

STUDY OF ENZYMES IN CEREBROSPINAL FLUID
IN SCHISTOSOMIASIS

Thesis

Submitted to the Faculty of Medicine
University of Alexandria

In

Partial Fulfilment of the Requirements
For the Master Degree in Biochemistry

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1977

ACKNOWLEDGEMENT

It is a pleasure to take this opportunity to express my deepest thanks and gratitude to my teacher, Professor Dr. Mohamed S. Zein, Head of the Department of Biochemistry for his continued interest, constructive criticism and the many valuable suggestions given generously throughout this work which is the result of his constant encouragement and learned guidance.

Thanks also due to Dr. Mahmoud A. Zahran Ass. Prof. in the Biochemistry Department for his constant enthusiasm and precious help during the whole work.

I owe special thank to Dr. Momtaz El-Sawy Ass. Prof. in the Clinical Pathology Department for his help and advice during the whole work to bring this thesis into final form.

I am greatly indebted to Dr. Helmi H. Abaza Ass. Prof. in the Department of Tropical Medicine who kindly supplied the C.S.F. samples needed to carry out this thesis without which this work could not have been started.

I am very grateful to Dr. M. Taky Lecturer of Public Health; for his help and advice concerning the statistical part of this work.

ABBREVIATIONS

ADP	=	Adenosine diphosphate
ATP	=	Adenosine triphosphate
CPK	=	Creatine phosphokinase
C.S.F.	=	Cerebrospinal fluid.
G-6-P	=	Glucose -6- phosphate
G-6-PDH	=	Glucose -6- phosphate dehydrogenase
G.O.T.	=	Glutamic oxalacetic transaminase
G.P.T.	=	Glutamic pyruvic transaminase
H.K.	=	Hexokinase
L.D.H.	=	Lactate dehydrogenase
NADP	=	Nicotinamide - adenine dinucleotide phosphate
NADPH	=	Nicotinamide-adenine dinucleotide phosphate, reduced.

UNITS:

C & W	=	Cabaud and Wroblewski
O & R	=	Oliver and Rosalki
R & F	=	Reitman and Frankel

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INTRODUCTION

INTRODUCTION

The cerebrospinal fluid is a clear colourless solution which fills the ventricles of the brain, the central canal of the spinal cord and the subarachnoid space and is continuous with the interstitial fluid that bathes the neurons of the brain and the spinal cord. Studies made on the production, movement and reabsorption of the cerebrospinal fluid indicated that it is a closed biological system comparable with that of the blood vessels. It performs a multiplicity of transport and protective functions ensuring optimal conditions for the activity of the central nervous system.

Historical Review:

The discovery of the fluid in the ventricles and subarachnoid space dates for back in the history. Egyptian neurosurgeons encountered the fluid in their early surgical probings of the brain, as this was described in the Egyptian papirii 3000 B.C.

However, the first recorded evidence of its recognition is to be found in the writings of Hippocrates, who recognised it both in animals and man. At that time, its true nature was not understood and its presence was attributed generally to pathological conditions particularly hydrocephalus.

The French surgeon Ambroise Pare (1510-1590) mentioned that some "waterish moisture" was always present in the cerebral ventricles and he hinted at the functions of the choroid plexus.

Cutugno - an Italian anatomist and clinician - (1764) was the first to positively demonstrate that a clear fluid filled the subarachnoid space and ventricular passages, but this work was overlooked. It was not until the 18th and early 19th centuries that cerebrospinal fluid was assigned a significant position in neuro-anatomy, physiology and chemistry. Francois Magendi (1824) described the appearance of the fluid and gave an account on its physiological functions and its chemical composition. Laissaigne who made the analysis for Magendi at that time, recorded a water content over 98%, a protein content less than 100mg%, chlorides about 800mg% and the presence of bicarbonates, phosphates and calcium. Hoppe, shortly thereafter observed that the cerebrospinal fluid reduced copper oxide, a reaction which was attributed soon to its sugar content.

Henirich Quincks (at the end of the 19th century) was the first to perform spinal puncture without incising the skin. This provided a portal of entry for the cerebrospinal fluid, which led to advance in the full scale of investigations on the fluid and became an indispensible neurological tool in diagnosis, therapy and research.

The begining of this century was especially distinguished by a series of investigations made on the C.S.F. In 1901, the cytological studies of Widai, Sicard and Ravaut appeared. In

1903, Froin described this syndrome of fluid block. In 1909, Plant made his serological studies and in 1912 Lang announced his colloidal gold reaction. In 1914, Retzius and Key demonstrated a view that the cerebrospinal fluid escapes from the subarachnoid space through the pachinian bodies into the subdural space, then into the venous sinuses and that some of it reaches the cervical lymphatics. In 1916 Quickenstedt added on additional diagnostic and research procedure through his work on the dynamics of the cerebrospinal fluid. In 1920 Ayer presented his method for cysternal puncture. In 1921 Sicard, in collaboration with his pupil Jaques Forestier, introduced lipiodol injection for the diagnosis of intraspinal lesions.

Production of the cerebrospinal fluid:

Site of production:

In all probability, the cerebrospinal fluid under normal conditions is almost exclusively formed by the choroid plexus, especially that of the lateral ventricles. However, other sources are mentioned to play, if at all, a minor role. These are:

- 1- Ependymal cells.
- 2- Perivascular spaces.
- 3- Parenchyma of the nervous system.
- 4- Meninges.

Under pathological condition any part of the lining of the ventricles and arachnoid space is capable of increasing the quantity of the fluid by either exudatory or transudatory mechanism.

Mechanism of production:

Several theories have been advanced in this respect pointing that the fluid is produced by one of the following mechanisms:

- | | |
|----------------|------------------|
| 1. Secretion. | 2. Dialysis |
| 3. Filtration. | 4. Transudation. |

For many years, there was controversy whether the cerebrospinal fluid is a secretion or transudate. Recently it should be realised that different components of the cerebrospinal fluid enter the system in different ways and places at different levels.

Rate of formation:

It has been estimated by isotope techniques that as little as 15ml. of C.S.F. could be produced per day. However some authors reported different figures: 40, 50 and even up to 400ml. per day.

Circulation and absorption:

The fluid, mostly formed in the lateral ventricles, traverses the third ventricle, the sylvian duct, and fourth ventricle to reach the subarachnoid space through the foraminae of Magendie and Lushka. Little enters the central canal of the

spinal cord, and in adults this is usually occluded by proliferation of the ependymal cells. The pulsations of the brain serve to drive the fluid around the base over the vertex, and downward around the spinal cord. It is probable that about four-fifths of the fluid is reabsorbed into the blood stream in the region of the vertex. Absorption in the veins is facilitated by the presence of the arachnoid villi which project through the dura into the lumen of the venous sinuses. This absorption is rendered possible by the fact that the pressure of the fluid is 5-15m.m. water above that in the veins.

