+	+	
Somalia		+	
Sudan	+	+	
Syrian Arab Republic		+	
Tunisia		+	
Yemen	+	+	
European Region			
Morocco		+	
Turkey		+	

South-East Asia Region	S. japonicum
Indonesia	+
Thailand	+
India	(S. haematobium?)

Western Pacific Region	S. japonicum
China	+
Democratic Kampuchea	(S. mekongi)
Lao People's Democratic Republic	(S. mekongi)
Japan	+
Malaysia	+
Philippines	+

S. intercalatum occurs in the forest areas of West and Central Africa and the Islands of Sao Tome (WHO, 1990).

In Kenya *S. haematobium* is found in the coastal districts of Kilifi, Kwale and the lower reaches of River Tana in Taveta. It is also found in Machakos and Kitui district. There are also scattered foci in Murang'a, Kiambu and Western Kenya. *S. mansoni* is mainly found in Machakos and Kitui districts. It is also found in Murang'a, Kiambu, Kirinyaga and along the shores of lake Victoria (Highton, 1974).

1.4.0   SCHISTOSOME METABOLISM

The study of metabolic pathways in the worms is essential in the understanding of the pathophysiology of the infection. It may lead to the application of more effective chemotherapeutic agents after targeting of novel pathways by novel drugs. Control measures so far have aimed at eradication of the snail vector by molluscides or manipulation of their water environment, treatment of cases, and health education (WHO, 1985). These control measures have so far not been very effective. Hence further studies in schistosome metabolism may be useful in terms of providing a rationale for design of effective drugs for chemotherapy or prophylaxis.

1.4.1   SHCISTOSOME CARBOHYDRATE METABOLISM

Schistosomes have a high rate of anabolism, with the female converting the equivalent of nearly its own body dry w 't to

eggs each day (Becker, 1977). Adult paired worms are estimated to utilize glucose at the rate of 20% of their dry weight/hour (Musa et al, 1969).

The glucose required for energy production is taken up primarily through the tegument (Rogers and Bueding, 1975) by both Na^+-dependent mediated transport (Isseroff et al, 1972; Podesta and Dean 1982a,b),and diffusion (Uglem and Read,1975). Insulin has no effect on the uptake of glucose into the worms *in vitro* (Clements and Bosch, 1989; Tielens et al, 1989b).

Schistosomes were initially considered to be homolactic fermenters (Bueding, 1950), however, there is now evidence that they are capable of aerobic metabolism. Foster *et al* (1989) and Tielens *et al* (1989a) have demonstrated the liberation of labelled CO_2 following culturing of adult worms in a medium containing labelled glucose (^{14}C – glucose), suggesting oxidation of either pyruvate or Kreb's cycle intermediates. Smith and Brown (1977) demonstrated the activities of the tricarboxylic acid cycle enzymes; citrate synthase, Isocitrate dehydrogenase, malate dehydrogenase and succinate dehydrogenase in *S. mansoni* and *S. japonicum* adult worms. The specific activities of these enzymes ranged between 6-30% of the activities of the same enzymes in cercariae, suggesting that anaerobic glycolysis is the main source of energy in adult schistosomes. There are no reports on the activities of the rest of the Kreb's cycle enzymes.

In general, the schistosome enzymes involved in glucose metabolism do not differ from the host enzymes. Pyruvate kinase

of *S. mansoni* not only has a molecular weight similar to that of rabbit muscle (270,000 daltons) but also resembles it in response to purine and pyrimidine nucleotide diphosphates (ADP, dADP, UDP, CDP) and divalent cations Mg^{++}, Mn^{++}, Co^{++} (Brazier and Jaffer, 1973). Malate dehydrogenase also has a molecular weight similar to the mammalian enzyme (Bout *et al*, 1978). Tielens *et al*, (1991) demonstrated the presence of all four gluconeogenic enzymes; (Glucose-6-Phosphatase, Fructose-1,6-biphosphatase, phosphoenol-pyruvate carboxykinase and pyruvate carboxylase) in adult *S. mansoni*. However they were unable to demonstrate actual gluconeogenesis using ^{14}C labelled substrates ([^{14}C]-NaHCO$_3$, D-(1-^{14}C)-glucose and D-[U-^{14}C]-glucose). They demonstrated substrate cycling between fructose-6-phosphate and fructose-1,6-biphosphate.

Female adult worms utilize more glucose than males, (Conford and Oldendorf, 1979), but isolated females have a lower uptake than males. This and the large amounts of glycogen found in single females, compared to females paired with males, may be explained by the transfer of glucose from the male to the female worms (Conford and Hout, 1981). Cercariae are known to rely on glycogen reserves, about half of which are stored in the tail (Lawson and Wilson, 1980). The tail uses relatively more oxygen than the rest of the body (Lawson and Wilson, 1980). Loss of the tail appears to cause the switch from aerobic metabolism to production of mainly lactic acid in the schistosomulum (Von Kruger *et al*, 1978; Van-Oordt *et al*, 1989). The mechanism of the change from aerobic

metabolism in the cercariae to anaerobic metabolism in the schistosomulum has not yet been established.

1.4.3.   SCHISTOSOME LIPID METABOLISM

Phospholipids of adult *S. mansoni* worms are mainly glycerophospholipids (phosphatidylserine, phosphatidylinositol, phosphatidyl choline, phosphatidylethanolamine and cardiolipin), and sterols (cholesterol) (Smith *et al*, 1970; Fried et al 1980; Meyer *et al*, 1970; Furlong and Caulifield, 1988). Both the triacylglycerols and phospholipids contain fatty acids with chain length of 12 to 24 carbons, (Smith *et al*, 1969). These lipids are absorbed from the mammalian host by the worms. The complex ones (triacylglycerols, phospholipids and Cardiolipins) be inghydrolyzed to free fatty acids, mixed acylglycerols and glycerol prior to incorporation (Smith *et al* 1969).

1.4.4.   SCHISTOSOME AMINO ACID METABOLISM AND PROTEIN SYNTHESIS

In vitro, free amino acids enter through the tegument by both diffusion and mediated transport. Five transport systems are probably involved (Chappell, 1974; Asch and Read, 1975a, b; Isseroff *et al*, 1976).

Glycine uptake and incorporation has been studied in a complex medium and resembles that in mammalian cells (Walker and Chappel, 1982). Alanine, arginine, aspartate, glutamate, leucine, methionine, phenylalanine and valine are taken up by schistosomes

via mediated carrier systems (Isseroff *et al*, 1976). Proline enters the schistosome by diffusion (Asch and Read, 1975a).

In general schistosomes depend on preformed amino acids from the host. Only 7 out of the 16 amino acids, alanine, arginine, proline, glutamate, aspartate, glycine and serine which have been investigated are converted to other amino acids and are incorporated into protein, and only two of these; glutamate and arginine appear to be of much importance (Chappel and Walker, 1982). Carbon dioxide is released from four of these amino acids (arginine, alanine, aspartate and glutamate (Bruce *et al*, 1972), suggesting they are degraded by the worms after transamination via the Kreb's cycle.

S. mansoni and *S. japonicum* have sex, strain, and developmental stage specific proteins. In the Japanese, Phillipine and Formosan strains of *S. japonicum*, the number of proteins present in the females are different from those in males. In the Japanese and Phillipine strains, some proteins are only present in males while others are only in females. With male extracts, 29, 28 and 29 distinct bands were resolved for the Japanese, Phillipine and Formosan strains respectively. Female extracts gave 31, 26 and 25 distinct bands for Japanese, Phillipine and Formosan strains respectively (Ruff *et al*, 1973).

In *S. mansoni* a protein of molecular weight 160,000 daltons is present only in cercariae, others with molecular weights 58,000, 23,000 and 11,000 daltons are present only in the adult worms, while one with a molecular weight of 29,000 daltons is found

only in females (Ruppel and Cioli, 1977).

Protein synthesis by *S. mansoni* has been investigated in infected snails. Three days prior to emergence, cercariae actively synthesize actin and myosin-like molecules, which are contractile proteins and may be useful for the locomotion of the cercriae. Four hours before emergence, polypeptides are synthesized, some of which might be enzymes used in the escape from the snail (Atkinson and Atkinson, 1981). Six hours after skin penetration by the cercariae, schistosomula protein synthesis increases (Nagai *et al*, 1977).

1.5.0. PATHOLOGY OF S. MANSONI INFECTION

1.5.1. PATHOPHYSIOLOGY.

The eggs which are retained in the liver cause an immunological response leading to granuloma development around them (Warren and Dewitt, 1958; DeWitt and Warren, 1959; Warren, 1966). Gradual arrangement of granulomas into strands surrounding the portal branches, followed by the addition of more granuloma at the periphery leads to the reduction of portal canals or at times closure. This leads to an increase in portal blood flow in the liver, causing congestive enlargement of the spleen. Reduced and/or blocked flow from the portal vein into the liver is overcome by the opening of veins from the venous plexus around the stomach, spleen liver and the serosal surface of the oesophagus. These join the systemic circulation, either via the inferior vena cava or the pulmonary veins. *S. mansoni* infection,

therefore, results in the development of hepatomegally, spleenomegally, and oesophageal varices due to portal-venous congestion (Warren and DeWitt, 1958; DeWitt and Warren, 1959; Warren, 1966).

1.5.2.   BIOCHEMICAL PATHOLOGY

1.5.2.1   EFFECTS OF S. MANSONI INFECTION ON HOST PROTEIN, AMINO ACID, AND AMMONIA METABOLISM

S. Mansoni infection has been shown to be associated with increased total serum protein. This has been electrophoretically shown to be due to increased proportion in beta and gamma globulins, and decreased serum albumin, resulting in an increase in the total serum proteins (DeWitt and Warren, 1957; Bruce *et al* 1963; Evans and Stirewalt, 1957; Ramirez et al, 1961; Sadum and Walton, 1958; Warren *et al*, 1965). Serum globulins are synthesized in the lymphoreticular system and their increase may be in response to the infection, whereas serum albumin is synthesized in the liver, an organ whose function is compromised in *S. mansoni* infection.

Serum aminotransferases, alkaline phosphatase and lactate dehyrogenase are increased in serum of patients and experimental animals infected with S. mansoni. This reflects liver cell damage due to this infection (Awadallah *et al*, 1975; Shakir *et al*, 1964; Khattab *et al*, 1970a,b,c; Higazi *et al*, 1960; Garson and Williams, 1957; Ghanem *et al*, 1967). Serum pseudocholinesterase and cholinesterase are decreased in *S. mansoni* infected animals and

people (Awadallah *et al*, 1975; Shakir *et al*, 1964; Khattab et al, 1967; Ghanem *et al* 1970a,b; Abdel-Rahim *et al* 1990).

In patients with schistosomal hepatic fibrosis, serum albumin is decreased, albumin synthesis is increased, although albumin catabolism is normal. From this and the decrease in serum cholinesterase and pseudocholinesterase, it has been proposed that an increase in plasma volume may occur during this infection(El-Saadan *et al*, 1968; Fayez *et al*, 1990). Indeed El-Saadan *et al*, (1968), demonstrated increased plasma volume in *S. mansoni* infected patients by injecting intravenously Radio-active labelled human serum albumin(RIHSA), and calculating plasma volume from the radioactive counts in the plasma from subsequent blood samples. The increase in total serum proteins may be the reason for the increase in total plasma volume due to the osmotic pressure exerted by the extra proteins. There are no reports on the osmolality of plasma in *S. mansoni* infection.

Dunn *et al* (1979), demonstrated increased levels of plasma proline in *S. mansoni* infection. Accumulation of ornithine or proline may be caused by decreased activity of some of the urea cycle enzymes. Tanabe *et al* (1989) showed that the total and specific activities of carbamoyltransferase were significantly decreased in livers of *S. mansoni* infected mice. Arginase was not affected by infection. The increased proline levels were used in the synthesis of collagen (Dunn *et al*, 1979). Collagen and collagen synthesis are increased in *S. mansoni* infected people and experimental animals (Dunn *et al*, 1977; Dunn *et al*, 1979; Takahashi

et al, 1980). Collagen is the main constituent of granulomata/fibrotic liver tissue. The increased proline is synthesized from arginine but not glutamate (Dunn *et al*, 1978).

Alterations in serum levels of amino acids in S. mansoni infection could be due to increased levels of serum ammonia. DeWitt and Warren (1959) demonstrated a six fold increase in ammonia levels in mice infected with *S. mansoni*. Other workers have also demonstrated an increase in serum ammonia and decreased tolerance to oral ammonia salts (Daugherty *et al*, 1954; Warren and Reboneas, 1964; Senft, 1967).

Ammonia could increase in plasma due to portal bypass of the liver parenchyma through portal systemic collateral circulation or due to increased metabolism of amino acids by schistosomes or their eggs within the host (Tao and Haug, 1965; Senft, 1966; 1963; Stjerholm and Warren 1974). Warren *et al*, (1965), showed extensive portal systemic collateral circulation accompanied by only minimal liver parenchyma damage in *S. mansoni* infected patients. This resulted from deposition of *S. mansoni* eggs in the portal areas of the liver, leading to extensive granuloma development which led to intrahepatic blockage of blood flow and the development of portal hypertension. This could also be attributed to decreased flux of the urea cycle enzymes (Tanabe *et al*, 1989).

High blood ammonia stimulates secretion of glucagon from the alpha-cells of the pancreas which promotes gluconeogenesis from amino acids, especially in the kidneys and hence increases the production of ammonia. Thus high blood ammonia levels tend to

be self propagating (James *et al*, 1979). The increased synthesis of glucose stimulates the secretion of insulin from the beta-cells of the pancreas, at a time when the liver's capacity to clear the hormone from the circulation is impaired, so that there is some degree of hyper-insulinaemia (Ghanem *et al*, 1973). Increased insulin secretion increases the uptake and hence the catabolism of the branched chain amino acids (leucine, isoleucine, valine) by the muscles. This increased catabolism of branched chain amino acids will increase further the burden of ammonia to be metabolized (James *et al*, 1979).

1.5.2.2 EFFECTS OF S. MANSONI INFECTION ON HOST LIPID METABOLISM

Serum cholesterol is significantly decreased in *S. mansoni* infection, together with both high and low density lipoproteins (El-Kharbotyly *et al*, 1965). They explained these results as reflecting the liver's impaired ability to catabolize oestrogens in the circulation. High plasma oestrogen levels have been associated with low plasma cholesterol (Cantarow and Trumper, 1962).

The levels of total, free and esterified cholesterol and the different cholesterol ester classes in the liver and plasma of *S. mansoni* infected mice are significantly decreased (De Oliviera and Costa, 1977). De Oliviera and Costa (1977), explained the decrease in total and esterified cholesterol as being due to the decreased

activity of lecithin:cholesterol acyl-transferase (LCAT), which catalyzes the esterification of cholesterol in the blood vessels and liver (Glouset *et al*, 1962). LCAT activity was shown to be reduced in chronic *S. mansoni* infected patients (Medeiros, 1975; as cited by Gillet and Carvalho, 1978).

However, adult *S. mansoni* do not synthesize cholesterol *de novo* from [^{14}C] acetate or mevalonate (Smith *et al*, 1970; Meyer *et al*, 1970) but it is acquired by the adults from the host (Smith and Brooks, 1969). The explanation of the low plasma cholesterol seen in *S. mansoni* infection may, therefore, be due to both uptake by the worms and depressed LCAT activity in this infection.

1.5.2.3. EFFECTS OF S. MANSONI INFECTION ON HOST VITAMIN NUTRITIONAL STATUS

Reduced levels in liver thiamin, niacin, pantothenic acid, have been demonstrated by Saleh and Shehata (1978), in mice and hamsters infected with *S. mansoni*. They showed no effect of the infection on host liver levels of riboflavin and pyridoxine. Samir-Amer *et al*(1967) demonstrated decreased levels of liver pyridoxal phosphate in mice infected with *S. mansoni*. Njagi (1990) showed decrease in the levels of pyridoxal phosphate in livers, kidneys and spleen, and increased levels in skeletal muscles of mice infected with *S. mansoni* compared to normal controls. He concluded that, whereas theres is no decrease in total body pyridoxal phosphate, there is redistribution in the various organs.

1.5.2.4.   **EFFECTS OF S. MANSONI INFECTION ON HOST GLUCOSE METABOLISM**

Glycosuria which disappeared after treatment of S. mansoni infection was demonstrated by Erfan and Camb (1933) in human patients. It was proposed that this observation could be due to elevated blood glucose in the host during this infection. Saleh et al (1976) showed elevated fasting bloodglucose in mice infected with *S. mansoni*. Other workers later reported that *S. mansoni* infection does not affect the blood glucose in mice (Sadun and Williams, 1966). Saleh *et al* (1976) suggested that the results of Sadum and Williams (1966) in respect to host blood glucose could be due to the short duration of the infection, or the lower parasite burden of infection.

Other investigators, (Ghanem *et al*, 1971, 1973 Hassabala *et al*, 1964; Sukkar *et al*, 1974, El-Hawary *et al*, 1973) have also found normal fasting blood glucose levels in *S. mansoni* infected patients but abnormal oral and intravenous glucose tolerance. The glucose disappearance rate is decreased in the infected patients compared with normal controls, suggesting that *S. mansoni* infection causes impairment in the utilization of glucose by the host.

The high rate of glucose utilization (20% of their dry weight/hour for coupled worms) by the worms (Musa *et al*, 1969) does

not seem to account for increased or normal levels of fasting glucose in man and experimental animals during this infection. Infection should indeed cause hypoglycaemia.

Saleh *et al* (1967), showed a very low incidence (4.2.%) of pancreatic involvment in schistosomiasis. This affects mostly the periductal tissues of the pancreas and only very rarely the parenchymal and Islet tissues. Ghanem *et al* (1973), demonstrated hyperinsulinaemia in schistosomiasis patients after intravenous glucose administration. The low pancreatic involvement shows that pancreatic function is unlikely to be affected by *S. mansoni* infection. The abnormal glucose metabolism observed in this infection is therefore unlikely to be due to the under production of insulin or glucagon. Indeed hyperinsulinaemia was demonstrated by Radioimmunoassay in humans infected by *S. mansoni* by Ghanem *et al* (1973), suggesting normal pancreatic response to hyperglycaemia. However, there are no reports on the bioactivity of insulin in *S. mansoni* infection.

Ghanem *et al* (1971) showed that insulin administration caused a smaller decrease of blood glucose in schistosomiasis infected patients with ascites compared with healthy subjects. Stimulation of insulin secretion with sulphonylureas, glycodiazine and tolbutamide also resulted in impaired lowering of blood glucose. Their observations may suggest an impairment of the cellular uptake of glucose in response to insulin.

Shaheen *et al* (1989) demonstrated decreased blood lactate, increased pyruvate, increased liver glucose-6-phosphatase and

glucose-6-phosphate dehydrogenase in mice infected with *S. mansoni*. These authors attributed the increase in blood pyruvate to decreased utilisation of pyruvate and other Kreb's cycle intermediates. This could be due to decreased thiamin and niacin levels in this infection. This was supported by Saleh and Shehata (1978) who demonstrated reduced thiamin and niacin levels in mice and hamsters infected with *S. mansoni*. Decreased pyruvate utilisation would increase the $NAD/NADH^+$ ratio which would inhibit the conversion of pyruvate to lactate and increase the pyruvate/lactate ratio. El-Hawary *et al* (1973) also demonstrated increased blood pyruvate in patients infected with *S. mansoni*, while Ezz *et al* (1971) and Saleh *et al* (1976) showed increased lactate in this infection.

In conclusion there appear to be conflicting reports on the effects of *S. mansoni* infection on blood sugar level . Some workers have found hyperglycaemia, (Erfan and Camb, 1933, Saleh *et al*, 1976), while others have found low blood glucose, (Sadum and Williams, 1966) and others have found normal fasting blood glucose levels in infected patients (Ghanem *et al*, 1971, 1973, Hassabala *et al*, 1964; Sukkar *et al*, 1974 and El-Hawary *et al*, 1973). There appear to be few, if any, reports on the effects of *S. mansoni* infection on the host's carbohydrate metabolic enzymes and their substrates.

1.5.2.5.   EFFECTS OF S. MANSONI INFECTION ON HOST TRACE

METAL IONS (Zn^{++} and Cr^{+++})

Zinc is a constituent of over 20 metallo-enzymes; including both RNA and DNA polymerases, Alchohol dehydrogenase, catalase, and alkaline phosphatase (Devlin, 1976). Zinc deficiency in children leads to dermatitis, poor growth, and impairment of sexual development. In both children and adults zinc deficiency is associated with poor wound healing and decreased taste acuity (Devlin, 1976).

Reduced serum zinc levels in *S. mansoni* infected patients and experimental animals have been reported by several investigators (Prasad *et al*, 1963; Mickhail *et al*, 1982). However, Soliman (1975), reported elevated serum zinc levels in patients with *S.mansoni* infection as compared to normal controls.

Zinc is also known to be required for the storage of pro-insulin in the pancreas. It is absorbed as a chelation product with picolinic acid in the gastrointestinal tract (Bender, 1982). Picolinic acid is a product of the main oxidative pathway of tryptophan, a pathway whose metabolic flux has been shown to be reduced in *S. mansoni* infection (Njagi, 1990). Reduced picolinic acid in *S. mansoni* infection could be the explanation for the reduced plasma zinc observed in the infection. Reduced plasma zinc could lead to poor storage of proinsulin in *S. mansoni* infection.
The reduced metabolic flux through the tryptophan oxidative pathway may lead to the reduction of nicotinic acid, a component

of the glucose tolerence factor, a chromium-nicotinic acid complex that enhances the action of insulin *in vitro* (Mertz, 1974; Toepfer *et al*, 1977). No literature exists on the serum levels of the trace element chrominum in *S. mansoni* infection in man and experimental animals.

1.6.0 **AIMS AND OBJECTIVES**

Broad objective.

To establish the effect of *S. mansoni* infection on mouse blood glucose levels, and determine its basis in terms of enzymes responsible for glucose metabolism, or an effect on the production or action of insulin.

Specific objectives

1) To determine the effect of *S. mansoni* infection on host blood glucose levels.

2) To establish the effect of a dose of exogenous insulin on the serum glucose concentration of *S. mansoni* infected mice.

3) To establish the effect or otherwise of *S. mansoni* infection on the pancreas by measuring serum concentrations of pancreatic lipase in *S. mansoni* infected mice.

4) To establish the serum concentration of the metal ions Zn^{++} and Cr^{+++} and the specific activities of enzymes whose activity is dependent on these ions in *S. mansoni* infected mice.

5) To determine the specific activities of the regulatory

enzymes of the glycolytic pathway and the Kreb's cycle in *S. mansoni* infected mice.

6) To establish if *S. mansoni* infection influences the host's end products of glycolysis by comparing serum concentrations of pyruvate and lactate and the specific activity of lactate dehydrogenase in *S. mansoni* infected mice.

CHAPTER 2

MATERIALS AND METHODS

2.1.0   ANIMALS

Male swiss albino mice were purchased from the animal house, Department of Zoology, University of Nairobi, at the age of 4 weeks. The mice were divided randomly into two groups, i.e. a control group, and those to be infected with *S. mansoni* (for method of infection see 2.2.0). Both groups were kept in mouse cages in the animal house of the Department of Biochemistry, University of Nairobi. They were fed the animal house diet of mouse pellets and water, *ad libitum*. The composition of the pellets was as shown in table 2.

2.2.0.   INFECTION OF MICE WITH S. MANSONI

Cercariae of *S. mansoni* were provided by the Kenya Medical Research Institute (KEMRI), Mbagathi, Nairobi. The infection is maintained in this Institute in the snail, *Biomphalaria pfeifferi*, which were caught in water bodies in the Eastern province of Kenya. The snails are infected with miracidium provided by the Institute of Primate Research (IPR), Nairobi, and they are hatched from eggs passed in stools of baboons, *(Papio anubis)*. The original infection was obtained in Machakos, Eastern Province of Kenya, in the form of S. mansoni eggs passed by a human patient.

The snails were induced to shed cercariae by placing them under bright light for about one hour. To ensure getting

Table 2. <u>Nutritional composition of mouse pellets.</u>

<u>(wt./wt).*</u>

Proteins	18.0
Fats	4.0
Carbohydrates	67.0
Vitamins	3.0
Minerals	4.0
Dietary fibres	4.0
Energy density(kJ/g)	15.73

Table 2 shows the ratio (wt/wt) of the various nutrients in the mouse pellets used in this study.

* Provided by Unga Feeds Ltd.

cercariae of both sexes, at least 10 snails were shed at the same time. (The snail phase of *S. mansoni* life cycle is asexual).

The cercariae were counted under the dissecting microscope, where 10 ul of water containing cercariae was placed on a plastic petri dish, and a drop of 1% iodine added to immobilize them. The total number of cercariae in 10 ul was multiplied by the volume of water containing the shed cercariae (in μl's) to give the total number of cercariae.

The mice were infected by the paddling method as described by Cowper (1971), within two hrs after collection of cercariae. The mice were first immersed in water reaching up to their bellies in a cage. They were left in this state for about 30 minutes. This was to induce micturition and defaecation, because these substances are lethal to cercariae. They were then transferred to another cage with a similar amount of water, and the cercariae were then added into the water. Mice were then left to get infected for 30 minutes, after which they were removed to their normal cages. The number of cercariae used was about 150 per animal.

2.3.0.   CONFIRMATION OF INFECTION

The presence of eggs in the stool confirmed the infection. The formalin-ether technique (Jordan and Webbe, 1982), was used to separate the eggs. Stool was obtained individually from each mouse, 50 days post infection, and homogenised in about 10 mls of

normal saline. The suspension was strained through two layers of wet gauze into a centrifuge tube. After centrifugation the supernatant fluid was pippetted off and the residue repeatedly resuspended in 10mls of normal saline until the supernatant was clear. The residue was then mixed with 10 mls of 4% formaldehyde and allowed to stand for 5 minutes. 3mls of ether was then added and the mixture capped and shaken vigorously. This was then centrifuged at 4,000 r.p.m. for 5 minutes with an MSE bench centrifuge (Fisons - England). This centrifugation resulted in four layers; the topmost clear layer of ether with dissolved fats, a plug of feacal debris, a clear layer of formaldehyede and the sediment containing the eggs. The sediment was placed on a glass slide and examined for *S. mansoni* eggs under the microscope.

2.4.0. CHEMICALS AND ENZYMES.

The reagents in this study were of analytical grade. They were obtained from British Drug House, Poole England; Sigma Chemicals, St.Louis, MO, U.S.A; and Boehringer Manheim GmBH, Germany. Lente insulin was obtained from Novo Industri A/S Copenhagen, Denmark.

2.5.0 PREPARATION OF TISSUES FOR ENZYME AND METABOLITE ASSAYS

The mice were sacrificed by anaesthesia. This was done by soaking cotton wool in diethyl ether placed in a large open mouth glass container. The mouse was then dipped into the bottle and

removed when anaesthetized. The mice were then pinned on a dissection board with the ventral side up. The abdomen was opened with a single median ventral incision and blood obtained from the inferior vena cava by needle aspiration. The liver, heart, lungs, spleen, kidneys and testes and the hind biceps skeletal muscle were dissected out. The brain was obtained by a craneotomy. The tissues were immediately immersed in ice cold 0.25 M sucrose.

The tissues were homogenized in the cold room (+4°C) using a teflon pestle operated at low and constant speed in order to achieve a gentle homogenization of the tissue. Homogenization conditions were the same for all preparations.

2.6.0 ENZYME ASSAYS

Except where otherwise indicated, all enzyme assays were carried out at 25°C, using a Perkin Elmer 550S uv/vis spectrophotometer. The appearance or disappearance of NADH, NAD$^+$ or NADP$^+$ was monitored at 340 nm. In these assays, 1 cm light path cuvettes were used and in all cases initial rates of reaction were determined. The molar extinction coefficient at 340 nm was taken as 6.22×10^3 L.mole^{-1}.cm^{-1}. A unit of enzyme activity was defined as the amount of enzyme that catalyzed the conversion of 1 nmole of substrate to products per minute at 25°C under the specified conditions of the assay system, while the specific activity of an enzyme is the activity/mg protein.

2.6.3.   ALCOHOL DEHYDROGENASE (ADH) (EC 1.1.1.1)

Alchohol dehydrogenase activity was assayed in 3 mls of 50 mM tris-HCl buffer, pH 8.4, containing 5% ethanol and 0.8 mM NAD.

The principle of the assay was based on the increase in absorbance at 340 nm due to the reduction of NAD according to the following reaction:

$$\text{Ethanol} + \text{NAD}^* \xrightarrow{\quad \text{ADH} \quad} \text{Acetaaldehyde} + \text{NADH}^* + \text{H}^*$$
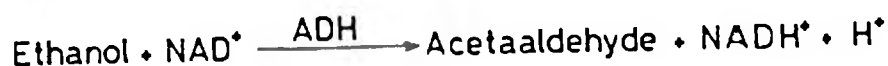
The reaction was started by the addition of 100μl of the extract.

2.6.4.   SUCCINATE DEHYDROGENASE ACTIVITY (E.C. 1.3.99.1)

The enzyme was assayed essentially as described by Pennington (1961). The assay was based on the reduction of 2-p-Iodophenyl-3-p-nitrophenyl-3-phenyl monotetrazolium chloride (INT). The assay medium contained 50 mM pottasium phosphate buffer, pH 7.4, 50 mM sodium succinate, 25 mM sucrose, and 100-200 ug mitochondrial protein. The reaction was initiated by addition of 100μl stock phenazine methosulphate (10 mg/ml) followed by 200 ul of stock INT. The final volume of incubation medium was 1.0 ml. After an incubation period of 5 min at 25°C, 1.0 ml of 10% trichloroacetic acid was added to the medium to terminate the reaction. Ethylacetate (4.0 mls) was then added to each tube, the contents shaken vigorously for 30 seconds, and the tubes left to stand until the ethylacetate layer cleared. This layer was then

carefully removed with a pasteur pipette and the absorption measured at 490 nm against ethylacetate. Control samples were treated in a similar manner except for the omission of succinate from the medium. Molar extinction coefficient for the reduction of INT in ethylacetate was taken to be 20.1×10^3 L. $mol^{-1}Cm^{-1}$.

2.6.5 Alpha-KETOGLUTARATE DEHYDROGENASE (E.C.1.2.4.2)

The enzyme was assayed according to Gibson $et\ al$(1984) in 50 mM MOPS adjusted to pH 7.6 with trizma base, 0.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 50 mM ethelenediamine tetracetic acid (EDTA), 0.5 mM dithiothreotol, 0.15 mM thiamin pyrophosphate, 0.5 mM 2-oxoglutarate, 1 mM NAD^+ 0.12 mM CoA, 40uM rotenone, 1mg/L.Triton X-100, and 25μl of the extract. The assay is based on the increase in absorbance at 340 nm due to the formation of NADH according to the following reaction:

$$\text{$\propto$-ketoglutarate} \xrightarrow[\text{NAD}]{\text{CoASH}} \text{Succinyl CoA} \quad \text{(+ } CO_2 \text{, } NADH^+ + H^+)$$
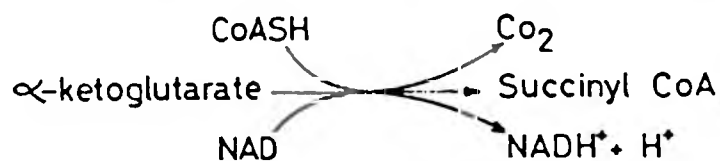
The reaction was started by the addition of NAD.

2.6.6   PYRUVATE DEHYDROGENASE(PDH)

The enzyme was assayed as described by Willimans and Hager (1966); by the measurement of CO_2 released from pyruvate during oxidative decarboxylation. Ferricyanide served as the terminal electron acceptor, so that the flavoprotein served in a catalytic

fashion.

Each Warburg cup contained 100 mM phosphate buffer, 10 mM of $MgCl_2$, 0.1. μM of thiamin pyrophosphate, 50 μM of pyruvate, 0.01M sodium lauryl sulphate, 0.5 mg ovalbumin, and 100 ul of the tissue extract in the main compartment of the flask, plus 25mM of ferricyanide in the side arm of the flask.

After temperature equilibration at 30°C the ferricyanide was tipped into the main compartment of the Warburg flask and CO_2 evolution was measured at 5 min intervals. One unit of enzymatic activity is defined as the evolution of 1 nmol of CO_2 per 30 minutes under above conditions. Specific activity is defined as units per milligram proteins.

2.6.7 HEXOKINASE (H.K.) (EC 2.7. 1L)

The enzyme was assayed according to Bergmeyer (1974) in 50 mM Triethanolamine buffer, pH 7.5, containing 10 mM $MgCl_2$, 1 mM $NADP^+$, 4 mM ATP, 5 ug/ml glucose-6-phosphate dehydrogenase and 1 mM D-glucose. The assay is based on the increase in absorbance caused by the formation of NADPH according to the following reactions:

$$\text{Glucose} \xrightarrow{\text{H.K.}} \text{Glucose - 6 - Phosphate} \xrightarrow{\text{G-6-PDH}} \text{6-Phospho-Gluconolactone}$$

ATP   ADP   NADP   NADPH
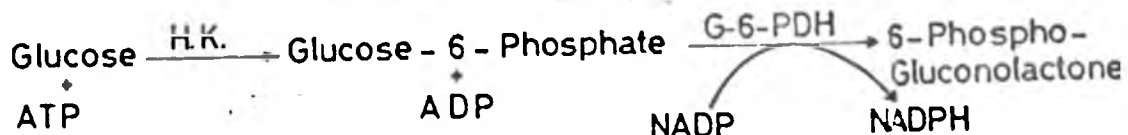
The reaction was started by the addition of 30 or 50 μl the extract.