The mechanism of absorption is dependant upon the difference in pressure between the intracranial venous sinuses and the cerebrospinal fluid as well as upon the effective colloid osmotic pressure across the blood cerebrospinal fluid barrier. The mechanism of absorption of that part of the fluid which circulates around the spinal cord is not so clear. The perineural lymphatics have been thought to drain it away, but these lymphatics have never been shown to communicate directly with the subarachnoid space. Small clusters of arachnoid cells do occur along the spinal cord, especially in the angle of reflection of the leptomeninges from the nerve roots, and it is possible that these filter the fluid into the small venous plexuses which are found in this region. Such a hypothesis is supported by studies using radioactive isotopes.

The rate of absorption of various constituents vary: water enters and leaves the cerebrospinal fluid-containing cavities with great rapidity across the lining memberanes and lining epithilia. Electrolytes and proteins depart from the cerebrospinal fluid at rates depending on their particle size.

The blood-fluid barrier:

This concept was induced by Erlich and meant that a certain mechanism must exist between the blood circulation and the fluid system and that this operates as regulator of the passage of substances from the blood to the fluid. In some way it resembles that of the cell memberane, though it may not be possible to explain its function on purely chemical and physical laws but should be accepted to be due to biological and vital forces. As there is a blood-fluid harrier, there exists a blood brain barrier for the substances that enters the central nervous system from the blood directly to the brain. The site of the blood-fluid barrier is the choroid plexus under normal conditions, but under abnormal conditions this can be extended to the entire lining of the arachnoid space and the ventricles with their lining.

C.S.F. ENZYMES IN HEALTH AND DISEASE

TRANSAMINASES:

In recent years there has been a major revision of our concepts about the formation of cerebrospinal fluid. C.S.F. is

not only an ultrafiltrate formed by way of the choroid plexuses in the ventricles, but is also the product of an exchange between the blood and the central nervous system at all levels of the subarachnoid space. The isotopic work upon which this concept is founded has, in part, been summarized by Sweet and his co-workers (1954). It may be deduced from this that the C.S.F. is in a state of dynamic equilibrium, not just with blood, but also with interstitial fluid of all parts of the central nervous system. Thus the C.S.F. is in a unique position to reflect such changes in the properties of neutral tissue as may occur in disease. This serves to emphasize the important contributions made by the many recent chemical studies of the C.S.F. The application of modern laboratory techniques, such as electrophoresis, chromatography, spectroscopy, and flame photometry, to the investigation of the C.S.F. has yield a better understanding of the diseases, as well as information of practical value in their diagnosis.

There is increasing interest nowadays in estimation of cerebrospinal fluid enzyme kinetics in several pathological condition.

Several enzymes present in the blood plasma occur also in the cerebrospinal fluid.

Kaplan and his co-workers (1940) were among the first to study the enzymes of the C.S.F. in health and disease. They

determined trypsin, antitrypsin, phosphatases, amylase, lipase, tributyrinase and esterase. Phosphatases were the only enzymes consistently present in normal fluids, the others were present in meningitis and could be related to the polymorphonuclear cell count in the C.S.F.

Glutamic oxalacetic transaminase and lactate dehydrogenase are present in high concentration in nervous tissue. Reports concerning the presence of these enzymes in the cerebrospinal fluid in various neurological diseases began appearing about the mid-1950, and numerous publications have since documented changes in the G.O.T. and L.D.H. activity of the C.S.F. that have accompanied some diseases of the nervous system (Davies-Jones, 1969).

The measurement of glutamic oxalacetic transaminase and lactic dehydrogenase activity in serum has been valuable for the assessment of cellular damage in a variety of tissues. In studies of cerebrospinal fluid from patients with neurologic disorders, however these enzymes have been of limited diagnostic value (Bentley et al., 1968).

Transaminases catalyse reactions involved in the transfer of an amino-group from an amino-acid to a keto-acid. The overall process is called transamination. The enzymes concerned with transamination reactions have been termed transaminases, these include:

- 1- Glutamic oxalacetic transaminase.
- 2- Glutamic pyruvic transaminase.

Transaminase activity is widespread in animals, plants and micro-organisms. It is increased in serum in conditions characterised by cell injury or tissue necrosis of many organs. Many reports emphasized the diagnostic value of transaminases, in cardiac, hepatic, skeletal muscle and renal conditions.

Drug induced transaminase alternations are usually characterized by greater increase in S.G.P.T. than S.G.O.T. activity contrary to infarction. The existence of the so-called blood brain barrier is a well-documented phenomenon. The presence of the blood brain barrier with respect to G.O.T. was mentioned in a report by La Due and Wroblewski (1955) in which an elevation of the enzyme in the serum was not reflected in the spinal fluid. Wakin and Fleisher (1956) showed evidence of a barrier in dogs following experimental cerebral infarction in which marked elevation of spinal fluid transaminase was associated with only minimal changes in serum level. High serum levels resulting from acute hepatic poisoning were not reflected in the spinal fluid in a subsequent report. These studies intimated that elevation of the serum transaminase should not be expected to follow acute cerebro-vascular accidents because of the blood brain barrier.

It was shortly thereafter that moderate rise of transaminase activity in both serum and spinal fluid was reported in a study of cerebrovascular disease in human being (Fleisher et al., 1957). This differed from the result of their previous work with dogs in that the marked elevations of the spinal fluid activity after experimental cerebrovascular damage did not occur.

Green et al., (1957) observed no elevation of transaminase in the serum after cerebral infarction, but found moderate increase in the activity in the spinal fluid in seven of eleven patients.

In another study transaminase activity was measured in the C.S.F. of 44 normal subjects (Lieberman et al., 1957a): ranging from 0-24 unit/ml. were found with a mean of 12.1 units. The level of transaminase activity in the spinal fluid of males was slightly higher than that of females. They also reported that the level of transaminases in the C.S.F. is approximately half the level found in the serum in the same laboratory.

Wakin and Fleisher (1956) demonstrated that the high serum transaminase level associated with hepatic necrosis in dogs was not transmitted to the C.S.F. Similarly there was only minimal transfer of the enzyme between the vascular and sub-arachnoid spaces after transaminase concentrates were injected intravenously

or intracisternally. The fact that the enzyme values in the C.S.F. were less elevated than those in the serum, was regarded as proof that the increase was not derived from damaged nerve cell.