2.6.8 PHOSPHOFRUCTOKINASE (P.F.K.) (EC 2.7.1.11)

The enzyme was assayed in 50 mM Tris-HCl buffer, pH 8, containing 10 mM MgCL2, 6 mM KCl, 0.1 mM NADH, 2 mM ATP, 1.5 mM cysteine, 5 µg/ml glycerophosphate dehydrogenase, 5 µg/ml triose phosphate isomerase, 5 µg/ml aldolase and 5 mM fructose-6-phosphate. (Storey & Bailey, 1978). The assay was based on the decrease in absorbance at 340 nm as NADH is oxidised according to the following reactions:

Fructose - 6 - phosphate
ATP
PFK
ADP

Fructose, 1,6 diphosphate

Aldolase

Dihydroxyacetone phosphate ⇌ Glyceraldehyde 3-phosphate
Triose-P
isomerase

NADH
Glycerolphosphate
NAD
dehydrogenase

α- glycerolphosphate
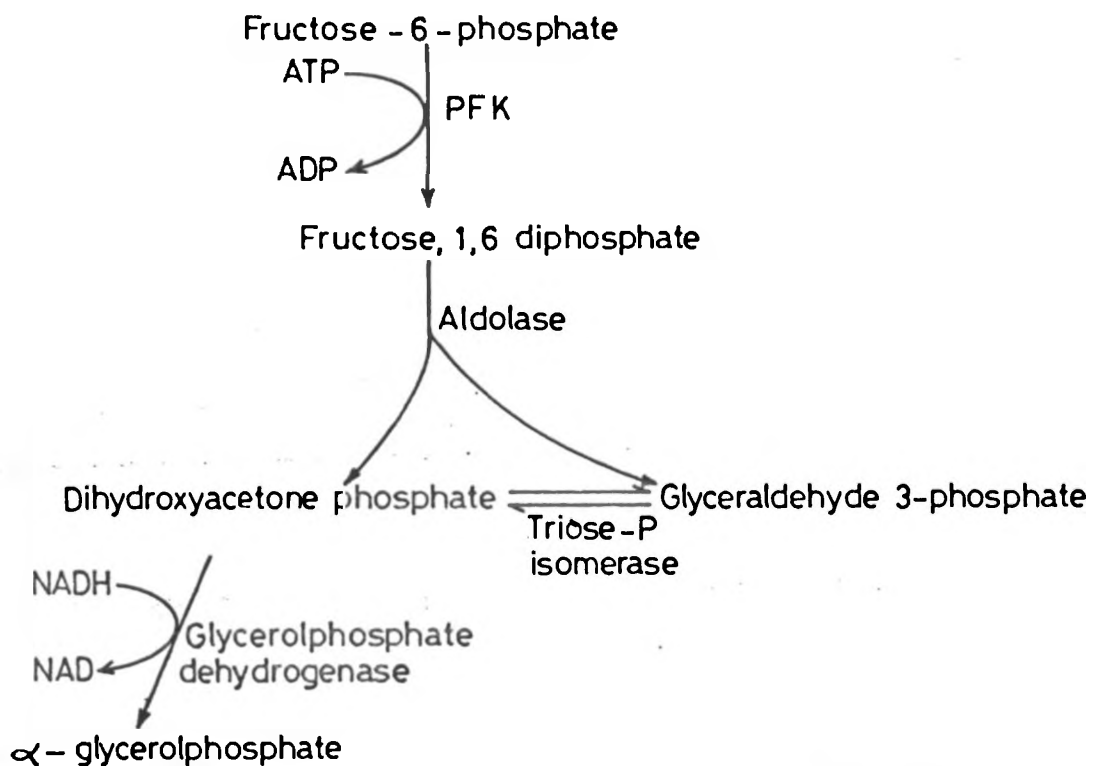
The reaction was started by the addition of 50 µL the extract.

2.6.9   PYRUVATE CARBOXYLASE(P.C. YR C.6.4.1.1.)

The enzyme was assayed in a malate dehydrogenase coupled reaction according to Weeda *et al* (1980) in 75mM Tris-HCl buffer (pH 7.4), 0.2mM NADH, 10mM $KHCO_3$, 10mM $MgCl_2$, 0.15mM acetyl CoA, 5ug/ml malate dehydrogenase (M.D.H.), 40mM ATP and 20 or 40μl of the extract. The reaction was started by the addition of 10mM pyruvate. The reaction sequence was as follows:

$$\text{Pyruvate} + HCO_3^- \xrightarrow[\text{ATP}\quad\text{ADP}]{\text{P. C.}} \text{Oxaloacetate} + Pi + H^+ \xrightarrow[\text{MDH}]{\text{NADH}\quad NAD^+} \text{Malate}$$
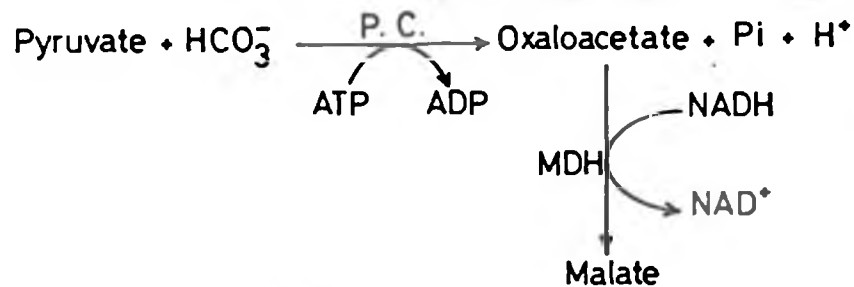
2.6.10.   CATALASE ACTIVITY (EC 1.11.1.6)

The activity of catalase, in red blood cells, was assayed by the method described by Bergmeyer (1974), based on the principle that in the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. This allows the decomposition of H_2O_2 to be followed directly by the decrease in extinction at 240 nm. Thus the difference in extinction (ΔE240 nm) per unit time is a measure of catalase activity.

Blood was collected as described in 2.5.0 in heparinized containers and centrifuged at 13,000 r.p.m for 5 min on an MSE microfuge (Fisons-England)and the plasma and leucocyte layers removed with a pasteur pipette. The erythrocyte sediment was then washed 3 times with isotonic saline. A stock haemolysate was prepared by addition of four parts by volume of distilled water.

The haemolysate was diluted 1:500 with phosphate buffer (50 mM, pH 7.0) immediately before the assay. The haemoglobin content of the stock haemolysate was determined in duplicate by the cyanomethaemoglobin method described 2.6.10.1.

The assay was carried out in a Perkin Elmer S 550 uv/vis spectrophotometer, in a 3 mls quartz cuvette, light path 1 cm. The test cuvette contained 2.0 mls diluted haemolysate, and 10 mM H_2O_2. This was read against the reference cuvette which contained 50 mM phosphate buffer, pH 7.0, and 2.0 mls of haemolysate. The reaction was started by addition of H_2O_2. Readings were taken at 15 second intervals for 1 minute.

Catalase activity was calculated by the formula:

$$\frac{k/g.Hb}{15} = \frac{2.3}{b} \times a \times \log \frac{E_1}{E_2} \ [sec.^{-1}]$$

where

E_1 is E_{240} at $t = 0$

E_2 is E_{240} at 15 sec.

a = $\dfrac{\text{Hb concentration in erythrocyte sediment (mg.Hb/ml)}}{\text{Hb concentration in cuvette (mg.Hb/ml)}}$

b = Hb content of blood or erythrocyte sediment (mg/ml)

k = rate constant of a first order reaction.

2.6.10.1 HAEMOGLOBIN DETERMINATION

Haemoglobin was determined by the cyanomethaemoglobin method. The reaction mixture contained 20µl of the sample and 5mls of ferricyanide reagent. The ferricyanide reagent was made by dissolving 1g $NaHCO_3$, 200mg pottasium ferricyanide, and 50mg pottasium cyanide in 1L of water. This was allowed to stand for 10

minutes and the absorbance was read at 540nm.

2.6.11. SERUM PANCREATIC LIPASE

Serum pancreatic lipase was assayed essentially by the method of Cherry and Crandall (1976). Pancreatic lipase hydrolyses esters of fatty acids to yield mixed acylglycerols and free fatty acids. The liberated free fatty acids can be quantitated titrimetrically with sodium hydroxide.

To two test tubes 0.75 mls of distilled water and 0.25 mls of serum were added. One of the above tubes was placed in a water bath at 100°C to inactivate the lipase and then cooled. This tube acted as the control. To both tubes were added 0.125 mls of 0.25 M phosphate buffer, pH 7; and 0.5 mls of 50 per cent olive oil emulsion (prepared by homogenising a mixture of equal parts of olive oil and a 5% solution of gum acacia in water containing 0.2 per cent sodium benzoate as preservative).

The test tubes were well shaken and incubated at 37°C for twenty four hours. Then, after adding 0.75 mls of 95% ethanol and 2 drops of phenolphthalein, each tube was titrated with 0.05 N sodium hydroxide to a similar pink colour.

Results were expressed in terms of the volume of 0.05 N sodium hydroxide required to titrate the fatty acids liberated. Units of lipase activity per ml. of serum = (mls titration of the unknown – mls titration of control) x 4.

2.7.0.   PROTEIN DETERMINATION

Protein was determined spectrophotometrically by the procedure of Lowry *et al* (1951), using bovine serum albumin as a standard. The standard curve was constructed using serum albumin concentrations ranging from 10-200 μg made to a final volume of 0.6 ml. To each protein sample 3.0 mls of solution A (prepared freshly by mixing 0.5 mls, 1% $CuSO_4$, 0.5. mls 2% Na-K tartarate with 49 mls 2% Na_2CO_3 in 0.1 M NaOH) was added. The solution was allowed to stand for 10 minutes. 0.3 mls solution B (folin phenol reagent, diluted 1:4 with distilled water) was then added and thoroughly mixed. The solutions were stored in the dark for 30 minutes and the optical density was then read at 750 nm in a Perkin Elmer 550S uv/vis spectrophotometer. Blanks were prepared using 0.6 mls of distilled water, and ran through the same procedure.

Sample aliquots were run through the same procedure and the protein concentrations read against the standard curve.

2.8.0   METABOLITE ASSAYS

2.8.1   GLUCOSE ASSAY

Serum glucose was assayed from blood obtained as described in 2.5.0. Red cells were separated by centrifuging at 13,000 r.p.m. for 5 minutes in the cold room(+4°C) using an MSE microfuge (Fisons-England) Glucose was determined immediately in 50 μl of serum in a reaction mixture containing 50 mM KCl, 10 mM $MgSO_4$, 0.2 mM EDTA, 1.0 mM $NADP^+$, 5 mM ATP, 1.5 I.U. hexokinase, 4.0 I.U.

glucose-6-phosphate dehydrogenase and 50mM Tris-HCl, pH 7.5. The assay was based on the increase in absorbance at 340 nm as NADP⁺ is reduced. This is shown in the following sequence of reactions:

$$\text{Glucose} \xrightarrow{\text{H.K.}} \text{Glucose - 6 - Phosphate} \xrightarrow{\text{G-6-PDH}} \text{6-Phospho-Gluconolacto}$$

with ATP → ADP, and NADP → NADPH
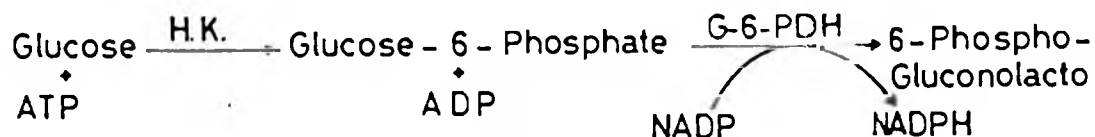
The reaction was started by the addition of ATP and the mixture was allowed to incubate at room temperature for 30 minutes. Absorbance was read at the start of the reaction and after incubation. The difference in absorbance (ΔE) was used to calculate the serum glucose concentration. The concentration of glucose in each sample was estimated using the formula:

$$C = \frac{E \times V}{6.22 \times v}$$

where: C = Concentration of glucose in mmol/l.

E = Change in absorbance during the assay.

v = Volume of sample.

V = Volume of assay mixture

An extinction coefficient of 6.22×10^{-6} per mole of NADP was used in all determinations at 340 nm.

2.8.2.   PREPARATION OF SAMPLES FOR DETERMINATION OF
LACTATE AND PYRUVATE

Blood was obtained as described in 2.5.0. Protein was precipitated in the cold room using perchloric acid (PCA), (3.15 mls of 7% PCA/ml of blood). The samples were then centrifuged at 13,000 r.p.m. in microfuge in the cold room ($+4^{\circ}C$). The supernatant was neutralized using 6 M KOH with methyl orange as indicator. Aliquots of the protein free, neutral extracts were used for lactate and pyruvate determinations.

2.8.3   LACTATE

Lactate was determined essentially as described by Bergmeyer (1965), at $25^{\circ}C$ using 3 mls cuvettes with 1 cm light path. An extinction coefficient of 6.22×10^{-6} per mole of NAD^+ was used for the determinations at 340 nm. The concentration of lactate in each sample was estimated using the formula:

$$C = \frac{E \times V \times D}{6.22 \times v}$$

where   C   = Concentration of lactate in moles/l.

E   = Change in absorbance during the assay (E_1-E_0).

D   = Total dilution.

v   = Volume of sample.

V   = Volume of assay mixture.

The reaction mixture contained 0.09 M hydrazine, 0.225 M glycine buffer, pH 9.5, 2.5 mM NAD^+. After preincubation for 5 minutes E_0 was read and the reaction was then started by the addition of 5.5 I.U. of lactate dehydrogenase, and allowed to go to

completion after which E_1 was read. The ΔE lactate was determined and used to estimate the concentration of lactate in each sample.

2.8.4   PYRUVATE

Pyruvate was determined essentially as described by Bergmeyer (1965), at 25°C using 3 mls cuvettes with 1 cm light path immediately after neutralization.

An extinction coefficient of 6.22×10^{-6} per mole of NADH was used in all determinations at 340 nm. The concentration of pyruvate in each sample assayed was estimated using the formula shown in section 2.8.3. for lactate.

The reaction mixture contained 300 mM triethanolamine buffer, pH 7.6, 3 mM EDTA, and 0.1 mM NADH. 2 mls. of the deproteinized sample were added to the reaction mixture. The reaction mixture was preincubated until a stable absorbance was obtained (E_0). This procedure allowed the reaction mixture to acquire a uniform temperature. After the preincubation period, the reaction was started by the addition of 5.5 I.U. of lactate dehydrogenase and allowed to go to completion and the absorbance (E_1) read. The ΔE pyruvate at 340nm. was determined and used to estimate the concentration of pyruvate in each sample assayed.

2.9.0   INSULIN CHALLENGE TEST

This experiment was carried out on the principles of the bioassay of insulin as described in the international pharmacopoeia (1967). Commercial insulin (40 I.U/ml) was diluted 500

times in 0.9 N saline, pH 2.5. 0.5 mls of the diluent was injected subcutaneously on the anterior abdominal wall of the mice, to give 0.04 I.U. of insulin per mouse.

Blood (50 μl) was obtained from a tail nick before and 30 minutes after injection of insulin. The blood was diluted in normal saline (500 μl) and centrifuged in the cold room for 5 minutes at 13,000 r,p.m. with an MSE microfuge (Fisons – England) to precipitate the red blood cells. Glucose was estimated in the supernatant according to the method described previously in section 2.8.1. The difference in glucose concentration (mmol/l) before and after insulin injection was taken as a measure of the responsiveness of the animal to exogenous insulin.

2.10.0 DETERMINATION OF SERUM CONCENTERATION OF ZINC AND CHROMIUM (USING X-RAY FLUORESCENCE SPECTROSCOPY)

Principle

The quantitative analysis of serum zinc and chromium was done by the method of Energy dispersive X-ray fluorescence spectroscopy (EXRF). The method used was a modification of the proton induced X-ray Emission spectroscopy (PIXE as described by Kinyua (1987) and Ishii *et al* (1975).

The technique is based on the principle that radiation, (for example X-rays), upon impacting on matter, causes excitation of the atoms in the sample. During the deexcitation of the atoms, characteristic radiation, i.e; emmision lines of elements are emitted. From measurements done on the yield and appropriate

energy calibration of the spectral lines, it is possible to do a qualitative and quantitative analysis of the elements in a given sample.

X-ray fluorescence spectroscopy instrumentation

A molybdenum X-ray tube (SIEMENS FK 60-04) was used to produce X-rays. The secondary target was molybdenum. The X-ray tube was operated at 40 kV and 35 mA.

A Silicon-Lithium (Si(Li)) X-ray detector (EG and G ORTEC) coupled to an ADC-multichannel analyzer (CANBERRA S100) was used for data collection and to analyze the X-ray spectra. The spectral data was quantitatively analysed by the AXIL fitting programme in a computer linked to the ADC-multichannel analyzer. The Si(Li) ORTEC detector used was of the following characteristics: active diameter of 6 mm, sensitive depth of 5 mm, and nominal berrylium window thickness of 0.025 mm. The detector was operated at a voltage of 1,500V, negative bias. The measured resolution prior to analysis was 200eV at Ful Wiidth Half Maximum (FWHM) at 5.9 keV of Mn K_α-line and at pulse shaping time constant of 10 μS. Figure 2 shows the geometrical set up of the X-ray tube, secondary target, sample and detector.

Sample preparation

The samples were doped on mylar, which are polyester, high purity, thin (3.6um in diameter) foils, supported on aluminium rings with adhesive material. For each sample 50 μl of 1% PVP (Polyvinylpyrolidone), 50 ul of 1000 ppm yttrium solution (which
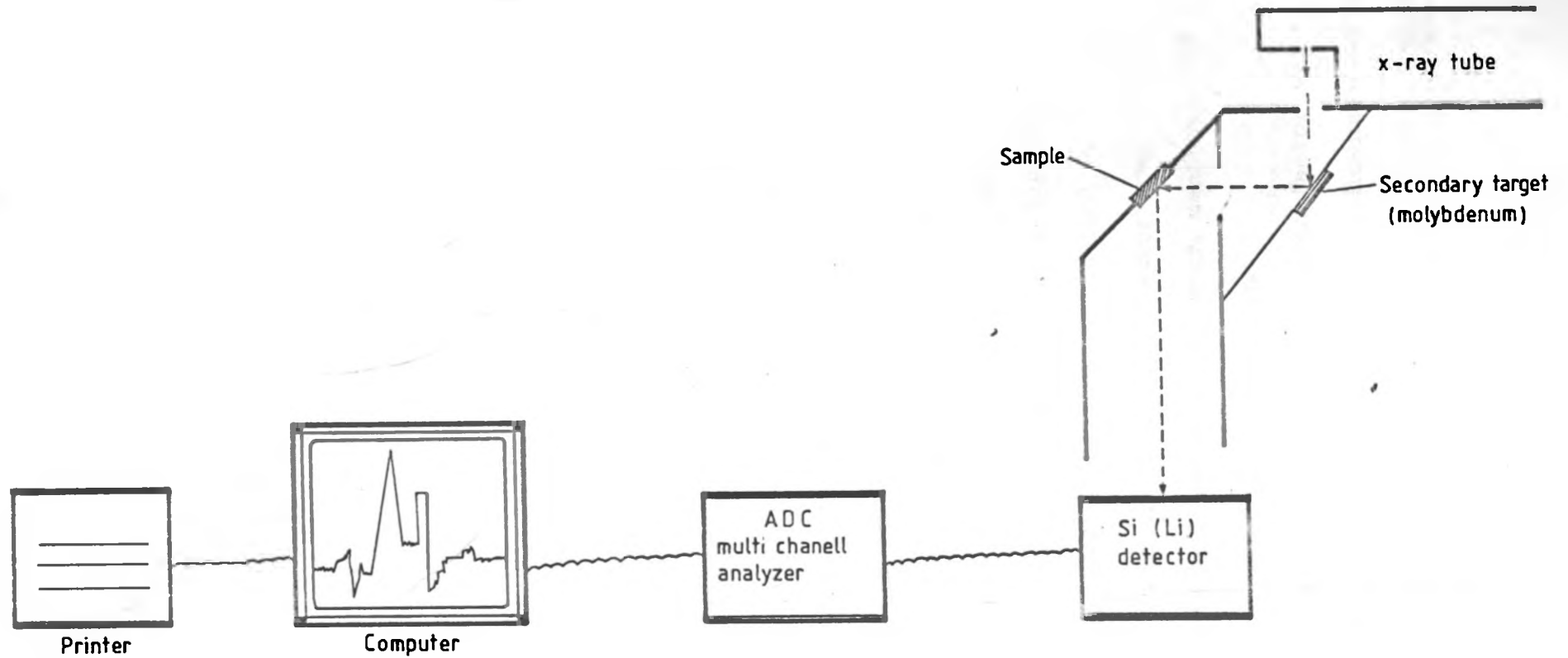
Fig. 2.

Geometrical set up of x-ray tube, secondary target, sample, detector, ADC analyzer and computer used for XRF analysis of serum trace elements.

acted as an internal standard), and 50 ul of sample material were pipetted onto each ring mounted mylar. PVP served to bind the sample and standard to the backing. The rings with samples were then dried in a dessicator at 50°C for two hours.

Optimization of the Technique

(a)   Construction of the calibration factor graph

The calibration factor graph is necessary in this technique to determine the calibration factors of the various elements (Kinyua, 1982; 1987). The calibration factor of an element is a measure of the sensitivity for the particular set up to detect that element. It is dependent on the atomic number of an element, the type of X-ray tube used, the secondary target, the operating voltage and current and the internal standard used. To determine the calibration factors, certified standards were used. These were serum trace elements (level II), urine toxic metals (elevated), supplied by the IAEA, and atomic absorption spectrophotometry (AAS) standards for titanium, vanadium, manganese, iron, nickel, copper, zinc and rubidium supplied by Sigma Pharmaceuticals. Yttrium was used as the internal standard. The elements used in construction of the calibration factor graph were: pottasium, calcium, titanium, vanadium, chromium, manganese, iron, nickel, copper, zinc, selenium, and rubidium.

For each element the calibration factor was calculated according to Kinyua,(1987),by the following equation:

Calibration factor = Element net counts/Element ppm (Certified)
                     Yttrium net counts/Yttrium ppm (Calculated)

Where:

Element net count = Area of elemental peak divided by

total time of data collection

(counts/seconds)

Element ppm = Concentration of the element in certified

sample in parts per million

Yttrium ppm = Concentration of yttrium in the internal

standard in parts per million.

The calibration factor graph was constructed using the element atomic number on the horizontal axis and the calibration factor on the vertical axis. For each element three determinations were made and the average calibration factor used in the plot. The graph was plotted as a third order polynomial. Figure 3. shows the calibration factor graph with 10% error bars.

(b) ACCURACY OF THE METHOD

The accuracy of the method was confirmed using a certified standard,(freeze dried animal blood) which was not used in the construction of the calibration factor graph.

Ig of freeze dried animal blood and 0.411 g. of yttrium nitrate (internal standard, 1000 ppm Y) were acid digested in a concentrated mixture of HNO_3 and HCl in the ratio 3:1(v/v). The digestion was done on a hot plate in a fume chamber until the solutions were clear. The final volume was 100 mls. Glassware used was first boiled in a concentrated mixture of HNO_3 and HCl in the ratio of 3:1(v/v) to remove residual metal elements, and then

CALIBRATION GRAPH

$y = -2.22 + .171x - .003x^2$

Calibration Factor (CF)

Atomic Number (Z)
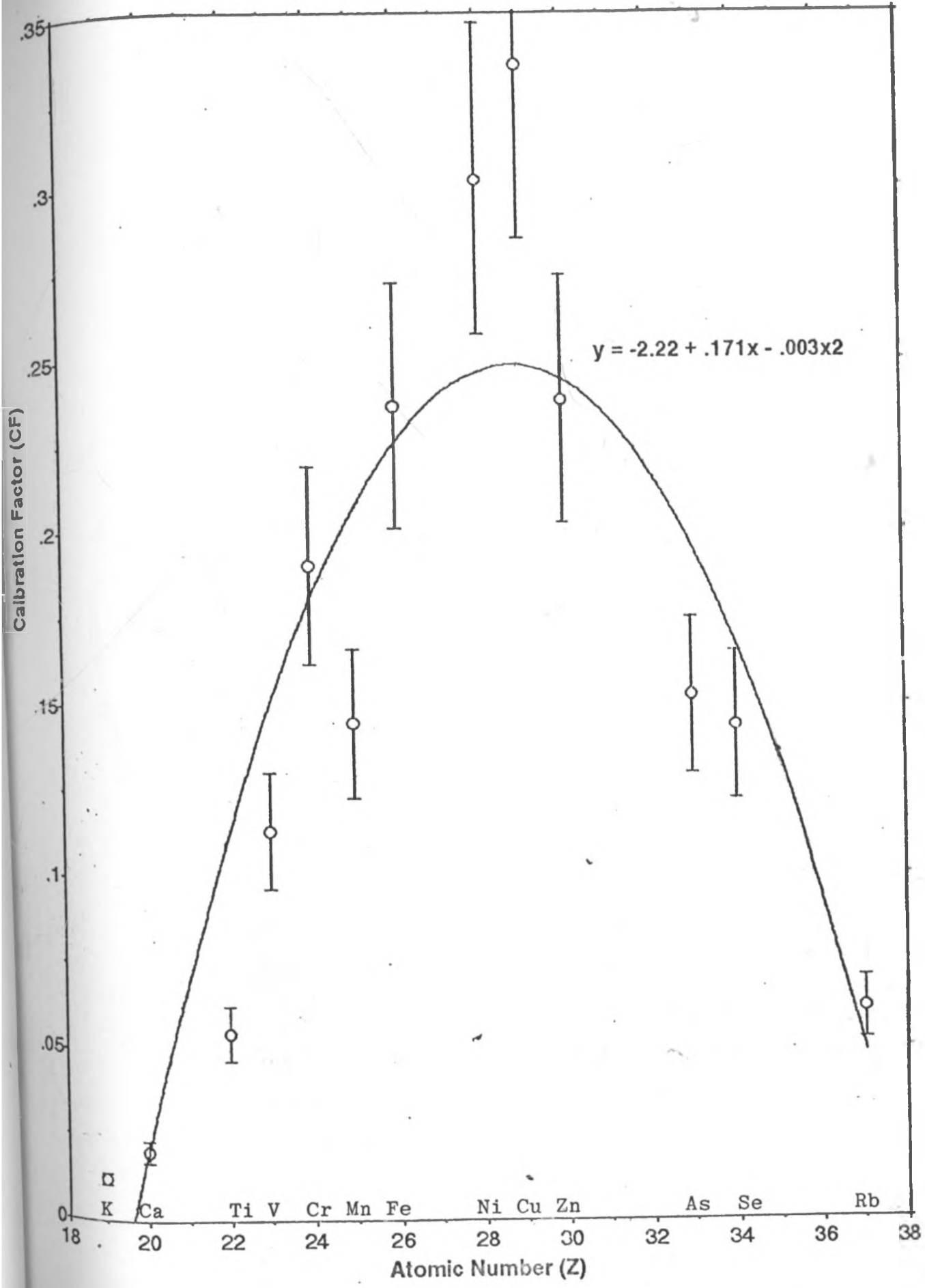
Fig. 3

rinsed in double distilled deionized water.

The samples were then prepared as explained before and a blank in duplicate was also prepared which contained 50 μl of 3:1, HNO_3:HCl and 50 μl of 1000 ppm yttrium and 50 μl 1% PVP.

The concentration of the various elements was calculated by the formula:

Element ppm = Element net counts (sample) X
yttrium net counts (sample)

Yttrium concentration (ppm) in sample
Element calibration factor

Where:

Element net counts = total area of elemental peak divided

by time of data collection (counts/seconds).

Yttrium net counts = total area of yttrium peak divided by

time of data collection (counts/seconds)

Element calibration factor = Calibration factor of the

element as obtained from the

calibration factor graph.

The concentration of the various elements in the blank sample was calaculated and subtracted from the respective elemental values obtained from each sample.

Since 1 g of freeze dried animal blood was dissolved in 100 mls, and 1 ppm = 1mg/l; to convert the concentration in ppm to μg/g dry weight the following formula was used.

Elemental concentration in μg/g = Element ppm x 0.1 x 1000

SERUM SAMPLES

Blood was obtained as described in section 2.5.0 from both infected and control mice. The blood was centrifuged at 13,000 r.p.m. with an MSE microfuge (Fisons-England) for 5 minutes. The serum was obtained from the clear supernatant, and stored at -20°C until time of assay. Samples were prepared and analysed as previously described.

Elements concentration was calculated by the formula previously given for standard sample. Blanks containing yttrium (1000 ppm) - 50 μl and 50 μL 1% PVP were also run and the concentrations of the various elements in ppm calculated. The average concentration of the various elements in the blank were subtracted from the final average concentration of the element in the sample.

CHAPTER 3

RESULTS

3.1.0   THE EFFECTS OF S. MANSONI INFECTION ON MOUSE

SERUM GLUCOSE CONCENTRATION

In order to determine whether *S. mansoni* infection in mice is associated with changes in serum glucose concentration as reported by Salehs *et al* (1976) and Shaheen *et al* (1989), serum glucose was determined in mice with 7-8 weeks infection.

Table 3 shows that the mean serum glucose concentration in *S. mansoni* infected mice was increased compared to the controls. The mean serum glucose levels being 8.24 mmol/l in the infected mice and 6.31 mmol/l in the control animals. This represented an increase of 30.7% in the mean serum glucose concentration in the infected mice, and was significant by the unpaired student's test.

In man, random serum glucose concentration between 3.0-8.0 mmol/l is considered normal. A serum glucose concentration above 8.0 mmol/l is considered as hyperglycaemia (W.H.O; 1980). The above results suggest that that S. mansoni infection causes a mild hyperglycaemia in mice.

3.2.0   EFFECTS OF S. MANSONI INFECTION ON SERUM

PANCREATIC LIPASE CONCENTRATION.

A small percentage (4.2%) of human patients with *S. mansoni* infection were found to have the worms directly infecting the pancreas, mainly the periductal tissues. (Saleh et al, 1967). To test if the mild hyperglycaemia described in section 3.1.0 is due to

Table 3.   Effects of _S. mansoni_ infection in mice on serum
            glucose concentration.

ANIMALS	SERUM GLUCOSE (mmol/1.)		
	Mean ±	S.D.	(n)
CONTROLS	6.31 ±	2.15	(17)
INFECTED	8.24 ±	2.29**	(15)

Values show mean ± standard deviation for the number of animals

shown in parenthesis. Significance of the differences of the means

was tested by the unpaired student's t-test.

[** 0.001<P<0.05]

schistosomal pancreatitis, serum pancreatic lipase activities were compared in *S. mansoni* infected and control mice.

Table 4 shows that the mean serum activity of pancreatic lipase was not significantly affected by *S. mansoni* infection. The mean activity was 0.77 U/ml and 0.78 U/ml in the control and infected mice respectively by the method of Cherry and Crandall (1976).

These results suggest that the mild hyperglycaemia seen in *S.mansoni* infection is not due to schistosmal pancreatitis.

3.3.0 **EFFECT OF EXOGENOUS INSULIN ON SERUM GLUCOSE CONCENTRATION IN *S. MANSONI* INFECTED MICE**

It was proposed that elevated serum glucose in *S. mansoni* infection could have been due to secretion of biologically inactive insulin due to the infection, or to blocked insulin receptors by toxins orginating from the infection.

In order to test this, blood was removed from both control and *S. mansoni* infected mice, before and 30 minutes after an exogenous dose of insulin. Serum glucose concentrations were determined at these two times, and the change in serum glucose concentration calculated.

As shown in Table 5, the change in serum glucose concentration in *S. mansoni* infected mice was significantly higher than in the control mice (2.07 mmol/l/30 min and 3.67 mmol/l/30 min in the control and infected mice respectively).

These results suggest that *S. mansoni* infected mice are

Table 4:   **Effect of _S. mansoni_ infection in mice on serum
pancreatice lipase concentration**

ANIMALS	ENZYME CONCENTRATION(U/ml serum)		
	Mean	±	S.D.
CONTROLS	0.77	±	0.15
INFECTED	0.78	±	0.23

Values are mean ± standard deviation for 6 animals in each group.
Significance of the differences in the means were tested by the
unpaired student's t-test.

Table 5. Changes in serum glucose concentration in control and
S. mansoni infected mice following sub-cutaneous
injection of exogenous insulin (0.04 I.U./animal)

ANIMALS	CHANGE IN SERUM GLUCOSE mmol/l/30 min
	Mean ± S.D.
CONTROLS	2.07 ± 0.97
INFECTED	3.67 ± 0.10*

Values are mean ± standard deviation for 4 animals in each group.
The differences in the means were tested by the unpaired
student's t-test. [*0.05<P<0.1].

more sensitive to exogenous insulin than normal controls. This precludes the hypothesis that insulin receptors may be blocked in this infection. The results raise the possibility that in *S. mansoni* infected mice there is secretion of less active insulin. This then may have caused the insulin sensitive tissues to respond by synthesizing more insulin receptors, and when an exogenous dose of biologically active insulin is administered, there is a greater response in change of serum glucose concentration. This would be in agreement with the elevated serum glucose concentration seen in this infection.