The similarity of the transaminase curves after surgical trauma to the brain, as compared with those associated with cerebro-vascular accidents warrants further comment. Such similarity suggests that the transaminase elevations associated with cerebro-vascular accidents may be explained on the basis of brain injury without the need for assuming damage to other organs. The greater elevations whether in blood or in spinal fluid occur within 3-5 days of the stroke. Further increase may be correlated with clinical extension of brain damage. Complicating cardiac disease usually produces an earlier or sharper elevation.

The glutamic oxalacetic transaminase (G.O.T.) activity in the C.S.F. and serum of patients with neurologic diseases has been surveyed by a number of workers. The G.O.T. level of the C.S.F. was increased in 64 per cent of patients with cerebral infarction (Green et al., 1957). Serum levels were normal, while the C.S.F. G.O.T. was elevated in a few cases of multiple sclerosis, it was normal in brain tumours. Patients with high serum G.O.T. activity and no evidence of central nervous system involvement, had normal C.S.F. levels. Fleisher and his co-workers

(1957) reported results which differed from the above in several respects. They found the G.O.T. of the C.S.F. to be almost the same as that of the serum in their control group. Green et al., (1957), Katzman et al., (1957); Lieberman et al., (1957), have variously estimated the C.S.F. G.O.T. level at $\frac{1}{2}$ to $\frac{2}{3}$ (approximately) of the serum activity in normal individuals.

Fleisher and his colleagues (1957) reported elevated G.O.T. activity in the C.S.F. of patients with cerebral atrophy and convulsive disorders. These, and other investigators (Katzman et al., 1957), did not find a high incidence of G.O.T. increases in the C.S.F. of patients with cerebral infarction. Some workers also noted more frequent serum elevations (Lieberman et al., 1957). There was agreement on the existence of a blood-brain barrier for G.O.T. and a lack of correlation between C.S.F. activity and protein content (Green et al., 1957; Lieberman et al., 1957). In a later communication (Green et al., 1958), dealing with 90 cases of cerebral infarction, it was noted increased G.O.T. in the C.S.F. in 66 per cent. There were occasional elevations of serum G.O.T. Elevated C.S.F. activity was found in a few cases of brain tumour, detracting from the value of the G.O.T. determination in the differentiation of cerebro-vascular disease from neoplasms (Green et al., 1958).

LACTATE DEHYDROGENASE:

Bruns, Jacob, and Weverinck (1956) were evidently the first to report a normal C.S.F. value for lactate dehydrogenase.

Interest in this enzyme stemmed from the demonstration of elevated serum levels in neoplastic disease (Hill and Levi 1954). Green et al., 1958) demonstrated the increased L.D.H. activity in the C.S.F. of all brain tumour patients studied with highest values in those with malignant tumours. Fleisher and his group (1957) reported elevations in a few cases of astrocytoma. Wroblewski, Decker, and Wroblewski (1958) found C.S.F. L.D.H. activity above the normal range in patients with intracranial neoplasms. Jakoby and Jakoby (1958), on the other hand, reported elevated activities in cerebrovascular accidents, but essentially normal C.S.F. levels in patients with brain tumours. It was also noted (Green et al., 1958) increased C.S.F. L.D.H. activity in patients with cerebrovascular disease, as well as in brain tumour patients. An independence of serum and C.S.F. L.D.H. activity was demonstrated (Green et al., 1958 and Wroblewski et al., 1958).

Similarly, Green et al., (1957), were not able to relate changes in the C.S.F. G.O.T. or L.D.H. level to the concentrations of these enzymes in brain tumours.

Fleisher et al., (1957) found normal C.S.F. L.D.H. and G.O.T. activities in four patients with metastatic carcinoma of the C.N.S., and Myerson, Hurwitz, and Sall (1957) obtained elevated transaminase activity in two of six such patients. In each patients with metastatic malignancy of the brain studied by Wroblewski et al., (1957), and by Wroblewski, Decker, and Wroblewski (1958), raised C.S.F. L.D.H. activity in four patients with cerebral metastases and Green et al., (1958) found raised C.S.F. L.D.H. and G.O.T. in three of four similar patients. Mann et al., (1960) and Mellick and Bassett (1964) in three patients with cerebral metastases obtained normal transaminase levels. In the group of 18 patients with metastatic carcinoma of the C.N.S. there was a high significant elevation of both the L.D.H. and G.O.T. of the C.S.F. in all but two patients (Davies-Jones 1969).

Although most previous workers have found no correlation between the increased enzyme activity of the C.S.F. and the degree of malignancy of a tumour the results of Mellick and Bassett (1964) do suggest this. Green, Oldewurtel, and Forster (1959) also found that the L.D.H. activity of the C.S.F. was greatest in patients with malignant anaplastic tumours.

The major source of these enzymes is almost certainly the tumours themselves in contact with circulating C.S.F.

Wroblewski (1959) has shown that tissue culture medium of malignant cells contain increasingly greater amount of L.D.H. with time (G.O.T. was not assayed), whereas tissue culture of benign cells does not produce an increase in the L.D.H. content of the medium. The destruction of nervous tissue by the tumour with the release of intracellular enzymes into the C.S.F. is another source. The cerebral oedema associated with very malignant growths results in a variable degree of cerebral hypoxia. This cerebral hypoxia, by producing incompetence of the cell memberane and blood/C.S.F. barrier (Lending et al., 1961; Slobody, et al., 1957), may also contribute to the increased enzyme levels in the C.S.F. through the accompanied by extensive cerebral oedema in eight of the nine patients who came to necropsy.

Beaty and Oppenheimer (1968) demonstrated that infection of the central nervous system is associated with an increased amount of L.D.H. in the C.S.F. Twenty-four of 25 patients with bacterial meningitis and nine of 11 with viral infections had enzyme level above normal. These results are consistent with the observations of several investigators who have found increased levels of L.D.H., isocitric dehydrogenase and G.O.T. in the C.S.F. of patients with meningitis or meningoencephalitis (Wroblewski et al., 1958; Lending et al., 1964; Van Ryment et al., 1966; Belsey 1966; Krogsgaard and Quaade 1963), but they do not simply reflect a

nonspecific response of all enzymes in the C.S.F. to infection. Lending et al., (1964) showed that during the acute stages of bacterial meningitis, the L.D.H. activity of the C.S.F. was universally increased whereas only a third of the patients had abnormalities in G.O.T. activity. None of the previous studies documented differences in enzyme levels according to the bacterial pathogen isolated, but, in general, viral infections were associated with either normal or only slightly increased levels of C.S.F. enzyme activity, where the increments in enzyme activity associated with bacterial meningitis were more dramatic.