3.4.0   THE EFFECTS OF *S. MANSONI* INFECTION ON SERUM CONCENTRATIONS OF ZINC AND CHROMIUM AND THE CORRELATION OF SERUM ZINC CONCENTRATION TO THE ACTIVITIES OF THE ZINC METALLOENZYMES; HEPATIC ALCHOHOL DEHYDROGENASE AND RED BLOOD CELL CATALASE.

Zinc is important in the storage of pro-insulin and insulin in the beta-cells of the pancreas. Insulin is stored as a hexamer stabilized by zinc in the secretory granules in the beta-cells of the pancreas (Bender, 1982; Newsholme and Leech, 1983). Zinc is absorbed as a chelation product with picolinic acid in the small intestine (Bender, 1982). It has been proposed that in *S. mansoni* infection, there may be deficiency of picolinic acid (Bender, 1982). This could arise due to the effects of the infection on Tryptophan dioxygenase, an enzyme involved in the tryptophan oxidative

pathway of which picolinic acid is a product (Njagi and Bender, 1990). Zinc deficiency could therefore lead to the poor storage of insulin and pro-insulin in the pancreas and so lead to hyperglycaemia in this infection. Serum zinc concentration was therefore compared in infected and control mice. Furthermore, the activities of hepatic alchohol dehydrogenase and red blood cell catalase, which are zinc metalloenzymes were compared in the *S. mansoni* infected and control mice to determine if the activities of these enzymes correlated to any changes in serum zinc concentration.

The "glucose tolerance factor" is a chromium-nicotinic acid complex which enhances insulin action *in vitro* (Bender, 1982). Nicotinic acid is a product of tryptophan oxidation, and it has been proposed that due to the effects of *S. mansonii* on Tryptophan Dioxygenase, there may be deficiency of nicotinic acid in this infection (Njagi and Bender, 1990). This would lead to a deficiency of the "glucose tolerance factor" and probably impair insulin action. To test if the mild hyperglycaemia seen in *S. mansoni* infection (section 3.1.0) is related to reduced chromium concentration, serum concentration of chromium was determined in *S. mansoni* infected and control mice.

As seen in table 6, the serum concentrations of zinc and chromium were not significantly affected by *S. mansoni* infection.That serum zinc concentration was not affected by *S.mansoni* infection was also reflected in the specific activities of hepatic alchohol dehydrogenase and red blood cell catalase,

Table 6.   Effects of _S. mansoni_ infection in mice on serum concentration of the trace elements zinc and chromium (mg/l)

ANIMALS	ELEMENT	
	CHROMIUM Mean ± S.D.	ZINC Mean ± S.D.
CONTROL	1.5 ± 1.13	7.00 ± 2.22
INFECTED	1.04 ± 1.11	6.50 ± 1.12

Values are mean ± standard deviation for 12 animals in each group. Significance of the differences in the means were tested by the unpaired student's t-test.

Table 7.   Effects of *S. mansoni* infection in mice on the specific activities of the zinc metalloenzymes: Hepatic alchohol dehydrogenase and red blood cell catalase.

ANIMALS	ENZYMES	
	Alchohol dehydrogenase (nmol/min/mg protein) Mean ± S.D.	Catalase (k/g.Hb.) Mean ± S.D.
CONTROLS	7.00 ± 1.00	0.11 ± 0.08
INFECTED	7.00 ± 1.00	0.13 ± 0.05

Values are mean ± standard deviation for 6 animals in each group. Significance of the differences in the means were tested by the unpaired student's t-test.

gluconeogenesis, while pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were taken to represent the Kreb's cycle enzymes.

Table 8 shows that the activities of liver hexokinase and pyruvate kinase in *S. mansoni* infected mice were significantly higher than in the control mice. Hexokinase activity was 87.5% higher, while pyruvate kinase activity was 115.7% higher in the infected mice. Phosphofructokinase activity in the liver was not significantly different in the two groups of animals. The increased activities of hexokinase and pyruvate kinase in liver of *S. mansoni* infected mice were probably due to the presence of the adult worms and eggs, as the assays were carried out on crude liver homogenates containing both adult worms and eggs. There are reports of much higher activities of hexokinase, phosphofructokinase and pyruvate kinase in adult worms compared to rat or mice liver (Doong *et al*, 1987; Brazier and Jaffe, 1973; Buending and Saz,1968 and Bergmeyer,1965). This shows that, the fact that phosphofructokinase activity was not elevated in liver of *S. mansoni* infected mice shows that host liver phosphofructokinase activity may be decreased in this infection. These results may tentatively suggest that liver hexokinase and pyruvate kinase activity are not affected by *S. mansoni* infection, whereas phosphofructokinase activity, which is the key regulatory enzyme of the glycolytic pathway (Southerland, 1990) may be depressed in S. *mansoni* infection.

From table 9 it is apparent that the activity of hexokinase,

which were not significantly affected by *S. mansoni* infection either (Table 7).

These results suggest that the mild hyperglycaemia seen in *S. mansoni* infection (section 3.1.0) is not due to zinc deficiency leading to poor storage of insulin, or due to deficiency of the "glucose tolerance factor" resulting from chromium deficiency.

3.5.1 EFFECT OF *S. MANSONI* INFECTION ON SOME ENZYMES INVOLVED IN GLUCOSE METABOL'SM IN MOUSE KIDNEY AND LIVER

The effects of *S. mansoni* infection on glucose metabolism in liver and kidney were investigated. The adult worms and their eggs are found mainly in the liver, and both liver and kidney are capable of gluconeogenisis (Southerland, 1990). The main end product of glycolysis in the adult worm is lactate (Bueding, 1950), and lactate, which can freely permeate the cell membrane is a precursor of gluconeogenesis. The lactate produced by the worms could have led to enhanced gluconeogenesis in these two organs, and lead to the mild hyperglycaemia observed in section 3.1.0. If *S.mansoni* infection induces gluconeogenesis in mice it is expected that glycolysis would be inhibited as the two pathways are "reciprocally regulated."

Key glycolytic enzymes that were selected for investigation were hexokinase, phosphofructokinase and pyruvate kinase. Pyruvate carboxylase was chosen to represent enzymes of

Table 8.   Effects of *S. mansoni* infection on the specific activities of key liver glycolytic enzymes, pyruvate carboxylase and some Kreb's cycle enzymes (Expressed in nmol/min/mg protein except for pyruvate dehydrogenase which is expressed in nmolCO$_2$/30 min/mg protein

ENZYME	CONTROLS Mean ± S.D. (n)		INFECTED Mean ± S.D.	(n)
Hexokinase	8 ± 3	(6)	15 ± 5**	(8)
Phosphofructokinas	8 ± 3	(6)	10 ± 3	(6)
Pyruvute kinase	15 ± 24	(6)	330 ± 157**	(6)
Pyruvate dehydrogenase	2 ± 1	(6)	1 ± 0.5	(6)
Alpha-ketoglutarate dehydrogenase	5 ± 1	(4)	5 ± 1	(4)
Succinate dehydrogenase	28 ± 12	(6)	28 ± 11	(6)
Pyruvate carboxylase	41 ± 9	(6)	61 ± 17**	(6)

Values show mean ± standard deviation for the number of animals shown in parenthesis.Significance of the differences in the means was tested by the unpaired student's t-test.

[** 0.001<P<0.05]

phosphofructokinase and pyruvate kinase in the kidneys were not significantly different in the S. mansoni infected and control mice. These results show that the key regulatory enzymes of the glycolytic pathway are not affected by S. mansoni infection in the kidneys.

Table 8 also shows that pyruvate carboxylase activity was significantly higher in liver of infected mice than in normal control mice. The increase was 48.8%. Other workers, (Tielens et al, 1990) have found that pyruvate carboxylase activity in adult S. mansoni is much less than that in rat liver. This suggests that the observed elevation in liver pyruvate carboxylase could have been due to increased activity of the host enzyme. This may suggest that gluconeogenesis, probably from lactate generated by the adult worms, may be enhanced in this infection and may have contributed to the mild hyperglycaemia observed in mice with this infection. Pyruvate carboxylase activity was not significantly affected by the infection in the kidneys (Table 9). These results suggest that there was no effect on gluconeogenesis in the kidney of S. mansoni infected mice.

The specific activities of the Kreb's cycle enzymes; pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were not significantly altered by the infection in the liver (Table 8). The results must be interpreted with care due to presence of the adult worms and eggs in the liver. The eggs have an active Kreb's cycle (Sterjernholm and Warren, 1974; Coles, Asch, 1975) but the activities of the different

Table 9.   **Effects of** *S. mansoni* **infection in mice on the specific
activities of key kidney glycolytic enzymes, pyruvate
carboxylase and some Kreb's cycle enzymes (Expressed
in nmol/min/mg except for pyruvate dehydrogenase
which is expressed in nmolCO$_2$/30 min/mg protein)**

ENZYME	CONTROL Mean ± S.D. (n)		INFECTED Mean ± S.D. (n)	
Hexokinase	12 ± 3	(6)	11 ± 1	(8)
Phosphofructokinase	21 ± 9	(6)	19 ± 2	(6)
Pyruvate kinase	219 ± 72	(6)	215 ± 11	(6)
Pyruvate dehydrogenase	3 ± 1	(6)	3 ± 1	(6)
Alpha-ketoglutarate dehydrogenase	5 ± 1	(4)	5 ± 1	(4)
Succinate dehydrogenase	43 ± 16	(6)	52 ± 15	(6)
Pyruvate carboxylase	54 ± 7	(6)	49 ± 7	(6)

Values are mean ± standard deviation for the number of animals
shown in parenthesis. Significance of the differences in the means
tested by the unpaired student's t-test.

enzymes have not been determined. In the the adult *S. mansoni*, the key enzymes of the Kreb's cycle are present only in small amounts (Smith and Brown, 1977). Given the presence in small amounts of the Kreb's cycle enzymes in both adult worms and eggs of *S. mansoni* it can be proposed with caution, that the presence of the worms and eggs in the homogenates did not alter the activities of the assayed Kreb's cycle enzymes in the liver. Therefore the finding that these enzymes were not signigicantly affected by *S. mansoni* infection is true for the host enzymes. This would imply that *S. mansoni* infection had no effect on the specific activity of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase in the liver. Table 9 shows that the specific activities of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase in the kidneys were not significantly different in the infected and control mice. These results show that these Kreb's cycle enzymes are not affected by *S. mansoni* infection in the kidneys.

3.5.2.   EFFECT OF S. MANSONI INFECTION ON SOME MOUSE
              ENZYMES INVOLVED IN GLUCOSE METABOLISM IN HEART
              AND SKELETAL MUSCLE.

It was hypothesized that *S. mansoni* infection may cause increased glycolytic and Kreb's cycle flux in the heart because the infection is associated with portal hyperternsion (Warren and Dewitt, 1958; Dewitt and Warren, 1959; Warren, 1966), which could result to increased heart muscle contractions. Heart muscle

requires a relatively high amount of energy in the form of ATP's which are generated mainly through the electron transport chain, with anaerobic glycolysis playing a minimal role (Southeland, 1990). Skeletal muscles also require a large amount of ATP, but unlike heart muscle, can obtain a substantial amount of ATP through anaerobic glycolysis. (Southeland, 1990).

The activities of the glycolytic enzymes; hexokinase, phosphofructokinase and pyruvate kinase, and those of pyruvate carboxylase, and the Kreb's cycle enzymes; pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were investigated in heart and skeletal muscle of infected and control mice.

Table 10 shows that *S. mansoni* infection resulted to a 37.5% elevation of hexokinase activity in heart muscle above the controls. However, the activities of phophofructokinase and pyruvate kinase were not significantly affected by the infection in the heart. It was suggested that the increase in glucose 6-phosphate through the increase in hexokinase activity could be catabolized adequately through the glycolytic pathway without increased activities of other glycolytic enzymes.

The mean specific activities of hexokinase, phosphofructokinase and pyruvate kinase were not significantly different in skeletal muscle of infected and control mice (Table 11), showing that glycolysis was not affected by the infection in this organ.

The specific activities of pyruvate carboxylase were not

Table 10.　Effects of *S. mansoni* infection on the specific
activities of key mouse heart glycolytic enzymes,
pyruvate carboxylase and some Kreb's cycle enzymes
(Expressed in nmol/min/mg protein except for pyruvate
dehydrogenase which was expressed in nmolCO₂/30
min/mg protein).

ENZYME	CONTROL Mean ± S.D	(n)	INFECTED Mean ± S.D.(n)
Hexokinase	16 ± 3	(6)	22 ± 1**(8)
Phosphofructokinase	22 ± 5	(6)	17 ± 3 (6)
Pyruvate kinase	169 ± 26	(6)	199 ± 81 (6)
Pyruvate dehydrogenase	2 ± 1	(6)	3 ± 3 (6)
Alpha-ketoglutarate dehydrogenase	7 ± 1	(4)	7 ± 3 (4)
Succinate dehydrogenase	20 ± 12	(6)	22 ± 13 (6)
Pyruvate carboxylase	30 ±ˋ 7	(6)	37 ± 12 (6)

Values are mean ± standard deviation for the number of animals
shown in parenthesis. Significance of the difference s in the means
was tested by the unpaired student's t-test.

[** 0.001<P<0.05].

Table 11.   Effects of *S. mansoni* infection on the specific activities of key mouse skeletal muscle glycolytic enzymes, pyruvate carboxylase and some Kreb's cycle enzymes (Expressed in nmol/min/mg protein except for pyruvate dehydrogenase which is expressed in nmolCO$_2$/30 min/mg protein)

ENZYME	CONTROL Mean ± S.D. (n)	INFECTED Mean ± S.D. (n)
Hexokinase	8 ± 3 (6)	10 ± 4 (8)
Phosphofructokinase	27 ± 16 (6)	32 ± 10 (6)
Pyruvate kinase	590 ± 174 (6)	517 ± 224 (6)
Pyruvate dehydrogenase	5 ± 2 (6)	5 ± 2 (6)
Alpha-ketoglutarate dehydrogenase	2 ± 0.4 (6)	2 ± 0.4 (6)
Succinate dehydrogenase	3 ± 3' (6)	2 ± 3 (6)
Pyruvate carboxylase	95 ± 36 (6)	121 ± 22 (6)

Values are mean ± standard deviation for the number of animals shown in parenthesis. Significance of the differences in the means was tested by the unpaired student's t-test.

significantly affected in heart and skeletal muscle of infected mice (Table 10 and 11). Since both heart and skeletal muscle do not carry out gluconeogenesis, the observed activity of pyruvate carboxylase could be responsible for the formation of oxaloacetate. The oxaloacetate may be used in various functions including transamination and formation of malate in the mitochondria.

Table 10 and 11 also show that the specific activities of the Kreb's cycle enzymes; pyruvate dehydrogenase, alpha-ketoglutarate dehydroganse and succinate dehydrogenase were not significantly affected by *S. mansoni* infection in heart and skeletal muscle. These results suggest that the observed hyperglycaemia was probably not due to decreased catabolism of glucose in the heart and skeletal muscle.

3.5.3. EFFECTS OF S. MANSONI INFECTION ON SOME ENZYMES INVOLVED IN GLUCOSE METABOLISM IN MOUSE LUNGS

The portal hypertension associated with *S. mansoni* infection (Warren and Dewitt, 1958; Dewitt and Warren, 1959; Warren 1966) may have led to hyperventilation and hence greater workload on the lungs. The effects of the infection on glucose metabolism in the lungs were therefore investigated. The glycolytic enzymes; Hexokinase phosphofructokinase and pyruvate kinase; the Kreb's cycle enzymes pyruvate dehyrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase, and pyruvate

Table 12. Effect of *S. mansoni* infection in mice on the specific activities of key lung glycolytic enzymes, pyruvate carboxylase and some Kreb's cycle enzymes (Expressed in nmol/min/mg protein except for pyruvate dehydrogenase which is expressed in nmolCO_2/30 min/mg protein.

ENZYME	CONTROL Mean ± S.D.	(n)	INFECTED Mean ± S.D.	(n)
Hexokinase	15 ± 6	(6)	20 ± 5	(8)
Phophofructokinase	10 ± 4	(6)	10 ± 2	(6)
Pyruvate kinase	235 ± 69	(6)	187 ± 61	(6)
Pyruvate dehydrogenase	3 ± 1	(6)	3 ± 1	(6)
Alpha-ketoglutarate dehydrogenase	2 ± 0.3	(4)	2 ± 0.2	(4)
Succinate dehydrogenase	N.D.		N.D.	
Pyruvate carboxylase	22 ± 8	(6)	27 ± 5	(6)

N.D. = Not detected.