Enzymes in the C.S.F. are derived from either the blood, C.N.S. tissue or cells in the C.S.F. Several studies have proved that when the blood-brain barrier is intact, C.S.F. enzyme activity is relatively unaffected by serum changes. For example, Fleisher and Wakin (1956) found that intravenous infusion of transaminase had little or no effect on the C.S.F. enzyme level in dogs. Wroblewski and his co-workers (1958) noted that normal levels of C.S.F. L.D.H. often were found in the presence of serum elevations, and Van der Helm, Zondag and Klein (1963) showed that the L.D.H. isoenzymes in the C.S.F. are not influenced by abnormal serum patterns. It has been assumed that certain C.N.S. diseases alter the permeability of the blood-brain barrier, allowing increased movement of enzymes from the serum to spinal fluid. Ursing (1965) did show that serum proteins enter the

C.S.F. of patients with C.N.S. infection, and Lowenthal and his colleagues (1961) found parallel isoenzyme patterns in the serum and C.S.F. of patients with neurological disease, but studies specifically designed to evaluate movement of enzymes into the C.S.F. have yielded contradictory results.

PHOSPHATASES:

From a clinical standpoint, interest is centered particularly upon the phosphatase activity of the blood plasma, serum or C.S.F.

Colling and Rossiter (1950), using phenyl phosphate as a substrate, determined the acid and alkaline phosphatase activity in the C.S.F. of normal individuals and patients with meningitis, poliomyelitis, and syphilis. While activity was found in less than half of normals, it was present and considerable in the patients with meningitis and poliomyelitis.

Kovacs (1953) discovered that when C.S.F. obtained from meningitis patients was incubated at 37°C there was a marked increase in inorganic phosphorus. This C.S.F. liberated phosphorus and sugar when incubated with bovine brain and cord emulsion (Kovacs 1953).

Many of the reports on C.S.F. enzymes do not define the type of the activity curve. This is particularly true of the

studies on phosphatases (Kaplan et al., 1939), nucleases (Kovacs 1953), tributyrinase (Kalsbeek et al., 1950), esterases (Kaplan et al., 1939; Tower and McEachern 1949), and even of recent surveys of G.O.T. and L.D.H. (Fleisher et al., 1957; Katzman et al., 1957 and Lieberman et al., 1957). There is no proof presented that the activity measured is completely due to the enzyme purported to be determined.

The source of C.S.F. enzymes is not always clear. Kaplan and his co-workers (1940) postulated three origins; from blood cells present in the C.S.F., from the blood due to an increased permeability of the blood-brain barrier, and from the tissues of the nervous system itself. To these we may add another source, the neoplastic cells of brain tumours. C.S.F. pleocytosis may partially account for the elevated activities of trypsin (Kaplan et al., 1940), phosphatases (Colling and Rossiter 1950; Kaplan et al., 1940), pseudocholinesterase (Colling and Rossiter 1949; Jefferson 1954), nucleases (Kovacs 1953 and Kovacs 1955), G.O.T. (Green et al., 1957), and L.D.H. (Green et al., 1958) reported in meningitis. A breakdown of the blood brain barrier is probably also responsible because in meningitis increased enzyme activity often is associated with an elevated total protein (Colling and Rossiter 1950; Jefferson 1954). The origin of elevated enzyme levels in degenerative and neoplastic diseases of the central nervous system is not so easily explained.

Changes in the blood-brain barrier for enzymes which have a high ratio of serum to C.S.F. activity may occur, but would hardly explain the occasional elevations of serum activity observed in these cases (Green et al., 1957 and Lieberman et al., 1957). Note that the demonstration of a "barrier" to the entrance of an enzyme into the C.S.F. has usually been based on conditions during health, or in certain systemic illnesses. Conclusions based upon such evidence do not necessarily apply to the blood-C.S.F. relationships in neurologic diseases.

CREATINE PHOSPHOKINASE:

Creatine phosphokinase is found chiefly in skeletal and cardiac muscle. It is virtually absent in liver, lung, kidney and erythrocytes. Creatine phosphokinase activity in serum increases markedly in myocardial infarction and most muscular disorders (Vincent and Rapaport 1965).

Mueller et al., (1975) have found C.P.K. levels measured in samples of serum from patients with elevated alkaline phosphatase activity were lower than expected.

To date, the clinical significance of cerebrospinal fluid creatine phosphokinase activity has not been established (Lisak and Graig, 1967).

ENZYMES IN SCHISTOSOMIASIS:

Epidemiologically, hepatic bilharziasis (Schistosomiasis) is the most common systemic disease in Egypt. The proper assessment of the activity of enzymes in cerebrospinal fluid in patients with schistosomiasis is essential as a guide to therapy, medical or surgical, and in evaluating the response to treatment, as well as the prognosis of liver involvement.

The liver function tests have been fragmentarily studied by various authors, but with variance of results.

There are many reports in the literature attempting to correlate function with altered histological changes of the liver, and invariably the correlation is most difficult. Recently, Talso and Remenchick (1968) stated that the study of function by biochemical techniques provides better correlation with the clinical phases of liver cirrhosis.

Patients suffering from this disease in this country are peasants, who commonly suffer from other factors that affect the liver such as malnutrition, infections (bacterial, viral, etc...) and other parasitic infestations.

Ghanem et al., (1970) demonstrated elevated values of S.G.O.T. occurred in 41.5% of non-ascitic and 56.81% of ascitic patients. In the early hepatomegalic stage, the level was very

slightly elevated. Apparent and significant increase occurred in the hepatomegalic-splenomegalic stage, but no further increase was noticed in the stage of shrunken liver and in ascitic cases having normal serum bilirubin levels. The highest values were observed when serum bilirubin exceeded 1.2mg% both in ascitic and non-ascitic cases, being more evident in the latter.

Dexamethasone produced an apparent decrease in the enzyme level especially in cases who had previously high values (Ghanem et al., 1970). The possible explanation is that dexamethasone, as Dahnus and Bonner (1965) stated, stabilises the lysosomes, and hence prevents the break-down of liver cells by the lysosomal enzymes. It also halts enzyme release through improving permeability of the cell memberane. Improvement of hepatic blood flow as a result of dexamethasone administration (Fayez et al., 1967) might also play a role.

Correlation between results of S.G.O.T. and S.G.P.T. revealed that high values of the former were accompanied by elevation of the latter in only 56.5% of cases (Ghanem et al., 1970).

Ghanem (1970) stated the rise of serum transaminases is usually considered to denote active hepatic cell damage and/or increased permeability of the cell memberane. Some of the sub-clinical causes of such damage are beyond detection by clinical or available laboratory procedures, such as the nonicteric type

of infectious hepatitis which constitutes as Grandy (1968) stated, about half of the total cases in adults. In bilharzial patients, the possibility of added viral and bacterial infection is rather great. Another contributing factor which might add to elevation of serum transaminases is antischistosomal therapy, but in their bilharzial patients, no specific treatment was given at least three months before examination. An extrahepatic factor which might contribute to the higher elevation of S.G.O.T. than S.G.P.T. in hepatic bilharziasis could possibly be related to skeletal muscle changes. This is postulated on the clinical observation of striking muscle wasting in the advanced cases, and on the finding of Mansour et al., (1965) that myopathy occurred in mice experimentally infested with *schistosoma mansoni*. This suggestion, however, needs further study and confirmation. (Ghanem et al., 1970).

The results of S.G.P.T. were parallel to those of S.G.O.T. (Ghanem et al., 1970), but with lower incidence and lower levels. High values were observed in 28% of non-ascitic and in 31.8% of ascitic patients.

Salah et al., (1976) found that the S.G.O.T. showed significantly higher on admission and after treatment with niridazole than control. Their level showed further increase at follow up but the difference was not significant perhaps due to the small number of patients included. All S.G.P.T. values fell within the normal range and showed no statistically significant change.

The significant elevation of S.G.O.T. and the lack of change of S.G.P.T. (Salah et al., 1976), provide additional evidence for the side effects of disease and niridazole on the mitochondria of the hepatic cells. This evidence is derived from the fact that the ratio of G.O.T. activity in mitochondria to that in the soluble fraction of liver cells is much higher than the corresponding ratio of G.P.T. (De Duve et al., 1962). This agrees quite well with previously mentioned studies (Kanani et al., 1970). Moreover, the presence in human hepatic tissue of considerably more G.O.T. (Wroblewski and La Due 1956) indicates that the released S.G.P.T. is too diluted in the extracellular compartment to cause significant increase in the G.P.T. activity. Variation in the release, destruction or excretion of the two enzymes, or an unknown metabolic aberration is probably an important contributory mechanism.

The relationship between parenchymal and serum enzyme activity may be altered by hepatic cellular injury. In bilharzias cases the total L.D.H. activity on admission was within normal range (Salah et al., 1976). Being one of the glycolytic enzymes, it has been found to be associated mainly with the supernatant fraction (Cytoplasm) (De Duve et al., 1962). Administration of niridazole however, could alter the cell membrane permeability leading to the leakage of the enzyme from liver cytoplasm to plasma. This would explain the significant increase

in total L.D.H. observed in patients after treatment (Salah et al., 1976). A similar finding has previously been reported by El-Hawary et al., (1970).

Popper and Schaffner (1957), found that 56% of non-jaundiced cirrhotics had raised serum alkaline phosphatase levels. El-Mofty and Khattab (1962) reported elevation of this enzyme in 30% of non-ascitic bilharzial patients. Whereas, Ismail and Sidky (1962) stated that the enzyme shows no increase except in the terminal stages. Wilkinson (1962), however, stated that severe malnutrition causes reduction of alkaline phosphatase activity.

The exact explanation of the raised serum alkaline phosphatase activity in liver disease, in absence of jaundice, is still a matter of speculation. According to the "hepatogenic" theory, the cells of the liver parenchyma or the cholangiolar cell may produce abnormally large quantities of enzyme in diseases of that organ (Freeman 1951). It has also been suggested that owing to changes in the permeability of the diseased liver cells, enzymes might leak out into the general circulation in much the same manner as transaminases (Wilkinson 1962). Although this theory was criticised by Gutman (1959) who favoured the "retention" theory, yet the marked dissociation between serum bilirubin and serum alkaline phosphatase, especially in cirrhosis, creates

difficulties with complete acceptance of the retention theory. Wilkinson (1962) revised the literature on alkaline phosphatase isoenzymes and their Michaelis constants as well as results of 5'nucleotidase and iso-citrate dehydrogenase, and stated that it seems likely that in liver cell disease, the slight or moderate increase in serum alkaline phosphatase activity may be largely due to a contribution from the liver itself. El-Fiky and Bolos (1967) noticed marked changes in the activity of alkaline phosphatase in the form of a generalised increase in the peribiliary area of murine liver cells infested with cercariae of schistosoma mansoni. Jackson (1952) suggested that in the rat, the intestine might be a possible source of the enzyme. Stolbach (1967) also reported that in a certain number of patients with hepatic cirrhosis, a major portion of the total serum alkaline phosphatase is of intestinal origin.

Although cast studies of the biliary system in hepatic bilharziasis revealed the presence of early anatomical changes in the form of constrictions and dilatation (Higazi 1960), yet elevation of serum bilirubin is known to be rare and very late (Mousa et al., 1958). The raised serum alkaline phosphatase observed could, therefore, be mainly explained according to the "hepatogenic" theory, rather than the "retention" theory and partly to the possible intestinal origin of the enzyme. Accordingly, the underlying mechanism for the noticed improvement after cortisone would be the same as mentioned for serum transaminases (Ghanem et al., 1970).

AIM OF THE WORK

AIM OF THE WORK

The aim of this work is:

- 1- To estimate certain enzymes including transaminases, lactate dehydrogenase, alkaline phosphatase, acid phosphatase and creatine phosphokinase in the cerebrospinal fluid of schistosomal patients with liver involvement.
- 2- To compare the results of the various enzymes in hepatic schistosomiasis with normal control.
- 3- To assess the changes of these enzymes in various stages of hepatic schistosomiasis.

MATERIAL AND METHODS

MATERIAL AND METHODS

MATERIAL

This study included 20 inpatients with Hepatic Schistosomiasis from department of Tropical Medicine Alexandria University. They included 18 male and 2 female. Their ages ranged between 15 and 50 years.

Confirmation of the nature of the disease was obtained from the history, clinical examination and rectal biopsy snipping. Patients were classified into 4 groups according to the stage of hepatic involvement:

Group 1: Schistosomal Hepatomegalic stage, includes 5 patients.

Group 2: Schistosomal Hepatosplenomegalic stage, includes 5 patients.

Group 3: Shrunken liver and splenomegaly, includes three patients.

Group 4: Late Ascitic stage, includes 7 patients.

Also ten healthy subjects were chosen as our controls.

Through clinical and laboratory investigations, lung, liver, gastrointestinal and muscle diseases which might affect our results, were excluded.

Fasting specimens of cerebrospinal fluid were aspirated. Special stress was taken to avoid trauma and samples contaminated with blood were discarded. All samples were kept at - 25°C until estimations were carried out.

The following battery of analysis were performed.