Each value is mean ± standard deviation for the number of animals given in parenthesis. Significance of the differences in the means was tested by the unpaired student's t-test.

carboxylase were selected as representative and their specific activities compared in crude lung homogenates of *S. mansoni* infected and control mice.

Table 12 shows that the specific activities of hexokinase phosphofructokinase and pyruvate kinase in the infected animals were not significantly different from those of control animals. These results suggests that glycolysis is not affected in the lungs by *S. mansoni* infection. It appears, therefore, that the portal hypertension associated with *S. mansoni* infection does not affect glycolysis in the lungs, and the mild hyperglycaemia noted in this infection is not due to effects of the infection on glycolysis in the lungs.

From table 12 it is apparent that the activity of pyruvate carboxylase was not significantly different from that of the control mice in the lungs. Since the lungs do not carry out gluconeogenesis, pyruvate carboxylase activity in the lungs is responsible for formation of oxaloacetate which can be channeled to the Kreb's cycle or undergo transamination to form aspartate. These results, therefore, show that these functions of pyruvate carboxylase are not affected by *S. mansoni* infection in the lungs.

The specific activities of the Kreb's cycle enzymes; pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and succinate dehydrogenase were not significantly affected by the infection in the lungs (Table 12). These results show that the mild hyperglycaemia described in section 3.1.0 is not related to an effect on the activities of these enzymes in the lungs of infected

mice.

3.5.4 EFFECTS OF *S. MANSONI* INFECTION ON SOME ENZYMES INVOVLED IN GLUCOSE METABOLISM IN MOUSE BRAIN

In trying to explain the mild hyperglycaemia observed in *S. mansoni* infection as described in section 3.1.0; the systemic effect of *S. mansoni* infection on selected glucose metabolism enzymes in the brain was investigated. The brain was chosen for investigation because of its characteristic dependence on glucose utilization for energy. Glycolysis in the brain is necessarily coupled to the electron transport chain (Southerland, 1990). To determine whether there is decrease in glucose utilization, the specific activities of hexokinase, phosphofructokinase, pyruvate kinase, pyruvate carboxylase, pyruvate dehydrogenase, alpha-ketoglytarate dehydrogenase and succinate dehydrogenase were compared in *S. mansoni* infected and control mice.

Table 13 shows that the specific activities of hexokinase, phosphofructokinase and pyruvate kinase were not significantly affected by *S. mansoni* infection. The results suggest that *S. mansoni* infection does not result to decreased glycolytic flux in the brain.

The specific activities of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were not significantly affected by *S. mansoni* infection in the brain (Table 13). This shows that *S. mansoni* infection does not affect

the Kreb's cycle at the level of these enzymes in the brain.

From table 13 it is apparent that the specific activity of pyruvate carboxylase is not significantly altered by *S. mansoni* infection in the brain. The observed activity of pyruvate carboxylase could be responsible for the formation of oxaloacetate, which could be used for transamination to aspartate or formation of malate in the mitochondria.

These results show that the mild hyperglycaemia observed in section 3.1.0. is not related to effects of *S. mansoni* infection on brain glycolysis or the Kreb's cycle enzymes pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase or succinate dehydrogenase. They also suggest that the mild hyperglycaemia is not related to effects of the infection on pyruvate carboxylase.

3.5.5.   **EFFECTS OF S. MANSONI INFECTION ON SOME ENZYMES INVOLVED IN GLUCOSE METABOLISM IN MOUSE SPLEEN AND TESTES.**

The spleen and testes of *S. mansoni* infected mice were investigated for decrease in glucose utilization. The spleen is part of the reticulendothelial system and is actively biosynthetic. It acts as a reservoir of venous blood. Spleenic tissue metabolizes glucose mainly via the glycolytic pathway, Kreb's cycle and electron transport chain (Southerland, 1990). Red blood cells, on the other hand, have no mitochondria and obtain energy through anaerobic glycolysis (Southerland, 1990). The

Table 13.   Effects of *S. mansoni* infection on key mouse brain glycolytic enzymes, pyruvate carboxylase and some Kreb's cycle enzymes (Expressed in nmol/min/mg protein except for pyruvate dehydrogenase which is expressed in nmolCO$_2$/30 min/mg protein)

ENZYME	CONTROL Mean ± S.D. (n)	INFECTED Mean ± S.D. (n)
Hexokinase	23 ± 2 (6)	26 ± 7 (8)
Phosphofructokinase	8 ± 4 (6)	9 ± 3 (6)
Pyruvate kinase	255 ± 73 (6)	190 ± 48 (6)
Pyruvate dehydrogenase	3 ± 1 (6)	3 ± 1 (6)
Alpha-ketoglutarate dehydrogenase	2 ± 0.3 (4)	2 ± 1 (4)
Succinate dehydrogenase	20 ± 9 (6)	18 ± 7 (6)
Pyruvate carboxylase	18 ± 4 (6)	24 ± 8 (6)

Each value is mean ± standard deviation for the number of animals given in parenthesis. Significance of the differences in the means was tested by the unpaired student's t-test.

testes are also active in biosynthesis especially spermatogenesis (Martin *et al*, 1985).

The effects of *S. mansoni* infection on selected spleen and testis glucose metabolism enzymes was therefore investigated in an effort to explain the mild hyperglycaemia noted during *S. mansoni* infection (Section 3.1.0). The enzymes, hexokinase phophofructokinase, pyruvate kinase, pyruvate dehydrogenase, pyruvate carboxylase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were selected and their specific activities determined in *S. mansoni* infected and control mice.

Table 14 and 15 show that the specific activities of the glycolytic enzymes; hexokinase, phophofructokinase and pyruvate kinase were not altered by *S. mansoni* infection in testis and spleen. Table 14 and 15 also show that the specific activities of the Kreb's cycle enzymes pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were not significantly affected by *S. mansoni* infection in spleen and testis. The abscence of detectable amounts of succinate dehydrogenase in the spleen may be due to the presence of large amounts of red blood cells which have no mitochondria. This could have diluted the succinate dehydrogenase present in the spleen. It was therefore concluded that *S. mansoni* infection has no effect on spleenic and testicular glycolysis and Kreb's cycle, and that the mild hyperglycaemia observed in this infection is not due to decreased glycolysis or Kreb's cycle flux in these two organs.

S. mansoni infection had no significant effect on spleen or

Table 14.   Effect of *S. mansoni* infection on the specific

activities of key mouse spleen glycolytic enzymes,

pyruvate carboxylase and some Kreb's cycle enzymes

(Expressed in nmol/min/mg protein except for pyruvate

dehydrogenase which is in nmolCO_2/30 min/mg protein).

ENZYME	CONTROL Mean ± S.D. (n)		INFECTED Mean ± S.D. (n)	
Hexokinase	18 ± 4	(6)	19 ± 4	(8)
Phosphofructokinase	11 ± 5	(6)	10 ± 5	(6)
Pyruvate kinase	192 ± 52	(6)	166 ± 66	(6)
Pyruvate dehydrogenase	2 ± 1	(6)	2 ± 1	(6)
Alpha-ketoglutarate dehydrogenase	1 ± 0.4	(4)	1 ± 0.3	(4)
Succinate dehydrogenase	N.D.		N.D.	
Pyruvate carboxylase	45 ± 28	(6)	55 ± 12	(6)

N.D. = Not detected.

Values are mean ± standard deviation for the number of animals

shown in parenthesis. Significance of the differences in the means

was tested by the unpaired student's t-test.

Table 15. Effects of *S. mansoni* infection on the specific activities of key mouse testes glycolytic enzymes, pyruvate carboxylase and some Kreb's cycle enzymes. (Expressed in nmol/min/mg protein except for pyruvate dehydrogenase which is in nmolCO_2/30 min/mg protein

ENZYMES	CONTROL Mean ± S.D. (n)		INFECTED Mean ± S.D. (n)	
Hexokinase	24 ± 8	(6)	28 ± 5	(8)
Phosphofructokinase	11 ± 6	(6)	10 ± 3	(6)
Pyruvate kinase	162 ± 52	(6)	178 ± 54	(6)
Pyruvate dehydrogenase	3 ± 1	(6)	3 ± 1	(6)
Alpha-ketoglutarate dehydrogenase	2 ± 1	(4)	2 ± 1	(4)
Succinate dehydrogenase	1 ± 1	(6)	1 ± 4	(6)
Pyruvate carboxylase	27 ± 4	(6)	24 ± 9	(6)

Values show mean ± standard deviation for the number of animals shown in parenthesis. Significance of the differences in the means was tested by the unpaired student's t-test.

testicular pyruvate carboxylase activity (Tables 14 and 15). This shows that formation of malate and oxaloacetate from pyruvate in the mitochondria is not affected by *S. mansoni* infection in the testis and spleen. The mild hyperglycaemia associated with this infection is, therefore, not due to an effect of the infection on this enzyme.

3.6.0   INFLUENCE OF *S. MANSONI* INFECTION IN MICE ON THE END PRODUCT OF GLYCOLYSIS AND THE ACTIVITY OF LACTATE DEHYDROGENASE IN VARIOUS TISSUES.

In order to test if the mild hyperglycaemia described in section 3.1.0. is related to an influence of *S. mansoni* infection on the end products of glycolysis, the serum concentrations of pyruvate and lactate were compared in *S. mansoni* infected and control mice. The specific activity of lactate dehydrogenase in the brain, heart, lungs, liver, spleen, kidney, testis and skeletal muscles were also compared in the infected and control mice.

Table 16 shows that the specific activity of lactate dehydrogenase in the lungs of infected mice was significantly decreasd. There was a 32.2% decrease in mean specific activity of lactate dehydrogenase in the *S. mansoni* infected mice. This decrease in lactate dehydrogenase activity in the lungs of infected mice was probably due to decreased blood flow to the lungs due to portal hypertension (Warren and Dewitt, 1958; Dewitt and Warren, 1959; Warren, 1966). Since the assays were performed on crude lung homogenates, containing the blood pooled in the

Table 16.   Effect of *S. mansoni* infection in mice on the specific
            activity of lactate dehydrogenase in various tissues
            (nmol/min/mg protein).

TISSUE	CONTROL Mean ± S.D.		INFECTED Mean ± S.D.	
Brain	179	± 67	220	± 93
Heart	198	± 59	252	± 65
Lungs	225	± 63	173	± 33**
Liver	360	± 196	336	± 123
Spleen	284	± 103	265	± 71
Kidneys	253	± 80	306	± 126
Testes	195	± 54	239	± 117
Skeletal muscle	1045	± 461	879	± 234

Values are mean ± standard deviation for 6 animals in each group.
Significance of the differences in the means was tested by the
unpaired student's t-test [**0.001<P<0.05].

Table 17.   Effects of *S. mansoni* infection in mice on serum

concentrations of pyruvate and lactate

ANIMALS	METABOLITES (mmol/l)	
	PYRUVATE Mean ± S.D.	LACTATE Mean ± S.D.
CONTROLS	0.20 ± 0.12	5.08 ± 1.66
INFECTED	0.15 ± 0.08	4.26 ± 1.00

Values show mean ± standard deviation for 6 animals in each group.
Significance of the differences in the means was tested by the
student's t-test.

lungs, with excess red blood cells which are known to rely on anaerobic glycolysis (Southerland, 1990), it is possible that reduced blood flow to the lungs of infected mice, resulted in smaller contribution of lactate dehydrogenase activity from red blood cells in the infected mice. Lactate dehydrogenase activity was not significantly affected by *S. mansoni* infection in the other tissues assayed. (Table 16).

Table 17 shows that serum levels of pyruvate and lactate were not significantly affected by S. mansoni infection. The observation that the serum concentrations of lactate and pyruvate were not significantly affected by the infection, suggests that the reduced lactate dehydrogenase activity in lungs of infected mice does not affect overall host end products of glycolysis. Indeed, there was a tendency for mean serum pyruvate levels to be lower in the infected mice compared to the controls (0.15 mmol/l and 0.20 mmol/l respectively, table 17), although the difference was not statistically significant.

From these results it was concluded that the mild hyperglycaemia noted in mice with *S. mansoni* infection is not related to an influence of the infection on host end products of glycolysis.

CHAPTER FOUR

DISCUSSION

The results in the present study show that *S. mansoni* infection in mice caused a mild hyperglycaemia (table 3). Saleh *et al* (1976) found a 54% elevation in fasting blood glucose in *S. mansoni* infected mice. Erfan and Camb (1933) demonstrated glycosuria in *S. mansoni* patients, which disappeared after treatment.

The elevated serum glucose concentration may arise from improper secretion of insulin by the pancreas due to parasitic infection of the pancreas. Saleh *et al* (1967) demonstrated a low incidence (4.2%) of pancreatic *S. mansoni* infection in human patients, which mainly affected the periductal tissues and rarely the parenchyma. In this study it was observed that *S. mansoni* infection did not cause any significant change in levels of serum pancreatic lipase, (Table 4) suggesting little or no pancreatic damage by the parasites occurred. Impaired production of insulin by the pancreas therefore did not lead to the observed mild hyperglycaemia in this infection.

The finding, (table 6), that zinc levels in serum as dertemined by X-ray fluorescence spectroscopy, and the activity of hepatic alchohol dehydrogenase and red blood cell catalase were unaffected by *S.mansoni* suggests the infection does not lead to zinc deficiency. This contradicts results by Mikhail *et al* (1982) and Prasad *et al* (1963) who demonsrated decreased serum zinc levels in patients infected with S. mansoni. The results also

contradict those of Soliman *et al* (1975) who found increased serum zinc levels in *S.mansoni* infected patients.

Zinc level analysis by the above authors was perfomed using the Atomic absorption spectrophotometer which has a better range of sensitivity than the X-ray fluoresce spectroscopy.

It was suggested from the decrease in the level of serum glucose 30 minutes after administration of an exogenous dose of insulin,(Table 5), that the insulin receptors were not impaired by S.mansoni infection. From these results it was speculated that the 'glucose tolerance factor', a chromium-nicotinic acid complex that enhances insulin action in vitro, (Bender,1982), is not affected in this infection. This is further supported by the finding that serum concentration of chromium was not significantly different in the infected and control mice (Table 6).

The finding of greater response to exogenous insulin also raises the possibility that there may be secretion of biologically inactive insulin during *S. mansoni* infection, such that insulin sensitive organs, respond by synthesizing more insulin receptors, so that when a dose of biologically active insulin is applied, more glucose enters the cells from the serum. This posssibility is supported by Ghanem *et al* (1973) who demonstrated hyperinsulinaemia in *S.mansoni* infected patients using radioimmunoassay. The high amount of immunoreactive insulin may have been biologically inactive.

The increased activities of liver pyruvate kinase and hexokinase shown in table 8, suggests that the mild

hyperglycaemia in mice with *S.mansoni* infection could have resulted from reduced flux through the glycolytic pathway as a result of inhibition of some key regulatoy enzymes of this pathway. Since liver tissue used for the enzyme assays contained the parasites and eggs, and the adult *S. mansoni* have been shown to contain between 10-50 times higher levels of hexokinase, phosphofructokinase and pyruvate kinase (Bergmeyer, 1965; Bueding and Saz,1968; Brazier and Jaffe,1973 and Doong *et al*, 1987) than mammalian liver, it is proposed that the higher activity of hexokinase and pyruvate kinase observed in liver of *S. mansoni* infected mice was due to the presence of the adult worms. It is also possible that host phosphofructokinase activity is depressed in the infected mice, since the high activity of this enzyme in the adult worm could have led to an increase in phosphofructokinase activity as well. Such a depression of phosphofructokinase activity, which is the key regulatory enzyme of the glycolytic pathway, (Southerland, 1990), would lead to decreased metabolic flux through the glycolytic pathway.

The elevation in hexokinase activity in the heart of infected mice is difficult to explain, but it appears unlikely to have contributed to the mild hyperglycaemia since the other regulatory enzymes of the glycolytic pathway in this organ were not significantly affected by the infection. It would appear that the increased glucose-6-phosphate generated by the higher activity of hexokinase is adequately catabolized by other glycolytic enzymes in this organ.

The activities of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were not significantly affected by the infection in the brain, heart, lungs, liver, spleen, testis, kidneys and skeletal muscle. It was therefore concluded that it is unlikely that metabolic flux through the Kreb's cycle is affected by *S. mansoni* infection to lead to the mild hyperglycaemia observed (Tables 8-15).

The finding that the serum concentrations of pyruvate and lactate were not significantly affected by *S. mansoni* infection (Table 17) is contrary to what was observed by Shaheen *et al* (1989), who reported decreased blood lactate and increased pyruvate concentrations. They also contradict those of El-Hawary *et al* (1973) who observed an elevation in blood pyruvate concentration in *S. mansoni* patients.

The decrease in lactate dehydrogenase activity in the lungs (Table 16) was probably due to the portal hypertension associated with this infection (Warren and Dewitt, 1958; Dewitt and Warren, 1959; Warren, 1966). It is speculated that portal hypertension could cause hyperventillation, which may have favoured aerobic glycolysis in the lungs generating more pyruvate and less lactate.

The specific activity of pyruvate carboxylase was found to be significantly elevated in liver of *S. mansoni* infected mice (Table 8). Despite the fact that the assays were performed on crude liver homogenates containing also the adult worms and eggs, it is likely that the observed elevation in pyruvate carboxylase activity was due to host enzyme activity, since it has been shown

that the specific activity of pyruvate carboxylase is about 10 times more in rat liver as compared to adult *S. mansoni* homogenates (Tielens *et al*, 1990).

Our results, however, show that pyruvate carboxylase activity was only elevated in liver, which is the most important gluconeogenic organ (Southerland, 1990). It is therefore most likely that the products of pyruvate carboxylase are channelled to gluconeogenesis in the liver. This is supported by our earlier finding that phosphofructokinase activity is depressed in liver of *S. mansoni* infected mice. Depression of the latter enzyme could lead to depression of glycolysis and elevation of gluconeogenesis as the two pathways are "reciprocally regulated". The above speculation is supported by the finding that some key Kreb's cycle enzymes are not affected by the infection. This is further supported by Shaheen *et al* (1989), who found that the specific activity of the last gluconeogenic enzyme, glucose 6-phosphatase was higher in liver of *S. mansoni* infected mice than in control animals.

Gluconeogenesis in liver of *S. mansoni* infected mice could be enhanced by the high lactate concentration found in liver of mice with this infection (Saleh *et al*, 1976; Ezz *et al*, 1971). Lactate is a substrate for gluconeogenesis (Southerland, 1990). It is speculated that gluconeogenesis could also be enhanced in *S. mansoni* infection by the high blood ammonia level during this infection (De Witt and Warren, 1959; Daugherty, *et al*, 1954; Warren and Reboucas, 1964 and Senft, 1967). High blood ammonia could

stimulate secretion of glucagon from the alpha-cells of the pancreas, which promotes gluconeogenesis from amino acids (James *et al*, 1979). Thus it appears gluconeogenesis may be enhanced in mice infected with *S. mansoni* and that it may have contributed to the mild hyperglycaemia observed in this infection. However, this assertion has to be confirmed by investigating the activities of phosphoenolpyruvatecarboxykinase,fructose-6-biphophataseand glucose-6-phosphatase which are the key regulatory enzymes of the gluconeogenic pathway (Southerland, 1990).

CONCLUSION

The present results show that *S. mansoni* infection in mice for 7-8 weeks causes a mild elevation in serum glucose. This was probably caused by decreased entry of glucose into the cells, probably due to production of biologically inactive insulin and/or inhibition of glycolysis and stimulation of gluconeogenesis. The results show no influence on respiratory behavior of the host during the infection, which could have led to an elevation in serum glucose as well.

Suggestions for further work

a) Assessement of the effect of *S.mansoni* infection on the other key enzymes of gluconeogenesis, i.e. Phosphoenolpyruvate carboxykinase, fructose-1,6-biphosphatase and glucose-6-phosphatase in liver.

b) Assessement of the effects of *S. mansoni* infection on

enzymes of the pentose phosphate pathway since the increased levels of glucose-6-phosphate arising from increased hexokinase activity may result in enhanced activity of this pathway.

c) Assessement of the biological status of insulin in the infected animals and seek for a possible mechanism leading to its inactivation if any.

REFERENCES

Abdel-Halim Saleh, S.; Higari, A.M.; Ata, A.A. (1967). Pancreatic Bilharziasis and its relation to *Diabetes mellitus.* Journal of the Egyptian Medical Association. *50:* 425-441.

Abdel-Tawab, G.A.; Kholief, T.S.; El-Sewedy, S.M.; Zeitoun, M.M. and Hassanein, E.A. (1969). Studies on tryptophan metabolism in bilharzial hepatic cirrhosis and non-bilharzial hepatic cirrhosis in children. Acta Pediatrica Scandinavia, *58:* 54-58.

Abdel-Rahim, I.M.; Kaiser-Ch; Homeida, M.; Elsheilch, M.; Schimidt, E.; Ehrich, J.H.H. and Doehring-Schwerdfeger, E. (1990). Enzyme activities and protein concentrations in serum of patients with hepatosplenic schistosomiasis. Tropical Medical Parasitology. *4:* 262-264.

Asch, H.L. and Read, C.P. (1975a). Transtegumental absorption of amino acids by *S. mansoni.* Journal of Parasitology. 61: 378-379.

Asch, H.L. and Read, C.P. (1975b). Membrane transport in *S.mansoni:* Transport of amino acids by adult males. Experimental Parasitology, *38:* 123-135.

Asch, H.L. (1975). Effect of selected chemical agents on longevity and infectivity of *S. mansoni* cercariae. Experimental Parasitology, *38:* 208-216.

Atkinson, K.H. and Atkinson, B.G. (1981). Protein synthesis *in vivo*

by *S. mansoni* cercariae. Molecular and Biochemical Parasitology, *4*: 205-216.

Awadallah,H.N; Sherif, A.F; Khalil, A.Z. and Guirgis, F.K.(1975). Enzyme levels in homogenates of liver from mice infected with *Schistosoma mansoni* and from uninfeced mice. International Journal for Parasitology, 5: 27-31.

Baron, E. (1939). Aspiration for removal of Biopsy material from the liver, report of thirty five cases. Archives of International Medicine, *63*: 276-289.

Becker, W. (1977). Zur stoff wechsel-physiologie der Miracidien von *S. mansoni*. Wahrend Aktivierung innerhalb der Eischale. Zeit schrift fur Parasiten kunde, *52*: 69-79.

Bergmeyer, H.U. (1965). Methods of Enzymatic Analysis. Academic Press. 2nd Ed. London/New York.

Bergmeyer, H.U. and Bernt, E. (1974).Methods of Enzymatic Analysis. Academic Press. London/New York.

Bender, D.A. (1982). Biochemistry of tryptophan in Health and disease. Molecular aspects of Medicine, *6*: 101-197.

Brazier, J.B. and Jaffe, J.J. (1973). Two types of pyruvate kinase in schistosomes and filariae. Comparative Biochemistry and Physiology, *44B*: 145-155.

Bruce,J.I; Warren,K.S. and Sadun,E.H.(1963). Observation on the Pathophysiology of Schistosomiasis mansoni in monkeys. Experimental Parasitology, 13: 194-198.

Bruce, J.I.; Ruff, M.D.; Belusko, R.J. and Werher, J.K. (1972). *S. mansoni* and *S. japonicum* Utilization of amino acids.

International Journal for parasitology, *2*: 425-430.

Bueding, E. (1950). Carbohydrate metabolism of *S. mansoni*. Journal of General Physiology, *33*: 475-495.

Bueding, E. and Saz, H.J.(1968). Pyruvate kinase and Phosphoenolpyruvate carboxykinase activities of Ascaris muscle, *Hymenolepsis diminuta* and *Schistosoma mansoni*. Comparative Biochemistry and Physiology. 24: 511-518.

Bout, D.; Dupas, H.; Capron, M.; El-Gezawi, A.; Carlier, Y; Delacourte, A.; and Capron, A. (1978). Purification, immunochemical and biological characterization of malate dehydrogenase of *S. mansoni*. Immunochemistry, *15*: 633-638.

Chappell, L.H. (1974). Methionine uptake by larval and adult *S. mansoni*. International Journal for Parasitology, *4*: 361-369.

Cantarow, A. and Trumper, M. (1962). Clinical Biochemistry. 6th. Ed. W.B. Saunders, Co. Philadelphia, London.

Chappell, L.H. and Walker, E. (1982). *S. mansoni*: Incorporation and metabolism of protein amino acids *in vitro*. Comparative Biochemistry and Physiology, *73B*: 701-707.

Cherry, L. S. and Crandall, L.A.(1969). In Practical Clinical Biochemistry. (By Harold Varley, Indian Ed.1988) 408-410. Publishers and distributors Jain Bhahawan. Bhola Nath. Nager Shandra Delhi- 110032, India.

Clements, L.E. and Basch, P.F. (1989). *S. mansoni*: Insulin Independence. Experimental Parasitology, *68*: 223-229.

Conford, E.M.and Hout, M.E.(1981). Glucose transfer from male to female schistosomes. Science, 213: 1269-1271

Coles, G.C. (1973a).   The metabolism of schistosomes: A review. International Journal of Biochemistry, *4*: 319-337.

Coles G.C.(1973). Enzyme levels in cercariae and adult *Schistosoma mansoni*. International Journal for Parasitology, *3*: 505-510.

Cowper, S.G. (1971).   A synopsis of African Bilharziasis.   London. H.K. Lewis and Co. Ltd.   Hazell Watson and Viney Ltd. Aylesbury, Bucks.   200.

Daugherty, J; Garson, S. and Heyneman, D.(1954). The effect of *Schistosoma mansoni* on Liver function in mice. 1. Amino acid oxidase and ammonia. American Journal of Tropical Medicine and Hygiene, 3: 511-517.

Devlin, T.M.(1976).   Textbook of Biochemistry with Clinical Correlations. 2nd. Ed. John Wiley and sons. New York.

DeWitt, W.B. and Warren, K.S. (1959). Hepatosplenic Schistosomiasis in mice. American Journal of Tropical Medicine and Hygiene, 8: 440-446.

De Oliviera, D.N.G. and Jose Carlos De M. Costa. (1977). *Schistosoma mansoni:* Cholesteryl esters profiles of plasma and liver in experimentally infected mice. Experimental Parasitology, 43:244-247.

Dunn, M.A; Rojkind, M; Warren, K.S; Hait, P.K; Rifas, L. and Seifter, S.(1977). Liver collagen synthesis in murine Shistosomiasis. Journal of Clinical Investigation. 59: 666-674.

Dunn, M.A; Rojkind, M; Hait, P.K. and Warren, K.S. (1978). Conversion of Arginine to Proline in murine schistosomiasis. Gastroenterology, 75: 1010-1015.

Dunn, M.A; Kamel, I.A; Biempica, L; Kholy,A; Hait, P.K Rojkind, M; Warren,K.S. and Mahmoud, A.(1979). Liver collagen synthesis in *Schistosomiasis mansoni*. Gastroenterology, **76:** 978-982.

Doong, Y.C; Wong, L; Bruce, J.I. and Wong S.S. (1987). Enzymatic differenes between Hyanthone resistant and sensitive strains of *Schistosoma mansoni*. Comparative Biochemistry and Physiology. 87B (3): 459-464.

El-Hawary, M.F.S.; Shakir, M.H.; Saif, M. and El-Mahrouky, S.F. (1973). Pyruvate metabolism test in bilharziasis. Journal of the Egyptian Medical Association, *56:* 303-312.

El-Kharbotly, M; Shalaby, E.S; Zaki, S.A. and Abdel-Salam, R.(1965). Serum lipids in bilharzial hepatic cirrhosis. Journal of the Egyptian medial Association, 48: 274 -281.

El-Saadani, A.M.; El-Sawy Habib, M.; El-Gengehy, M.T. and Fayez, M.A. (1968). Albumin turnover in schistosomal liver cirrhosis. The American Journal of Tropical Medicine and Hygiene, 17: 844-850.

Erfan, M. and Camb, H. (1933). Bilharziasis and *Diabetes mellitus*. Journal of Tropical Medicine and Hygiene, 36: 348-349.

Eshete, F. and Bennet, J.L. (1990). *S. mansoni*: Biochemical characteristics of antischistosomal effects of R₀15-5458. Experimental Parasitology, 71: 69-80.

Evans,A.S. and Stirewalt, M.A.(1957) Serological reactions in *schistosoma mansoni* infections.111.Ionographic fractionation of sera of mice with progressive disease. Experimental Parasitology, 6: 8-17

Ezz, E.A.; El-Bassiouni, E.A. and Gilazal, A. (1971). Effect of Bilharzial Infestation on the metabolism of the liver cells. Proceedings of the Afro-Asian Sympsium on chemotherapy of schistosomiasis. 611-612.

Fairley, N.H. (1931). Vesical schistosomiasis complicated by carcinoma. British Medical Journal, ii: 983-985.

Fayez, M.A; El-Sawy Habib,M; El- Saadany,A.M. and El- Gengehy, M.T.(1969). The rate of synthesis of albumin and its distribution in bilharzial hepatosplenomegaly, measured by I³¹ labeled human serum albumin. Journal of The Egyptian Medical Association, 50: 595-607

Foster, L.A.; Chen, G.Z.; Van de Waa, E.A.; Pax, R.A. and Bennett, J.L. (1989). Glutamine vs glucose supported motor activity in *S.mansoni*. Experimental Parasitology, 69(1): 44-53.

Garson, S; Durban, A. and Williams, J.S. (1957). Alterations in serum glutamic- oxaloacetic transaminase (SGO-T) in the course of experimental *Schistosomiasis mansoni* Journal of Parasitology,43: 27.

Ghanem, M.H.; Said, M. and Guirgis, F.K. (1971). Glucose intolerance in hepatic schistosomiasis. Journal of Tropical Medicine and Hygiene, 74: 189-194.

Ghanem, M.H.; Antoniades, H.N.; Habib, M.; Guirgis, F.K.; Zein, M.S.; Nofal, M. and El-Sawy, M. (1973). Immunoreactive insulin in schistosomal hepatic fibrosis. Journal of Tropical Medicine and Hygiene, 76: 16-18.

Ghanem, M.H.; Guirgis, F.K.; and El-Sawy, M. (1970a). Serum and

Ascitic fluid magnessium in Bilharzial (Schistosomal) hepatic fibrosis. Journal of Tropical Medicine and Hygiene: 73: 237-239.

Ghaneim, M.H;Guirgis, F.K. and EL-Zawy, M.(1970b). Assessment of liver function in Bilharzial hepatic fibrosis. Alexandria Medical Journal. 16:117-136.

Ghanem, M.H; Moufid, M.D; Fahmy, M.D; Aziz, T; Abdel-Malek, M.D; Fayek, K; Guirgis, M.D. and El-Lozy, M. (1970). Comparative study of cholestrol phpspholipids and their ratio in bilharzial hepatic fibrosis, Coronary heart disease and hypertensive cases. Alexandria Medical Journal. 16(1): 1-8.

Gibson, G.E; Ksiezak-Reding, H; Sheu, K.F.R; Mykyty, V. and Blass, J.P.(1984). Correlations of enzymatic, metabolic and behavioral deficits in thiamine deficiency and its reversal. Neurochemical Research, 9: 803-814.

Gillet, M.P.T. and Carvalho, V.C. (1978). *Schistosoma mansoni*: A comparative study of plasma and erythrocyte lipid alterations in the experimentally infected mouse and in selected human patients. Experimental Parasitology, 44:173-180.

Glinsmann, W.H. and Mertz, W. (1966). Effect of trivalent chromium on glucose tolerance. Metabolism, 15: 510-520.

Glouset, J.A; Parker, F; Tjaden, M. and Williams, R.H.(1962). The esterification *in vitro* of free cholesterol in human and rat plasma. Biochimia et Biophsica Acta, 58: 398-406.

Harris, A.R.C.; Russel, R.J. and Charter, A.D. (1984). A review of schistosomiasis in immigrants in Western Australia, demonstrating the unusual longevity of *S. mansoni.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 78: 385-388.

Hassaballa, A.; Kinawi, M.; Fahny, T. and Addel-Razak (1964). The carbohydrate metabolism in bilharzial hepatic fibrosis. Journal of the Egyptian Medical Association, 47: 353-359.

Higazi,A.M; Fahmy, A.R. and Kaddah, S.(1960). Serum glutamic-oxaloacetic transaminases in hepatic bilherziasis. Journal of The Egyptian Medical Association, 43: 923-928.

Highton, R.B.(1974). Schistosomiasis in Health and Disease in Kenya. East African Literature Bureau. NAIROBI.

Hopkins, L.L.; Ransome-Kuti, O. and Najay, A.S. (1968). Improvement of impaired carbohydrate metabolism by chromium (III) in malnourished infants. American Journal of Clinical Nutrition, 21: 203-211.

International Pharmacopoeia (1967). Specifications for the quality control of pharmaceutical preparations. WHO 2nd Edition. Geneva, 796-799.

Isseroff, H.; Bonta, C.Y. and Levy, M.G. (1972). Monosaccharide absorption by *S. mansoni.* Kinetic characteristics. Comparative Biochemistry and Physiology, 43B: 849-858.

Isseroff, H.; Ertel, J.C. and Levy, M.G. (1976). Absorption of amino acids by *S. mansoni.* Comparative Biochemistry and Physiology, 54B: 125-133.