- 1- Glutamate oxalacetate transaminase (Reitman and Frankel 1957).
- 2- Glutamate pyruvate transaminase (Reitman and Frankel 1957).
- 3- Lactate dehydrogenase (Cabaud and Wroblewski 1958).
- 4- Alkaline phosphatase (Kind and King 1954).
- 5- Acid phosphatase (Kind and King 1954).
- 6- Creatine phosphokinase (Oliver 1955 and Rosalki 1967).

METHODS

(1&2) ESTIMATION OF TRANSAMINASES

Colorimetric estimation of C.S.F. G.O.T./G.P.T. by the method of Reitman and Frankel (1957).

PRINCIPLE

Glutamic-oxalactic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) act in the following way:

GOT:

Aspartic acid + α -Ketoglutaric acid $\xrightleftharpoons{\text{GOT}}$ Oxalacetic acid + Glutamic acid.

GPT:

Alanine + α -Ketoglutaric acid $\xrightleftharpoons{\text{GPT}}$ Pyruvic acid + Glutamic acid.

The method of Reitman and Frankel consists of estimating the quantity of pyruvic acid or oxalacetic acid formed during the transamination, through 2,4,- dinitrophenyl hydrazine. The corresponding hydrazone which, in an alkaline solution gives a brown colour, can then be measured colorimetrically.

REAGENTS: (Using reagents from bio-Merieux Kit)

Reagent No. 1: GOT substrate.

Solution containing aspartic acid and α -ketoglutaric acid, buffered at pH 7.5.

Reagent No. 2: GPT substrate

Solution containing alanine and ~~α~~-ketoglutaric acid, buffered at pH 7.5.

Reagent No. 3:

2-4,- dinitrophenyl hydrazine solution.

Reagent No. 4: Standard solution.

This reagent is used to prepare the standard curve. It is a solution of sodium pyruvate.

Sodium hydroxide Solution 0.4 N.

METHOD

Preparation of the standard curve:

Set up a row of tubes in the following manner:

Tube number	1	2	3	4	5	6
Distilled water (in ml.)	0.2	0.2	0.2	0.2	0.2	0.2
Reagent No. 1 (in ml.)	1.0	0.9	0.8	0.7	0.6	0.5
Reagent No. 4 (in ml.)	-	0.1	0.2	0.3	0.4	0.5
Reagent No. 3 (in ml.)	1.0	1.0	1.0	1.0	1.0	1.0

Mix and leave at room temperature for 20 minutes. Add:

0.4 N sodium hydroxide	10	10	10	10	10	10
GOT units/ml.	0	22	55	95	150	215
GPT units/ml.	0	25	50	83	126	-

Mix and wait for 5 minutes before reading on the colorimeter. The brown colour which develops is stable for at least 1 hour. Read the optical densities at 505nm. (490-250nm.), against a blank of distilled water.

Draw the standard curve by plotting the percentages transmission against the number of units per ml. Carrying out the reaction: simultaneous estimation of GOT and GPT.

GLUTAMIC OXALACETIC TRANSAMINASE:

Pipette 1ml. of reagent No. 1 (GOT substrate) into a test tube of at least 15ml. capacity. Incubate at 37°C for 5 minutes. Add 0.2ml. of C.S.F. Mix and incubate at 37°C for exactly 1 hour.

GLUTAMIC PYRUVIC TRANSAMINASE:

20 minutes after adding the C.S.F., pipette 1ml. of reagent No. 2 (GPT substrate) into another tube and incubate 5 minutes. Exactly 30 minutes after incubating the tube, add 0.2ml. of C.S.F. to the GPT tube.

Exactly 30 minutes later; remove both tubes. Add 1ml. of reagent No. 3 (dinitrophenyl hydrazine) to each tube, mix and allow to stand at room temperature for 20 minutes.

Add 10ml. of 0.4 N sodium hydroxide solution to each tube. Wait 5 minutes before reading on the colorimeter. The colour is stable for at least 1 hour.

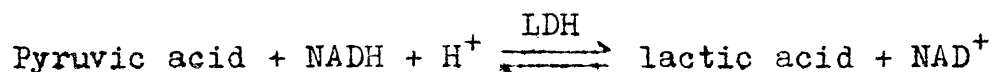
Read at the same wavelength, and use the same cuvettes as for the standard curve. Having found the percentage transmission, determine from the standard curve the number of units of GPT and GOT per ml. of C.S.F.

(3) ESTIMATION OF LACTATE DEHYDROGENASE:

Estimation of lactate dehydrogenases in C.S.F. by colorimetric method of Cabaud and Wroblewski (1958).

PRINCIPLE

Pyruvic acid is converted into lactic acid in the presence of lactate dehydrogenase (LDH) and reduced nicotinamide adenine dinucleotide (NADH). The reaction is summarized below:



The quantity of pyruvic acid converted into lactic acid is directly proportional to the activity.

In the method of Cabaud and Wroblewski, the pyruvic acid not converted, is measured by the colour reaction developed in the presence of 2-4 dinitrophenyl hydrazine.

Consequently, a pyruvate solution of known concentration can be used in conjunction with 2-4 dinitrophenylhydrazine to produce a known optical density. If LDH is added, it will cause conversion of the pyruvate and produce a drop in optical density. The decrease is proportional to the amount of LDH present.

REAGENTS:

(Using reagents from bio Merieux Kit).

Reagent No. 1: Pyruvate substrate: Sodium pyruvate solution buffered at pH 7.5.

Reagent No. 2: NADH

Each vial contains exactly 1mg. of nicotinamide adenine dinucleotide. The enzymatic reaction is carried out directly in the vial.

Reagent No. 3: 2-4 dinitrophenyl hydrazine solution in hydrochloric acid.

Sodium hydroxide Solution 0.4 N

Notes

- Reagent no. 1: Contains a preservative. However it is advisable to handle it as aseptically as possible.
- Reagent No. 3: To be stored in the dark.

METHOD:

Preparation of standard curve:

Set up 6 test tubes and add the reagents in the following order:

Tube numbers	1	2	3	4	5	6
Reagent No. 1 (in ml.)	1.0	0.8	0.6	0.4	0.2	0.1
Distilled water (in ml.)	0.1	0.3	0.5	0.7	0.9	1.0
Reagent No. 3 (in ml.)	1.0	1.0	1.0	1.0	1.0	1.0

Mix and leave at room temperature for 20 min. Add:

0.4 N sodium hydroxide (in ml.)	10	10	10	10	10	10
LDH units per ml. C.S.F.	0	280	640	1,040	1,530	2,000

Mix and allow to stand at room temperature for 30 minutes.