Ishii, K; Morita, S; Tawara, H; Chu, T.C; Kaji,H. and Shiokawa,T.

    (1975). Quantitative trace element analysis by proton-

    induced X-rays. Nuclear Instrumentation Methods, *128* 75.

James, J.H; Ziparo, V; Jeppson, B. and Fischer, J.E. (1979).

    Hyperammonaemia, plasma amino acid imbalance, and blood-

    brain amino acid transport: a unified theory of portal-

    systemic encphalopathy. Lancet, 772-775.

Jeebjeebhoy, K.N.; Chu, R.C.; Marliss, E.B.; Greenberg, G.R. and Bruce

    Robertson, A. (1977). Chromium deficiency, glucose

    intolerance and neuropathy reversed by chromium

    supplementation in a patient receiving long-term total

    parentral nutrition. American Journal of Clinical Nutrition.

    30: 531-538.

Johansson,S.A.E. and Johansson, T.B.(1976). Analytical application

    of particle induced X-ray emission. Nuclear Insrumentation

    Methods, *137.* 473

Jordan, P. and Webbe, G. (1982). Schistosomiasis; Epidemiology,

    treatment and control. William Heineman Medical Books Ltd.

    London 169.

Khattab, M; Zaki, A.H; El-Kammah,B.M. and Ayadi, A.(1967). Serum and

    ascitic fluid enzymatic studies in Bilharzial hepatic

    fibrosis. Journal of the egyptian Medical Association, 49:

    381-390.

Kinoti, G.K. (1971a). The epidemiology of *S. haematobium* infection on

    the Kano Plains of Kenya. Transactions of the Royal Society

    of Tropical Medicine and Hygiene, 65: 637-645.

Kinoti, G.K. (1971b). The epidemiology of *S. mansoni* infection on the
Kano Plains of Kenya. Transactions of the Royal Society of
medicine and Hygiene, 65: 646-649.

Kinyua, A.M.(1982). Multi-element analysis of solid and liquid
samples by X-ray Fluorescence Analysis (XRFA). MSc thesis,
University of Nairobi.

Kinyua, A.M.(1987). Trace Metal Analysis of Tissues from rats
treated with anti-convulsants. MSc thesis, University of
Florida, U.S.A.

Lawson, R.A. and Wilson, P.E. (1980). Metabolic changes associated
with the migration of the schistosomulum of *S. mansoni* in the
mammalian host. Parasitology, 81: 325-336.

Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. (1951).
Protein measurement with folin-phenol reagent. Journal of
Biological Chemistry, 193: 265-275.

Market, S.F.; Lo Verde, P.T. and Britt, E.M. (1978). Prolonged latent
schistosomiasis. Journal of the American Medical
Association, 240: 1746-1747.

Martin,D.W; Mayes,P.A; Rodwell,V.W. and Granner,D.K. (1985). Harper's
Review of Biochemistry. 20th Ed. Lange Medical
Publications.Los Altos, California. U.S.A.

McDaniel, H.G.; Reddy, W.J. and Boshell, B.R. (1972). The mechanism 6
inhibition of phosphoenol pyruvate carboxylase by quinolinic
acid. Biochemie Biophysique Acta, 276: 543-550.

Mertz, W. (1974). Biological function of nicotinic acid-chromium
complexes. Federal Proccedings. 33: 659.

Mertz, W. and Schwartz, K. (1959). Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diet. American Journal of Physiology, **196:** 318-332.

Meyer, F; Meyer, H. and Bueding, E. (1970). Lipid metabolism in the parasite and free-living flatworms, *Schistosoma mansoni* and *Dugesia Dorotocephala.* Biochimica et Biophysica Acta, **210:** 257-266.

Mikhail, M.M. and Mansour, M.M. (1982). Complications of human schistosomiasis and their effect on the levels of plasma copper, zinc and serum vitamin A. Human Nutrition: Clinical Nutrition, **36C:** 289-296.

Miller, P. and Wilson, R.A. (1980). Migration of the schistosomula of *S. mansoni* from the lungs to the hepatic portal system. Parasitology, **80:** 267-288.

Mousa, A.H.; Abdel-Wahab, A.F. and Mousa, W. (1967). Tryptophan metabolism in hepatosplenic bilharziasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, **61:** 640-647.

Musa, A.H.; Ata, A.A. and Ayad-Garem, A. (1969). A critic digest review in Bilharziasis in Africa. Chemical Industries Development. Dar-el-Maaref, Cairo, 14.

Nagai, Y.; Gazinelli, G. De Moraes, G.W.G. and Pellegrino, J. (1977). Protein synthesis during cercariae-schistosomulum transformation and early development of the *S. mansoni* larvae. Comparative Biochemistry and Physiology, **57B:** 27-30.

Njagi, E.N.M. (1990). Tryptophan metabolism in Schistosomiasis.
    Phd. Thesis. University College London.

Njagi, E.N.M. and Bender,D.A.(1990). *S. mansoni*: Effects on
    Tryptophan metabolism in mice. Experimental Parasitology.
    70:43-54.

Newsholme, E.A. and Leech, A.R.(1983) Biochemistry for Medical
    Sciences. John Wiley Sons Ltd. Chichester. England.

Pennington, R.J.(1961). Biochemistry of Dystrophic muscle.
    Mitochondrial succinate-tetrazolium reductase and
    adenosine triphosphatase. Biochemical Journal. 80: 649-654.

Podesta, R.B. and Dean, L.L. (1982a). A method for determining non
    electrolyte uptake by adult *S. mansoni* using compartmental
    analysis of hexose fluxes. Molecular and Biochemical
    parasitology, 5: 345-352.

Podesta, R.B. and Dean, L.L. (1982b). Uptake of galactose by
    epithelial syncytium of *S. mansoni*. Molecular and Biochemical
    Parasitology, 5: 353-360.

Prasad, A.S.; Miale, A.; Farid, Z.; Schulert, A. and Sandstead, H.H.
    (1963). Zinc metabolism in normals and patients with syndrome
    of iron deficiency anaemia, hypogonadism, and dwarfism.
    Journal of Laboratory Clinical Medicine. 61: 536-549.

Ramirez, E.A; Rivera de Sala, A; Serrona D. and Cancio, M. (1961).
    Electrophoretic serum protein studies in chronic human
    mansoni's Shistosomiasis. American Journal of Tropical
    Medicine and Hygiene, 10: 530-536.

Ruff,M.D; Davis, G.M. and Werner, J.K. (1973). *Schistosoma Japonicum:*

Disc electrophoretic protein patterns of the Japanese, Phiipine and Formosan strain. Experimental Parasitology, 1: 31-34.

Rogers, S.H. and Bueding, E. (1975). *Schistosomatium douthitti*: Carbohydrate metabolism in adults. Experimental Parasitology, **40**: 397-405.

Ruppel, A. and Cioli, D.(1977). A comparative analysis of various developmental stages of *Schistosoma mansoni* with respect to their protein composition. Parasitology, **75**: 339-343.

Sadun, E.H. and William, J.S. (1966). Biochemical aspects of *S. mansoni infection* in mice in relation to worm burden and duration of infection. Experimental Parasitology, **18**: 266-273.

Sadun, E.H. and Walton, B.C. (1958). Studies on the host-parasite relationsips to *Schistosoma japonicum*. 11. Quantitative changes in the concentration of serum proteins in humans and rabbits. American Journal of Tropical Medicine and Hygiene, **7**: 500-504.

Saleh, S.; Abdel-Aziz, M.T.; El-Sayed, H.M. and Khayyad, M.T. (1976). Effects of antischistosomal drugs on some aspects of carbohydrate metabolism in mice. Pharmacological Research

Saleh, S.A.H.; Higazi, A.M. and Ata, A.A. (1967). Pancreatic bilharziasis and its relation to *Diabetes mellitus*. Journal of the Egyptian Medical Association, **50**: 425-441.

Saleh,S. and Shehata,M.(1979). The effect of *Schistosoma mansoni* infection, and of Oxamniquine, a new antischistosomal drug,

on some vitamins in the livers of hamsters. Polish Journal
of Pharmacology and Pharmacy, 31: 555-561.

Shaheen, A.A; Ebeid, F.A. and Fahim, A.T. (1989). Effects of
Praziquantel on some aspects of carbohydrate Metabolism in
mice infected with *Schistosoma mansoni*. Pharmacological
Research, 21(3), 263-270

Shakir, M.H.; Saif, M. and Abdell-Fattah, F. (1964). The serum
cholinestrase levels in bilharziasis. Journal of Egyptian
Medical Association, 47: 122-126.

Samir-Amer, M; Abdel-Daim, M.H. and Abdel-Tawab, G.A.(1967).
Kynurenine metabolism in liver homogenates of normal and
Schistosoma mansoni infected mice. Biochemical Journal, 104:
656-662.

Samira, Saleh, M.T.; Abdel-Aziz; El-Sayed, H.M. and Khayal, M.T.
(1976). Effect of antischistosomal drugs on some aspects of
carbohydrate metabolism in mice. Pharmacological Research
Communications, 8(4): 359-368.

Sandstead, H.H.; Prasad, A.S.; Schulbert, A.R.; Farid, Z.; Miale, A.;
Bassilly, S. and Darby, W.J. (1967). Human Zn^{++} deficiency:
endocrine manifestations and response to treatment.
American Journal of Clinical Nutrition, 20: 422-442.

Schwartz, K. and Mertz, W. (1957). A glucose tolerance factor and
its differentiation from factor 3. Archives Biochemie et
Biophysique, 72: 515-518.

Schwartz, K. and Mertz, W. (1959). Chromium (III) and the glucose
tolerance factor. Archives Biochemie et Biophysique, 85:

292-295.

Senft, A.W. (1963). Observations on amino acid metabolism of

Schistosoma mansoni in a chemiacally defined medium. Annals

of the New York Academy of Sciences, 113: 272-278.

Senft, A.W. (1966). Studes in arginine metabolism by schistosomes.

1.Arginine uptake and lysis by Schistosoma mansoni.

comparative Biochemistry and Physiology, 18: 209-216.

Senft, A. W. (1967). Studies in arginine metabolism by

schistosomes. II. Arginine depletion in mammals and snails

infected with S.mansoni or S. haematobium. Comparative

Biochemistry and Physiology, 21: 299-306.

Smith, T.M; Brooks, T.J. and Lockard, V.G. (1970). In Vitro studies n

cholesterol metabolism in the blood fluke Schistosoma

mansoni.Lipids, 5: 854-856.

Smith, T.M. and Brooks, T.J.(1969). Lipid fractions in adult

Schistosoma mansoni. Parasitology, 59: 293-298.

Smith, T.M. and Brown, J.N. (1977). Tricarboxylic acid cycle enzyme

activities in adult Schistosoma mansoni and Schistosoma

japonicum. Tansactions of the Royal Society of Tropical

Medicine and Hygiene. 71(4): 329-330.

Smith, T.M; Brooks, T.J. and White H.B.(1969). Fatty acid composition

of adult Shistosoma mansoni. Lipids, 4: 31-36.

Stjernholm, R.L. and Warren, K.S. (1974). S. mansoni: Utilization of

exogenous metabolites by eggs in vitro. Experimental

Parasitology, 36: 222-232.

Storey, K.B. and Bailey, E. (1978). Intracellular distribution o the

enzymes associated with lipogenesis and gluconeogenesis in the adult male cockroach: *Periplaneta*.Insect Biochemistry, 8: 125-131.

Southerland, W.M.(1990). Foundations of Medicine- BIOCHEMISTRY. Churchill Livingstone Inc. U.S.A.

Soliman, L; El- Safouri, S; Megahed, G.M. and EL- Maola, G. (1975). Copper and Zinc levels in the blood serum and urine of Bilharzial hepatic fibrosis. Experientia. 31(3): 280-281.

Sukkar, M.Y.; Omer, A.H.S. and Nasr-el-Din Ahmed (197 4). Impaired glucose tolerance in hepatic schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 68(4): 327-332.

Takahashi, S; Dunn, M.A. and Seifter, S. (1980). Liver collagenase in murine Schistosomiasis. Gastroenterology, 71: 484-489.

Tanabe, M; Kaneko, N. and Takeuchi, T. (1989). *Schistosoma mansoni:* Suppresion of carbamoyl phosphate synthatase (ammonia) and ornithine carbamoyl transferase ativities in the liver of infected mice. Experimental Parasitology, 68: 432-442.

Tao, I.H. and Huang, T.Y. (1965). Studies on arginase of *Schistosoma mansoni*. Scientia Sinica, 14: 417-422.

Tielens, A.G; Celik, C; Van-den-Heuvel, J.M; Elfring, R.H. and Van-den-Bergh, S.G. (1989a). *Schistosoma mansoni*: Rapid turnover of glycogen by adult worms. Parasitology, 98(1): 67-73.

Tielens, A.G.; Van-der-Meer, P.; Van-den-Heuvel, J.M. and Van-den-Bergh, S.G. (1991). The enigmatic presence of all

gluconeogenic enzymes in *S. mansoni* adults. Parasitology, 102(2): 267-276.

Tielens, A.G.M.; Van Oordt, B.E.P. and Van Den Bergh, S.G. (1989b). Carbohydrate metabolism in adult schistosomes of different strains and species. International Journal for Prasitology, 19(4): 447-449.

Toepfer, E.W.; Mertz, W.; Polansky, M.M.; Roginski, E.E. and Wolf, W.R. (1977). Preparation of chromium containing material of glucose tolerance factor activity from brewer's yeast extracts and by synthesis. Journal of Agricultural Food Chemistry, 15: 162-166.

Uglem, G.L. and Read, C.P. (1975). Sugar transports and metabolism in *S. mansoni*. Journal of Parasitology, 61, 390-397.

Van Espen, P; Nullen, H. and Maenhaunt, W.(1979). Microbeam Analysis, E.E. Newbury(Ed). San Fransisco Press. San Fransisco.

Van-Oordt, B.E.; Tielens, A.G. and Van den-Bergh, S.G. (1989). Aerobic to anaerobic transition in the carbohydrate metabolism of *S. mansoni* cercariae during transformation *in vitro*. Parasitology, 98(3): 409-415.

Varley, H.; Gowenlock, A.H. and Bell, M. (1976). Practical Clinical Biochemistry. 5th Ed. Heinemann Medical Books. London.

Veneziade, C.M.; Walter, P.; Kneer, N. and Lardy, H.A. (1967). Influence of L-tryptophan and its metabolites on gluconeogenesis in the isolated perfused liver. Biochemistry, 6: 2129-2138.

Von Kruger, W.M.A.; Gazzinelli, G.; Figueiredo, E.A. and Pellegrine, J. (1978). Oxygen uptake and lactate production by *S. mansoni* cercarial body and tail, and schistosomulae. Comparative Biochemistry and Physiology, 60B: 41-46.

Walker, E. and Chappel, L.H. (1980). *S. Mansoni.* Comparison of the effect of cyclohexemide and Emetine on protein synthesis in adult worms. Comparative Biochemistry and Physiology, 67C: 129 - 134.

Walker, E. and Chappel, L.H. (1982). *Schistosoma mansoni: In vitro* uptake and incorporation of glycine by adult worms. Comparative Biochemistry and Physiology, 73B: 385-392.

Warren K.S.(1966). The pathogenesis of 'Clay-pipe Stem cirrhosis' in mice with chronic Schistosomiasis mansoni, with a note on the longevity of the schistosomes American Journal of Pathology, 49: 477-489

Warren, K.S. and Reboucas, G.(1964). Blood ammonia during bleeding from oesophageal varices in patients with hepatosplenic Schistosomiasis. New England Journal of Medicine, 271: 921-926.

Warren K.S. and DeWitt, W.B.(1958). Production of hypertension and oesophageal varices in the mouse. Proccedings of the Society of Experimental Biology and medicine, 98: 99-101.

Warren K.S; Reboucas, G. and Baptista, A.G.(1965). Ammonia metabolism and hepatic coma in hepatosplenic Schistosomiasis: Patients studied before and after portocaval shunt. Annals of Internal Medicine, 62: 1113-1133.

Weeda, E.; Koopmanschap, A.B.; De Kort, C.A.D. and Beenakker, A.M.
(1980). Proline synthesis in the fat body of *Leptinotassa
decemlineata*. Insect Biochemistry, 10: 631-636.

Williams, D.L. and Marks,V. (1990). Biochemistry in Clinical Practice.
William Heinemann Ltd. LONDON.

Williams, F.R. and Hager, L.P (1966). Pyruvic (cytochrome b,)
Dehydrogenase: Methods of Enzymology. Edited by Willis A.
Wood. Academic Pres. Pg 265 - 277.

World Health Statistics Annual (1991). Geneva pg. 15 - 17.

World Health Organization (1985). The control of schistosomiasis.
Report of a WHO expert Committee. Techinical report series,
728: 1 - 113.