Read the optical densities between 400 and 550 nm. against a distilled water blank.

Draw the standard curve on semi logarithmic paper.

Carrying out the reaction:

Add 1ml. of reagent No. 1 (Pyruvate substrate) to a vial containing reagent No.2 (NADH). Incubate at 37°C for 5 minutes.

Add 0.1ml. of C.S.F. into the vial. Close with a rubber stopper and mix well by inversion. Incubate at 37°C for exactly 30 minutes.

Remove the vial from the water-bath and immediately add 1 ml. of reagent No. 3 (2-4 dinitrophenyl hydrazine). Mix and allow to stand for 20 minutes at room temperature.

Add 10 ml. of 0.4 N sodium hydroxide. Mix and leave at room temperature for 30 minutes.

Transfer to a colorimeter tube (or cuvette) and read at the selected wavelength against a distilled water blank.

CALCULATION

Read the number of LDH units from the standard curve. The result is expressed in number of units of LDH per ml.

(4) ESTIMATION OF ALKALINE PHOSPHATASE

Colorimetric estimation of C.S.F. Alkaline Phosphatase by the modified method of KIND and KING (1954).

"PHOSPHATASE ALKALINE - KIT"

Allows the estimation of C.S.F. alkaline phosphatase using a simple and sensitive colorimetric technique which involves small volumes of C.S.F. and a limited number of ready - prepared reagents.

PRINCIPLE

The enzyme acts on the substrate (disodium phenyl phosphate) at pH 10 to liberate phenol which is estimated in the presence of 4-amino antipyrine and potassium ferricyanide. The

presence of sodium arsenate in the amino antipyrine reagent allows the enzyme reaction to be stopped after incubation at 37°C. Under the conditions of the estimation the red colour produced is proportional to the enzyme activity.

REAGENTS

(Using reagents from bio Merieux Kit).

Reagent No. 1: Substrate. Solution of disodium phenyl phosphate in a carbonate-bicarbonate buffer at pH 10.0.

Reagent No. 2: Standard phenol solution, 20 kind and king units.

Reagent No. 3: Solution of 4-amino antipirryne and sodium arsenate.

Do not pipette this reagent directly and avoid contact with the skin.

Reagent No. 4: Solution of potassium ferricyanide.

These reagents can be stored at + 4°C up to the date printed on the pack; they can only be used for the estimation of alkaline phosphatase.

METHOD

Make up the following range of tubes:

Reagents	C.S.F. Determination	C.S.F. Blank	Standard Determination	Reagent blank
Reagent No. 1	2 ml.	2 ml.	2 ml.	2 ml.

Incubate for 5 minutes in a water bath at 37°C

C.S.F.	0.05 ml.	-	-	-
Reagent No. 2	-	-	0.05 ml.	-

Incubate for exactly 15 minutes in a water bath at 37°C.

Reagent No. 3	0.5 ml.	0.5 ml.	0.5 ml.	0.5 ml.
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Mix well (see note 1)

Reagent No. 4	0.5 ml.	0.5 ml.	0.5 ml.	0.5 ml.
C.S.F.	-	0.05 ml.	-	-
Water	-	-	-	0.05 ml.

Mix and allow to stand for 10 minutes away from direct light; read the tubes against the reagent blank at 510 nm. The colour is stable for at least 45 minutes.

RESULTS

Definition of units:

KIND AND KING UNIT:

This is the amount of enzyme (contained in 100 ml. of serum) which liberates 1 mg. of phenol from a solution of phenyl phosphate buffered at pH 10.0 when incubated for 15 minutes at 37°C.

INTERNATIONAL UNIT:

This is the amount of enzyme contained in a litre of serum which liberates one micromole of phenol per minute under the conditions of the estimation.

- 1 Kind and King unit = 7.09 international units
- Alkaline phosphatase activity:
- In KIND and KING units/100 ml.:

$$\frac{\text{O.D. C.S.F. test} - \text{O.D. C.S.F. blank}}{\text{O.D. Standard estimation}} \times 20$$

- In international units/litre:

$$\frac{\text{O.D. C.S.F. test} - \text{O.D. C.S.F. blank}}{\text{O.D. Standard estimation}} \times 142$$

NOTES:

It is especially important to ensure complete mixing of reagent no. 3 after adding it to the other reagents: the presence of this reagent on the walls of the tube can result in cloudiness when reagent no. 4 is added.

(5) ESTIMATION OF ACID PHOSPHATASE:

Colorimetric Estimation of C.S.F. Acid Phosphatase by the modified method of KIND and KING (1954).

"PHOSPHATASE ACID-KIT":

Allows the estimation of C.S.F. acid phosphatases using a simple and sensitive colorimetric technique involving small quantities of C.S.F.

PRINCIPLE:

The enzyme acts on the substrate (disodium phenyl phosphate) at pH 4.9 to liberate phenol which is estimated in the presence of 4-amino antipyrine and potassium ferricyanide. The presence of sodium arsenate in the amino antipyrine reagent allows the enzyme reaction to be stopped after incubation at 37°C. Under the conditions of the determination the red colour produced is proportional to the enzyme activity.

REAGENTS:

(Using reagents from bio Merieux Kit).

Reagent No. 1: Substrate: disodium phenyl phosphate.

Reagent No. 2: Citrate - Citric acid buffer, pH 4.9.

Reagent No. 3: Standard phenol solution, 20 Kind and King units.

Reagent No. 4: Alkaline solution of 4-amino antipyrine and
sodium arsenate.

Do not pipette this reagent directly and avoid contact
with the skin.

Reagent No. 5: Solution of potassium ferricyanide.

SEPARATE REAGENT:

Solution of sodium L-Tartrate, pH 4.9.

These reagents can be stored at + 4°C up to the date
printed on the pack; they can only be used for the estimation
of acid phosphatase.

METHOD

Make up the following range of tubes:

Reagents	C.S.F. Determination	C.S.F. blank	Standard Determination	Reagent blank
Reagent No. 1	0.5 ml.	0.5 ml.	0.5 ml.	0.5 ml.
Reagent No. 2	0.5 ml.	0.5 ml.	0.5 ml.	0.5 ml.

Mix, incubate in a water bath at 37°C for 5 minutes

C.S.F.	0.05 ml.	-	-	-
Reagent No. 3	-	-	0.05 ml.	-

Incubate for exactly one hour in a water bath at 37°C

Reagent No. 4	1 ml.	1 ml.	1 ml.	1 ml.
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Mix well (See note 1)

Reagent No. 5	1 ml.	1 ml.	1 ml.	1 ml.
C.S.F.	-	0.05 ml.	-	-
Water	-	-	-	0.05 ml.

Mix and allow to stand for 10 minutes away from direct light; read the tubes against the reagent blank at 510 nm. The colour is stable for at least 45 minutes.

RESULTS:

Definitions of units:

KIND AND KING UNIT:

This is the amount of enzyme (contained in 100 ml. of serum) which liberates 1 mg. of phenol from a solution of phenyl phosphate buffered at pH 4.9 when incubated for one hour at 37°C.

INTERNATIONAL UNIT:

This is the amount of enzyme contained in a litre of serum which liberates one micromole of phenol per minute under the conditions of the estimation.

1 Kind and King unit = 1.77 International units.

- Acid phosphatase activity.

- In KIND and KING units/100 ml.:

$$\frac{\text{O.D. C.S.F. test} - \text{O.D. C.S.F. blank}}{\text{O.D. Standard estimation}} \times 20$$

- In International units/litre:

$$\frac{\text{O.D. C.S.F. test} - \text{O.D. C.S.F. blank}}{\text{O.D. Standard estimation}} \times 35.4$$

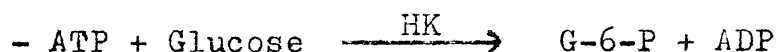
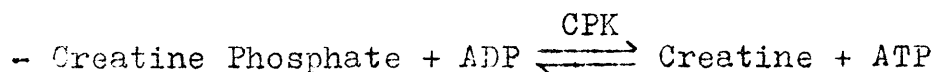
NOTES:

It is especially important to ensure complete mixing of reagent No. 4 after adding it to the other reagents: the presence of this reagent on the walls of the tube can result in cloudiness when reagent No. 5 is added.

(6) ESTIMATION OF CREATINE PHOSPHOKINASE:

Colorimetric Estimation of C.S.F. Creatine phosphokinase by the modified method of Oliver (1955) and Rosalki (1967).

TEST PRINCIPLE:



In this reaction, creatine phosphokinase (CPK) catalyzes the reaction in which ATP is formed from ADP and creatine phosphate. ATP subsequently phosphorylates glucose in the presence of hexokinase, and the formed glucose -6- phosphate (G-6-P) reduces NADP to give NADPH. The rate of formation of NADPH is measured photometrically and is proportional to the CPK activity of the sample.

REAGENT COMPOSITION: (Using reagents from Calbiochem Kit).

"A" vials (reagent) when reconstituted according to instructions, will yield a solution containing approximately:

- PIPES buffer 0.05 moles/liter
- Glucose 1.45×10^{-2} moles/liter
- AMP 9.1×10^{-3} moles/liter
- Creatine phosphate 1.8×10^{-2} moles/liter
- ADP 1.1×10^{-3} moles/liter
- G-6-PDH (microbial) 333 IU/liter
- HK (microbial) 1000 IU/liter
- Sulfhydryl activator
- Non-reactive stabilizer
- Activators
- pH 6.8

"B" vials (Cofactor) when reconstituted according to instructions, will yield approximately:

- NADP 5×10^{-4} moles/liter
- Non-reactive
- Stabilizer

PRECAUTIONS:

Vial contents may be under vacuum. If there is any indication moisture has penetrated the seal, discard the vial in question.

RECONSTITUTION OF THE REAGENTS:

Open one of the vials labeled A and one labeled B; dissolve all of the material from both vials in a total of 155 ml. of distilled water. Mix by gentle stirring.

DO NOT SHAKE.

STORAGE AND STABILITY:

Store unopened vials between 2° and 8°C. The reconstituted and combined reagent A and B is stable for at least 48 hours when stored in a glass container between 2° and 8°C.

In evidence of bacterial contamination appears, discard the solution.

If the well-mixed reagent shows an intial absorbance measured against distilled water of more than 0.6 at 340 nm., consider the reagent unsuitable for CPK assay and discard.

INTERFERING SUBSTANCES:

A number of drugs and substances affect the activity of CPK. Young, et al., (1975) have published a comprehensive list of such substances.

PREPARATION OF THE PATIENT:

No special preparation of the patient is required.

TEMPERATURE FACTORS:

The temperature is critical and must be kept constant during the reaction. At temperatures below 22°C, the reaction rate may be too slow, while above 37°C, the enzyme may be inactivated.

TEMP °C	FACTOR	TEMP °C	FACTOR
22	1.85	30	1.00
23	1.72	31	0.93
24	1.59	32	0.85
25	1.47	33	0.79
26	1.35	34	0.73
27	1.27	35	0.68
28	1.16	36	0.63
29	1.08	37	0.58

PROCEDURE:

Depending on the technical equipment available, specimen/assay volume ratios may vary.

- 1- Using a suitable pipet, dispense 3.0 ml. of the prepared reagent into a clean, dry cuvet with a 1 cm light path.
- 2- All readings should be made at 340 nm. using water as a blank.

- 3- Place the cuvet in a constant-temperature waterbath for three minutes or as long as needed to bring the reagent to the established temperature. Preincubate the C.S.F. for the same length of time in the same bath.
- 4- Add 0.100 ml. (100 ul) of C.S.F. to the cuvet. Mix quickly by gentle inversion with a square of parafilm over the mouth of the cuvet. Avoid shaking as this can trap air bubbles in the solution. Wipe the cuvet dry and immediately insert into the temperature-controlled cell compartment of the photometer. A constant temperature must be maintained while obtaining assay values.
- 5- Read the initial absorbance (A_0) approximately 6 minutes after placing the cuvet in the instrument. Simultaneously, start a timer.
- 6- Exactly 5 minutes after the initial reading, determine the final absorbance (A_5).
- 7- If the absorbance change of a sample is more than 0.25/5 minutes (CPK activity greater than 250 mU/ml.), repeat the test using less sample. Use dilution factor D to compensate for the dilution.

CALCULATION:

CPK activity in mU/ml. = ΔA (for 5 minutes) $\times$ 1000 $\times$ F $\times$ D.

ΔA is the observed change in absorbance in the 5-minute period.

1000 is the conversion factor whose derivation is described later.

F is a temperature factor and D is a dilution factor.

- 1- ΔA , the change in absorbance, is obtained by subtracting the initial absorbance (A_0) from the final absorbance (A_5).

$$\Delta A \text{ (for 5 minutes)} = A_5 - A_0$$

- 2- To obtain CPK values in International milli-units per milliliter, multiply ΔA by 1000 if a 5-minute incubation was used.

- 3- If the assay was not carried out at 30°C, multiply the value for ΔA obtained by the appropriate temperature correction factor F obtained from the temperature table.

- 4- If the sample is too active, use less sample. The actual amount of sample used determines the dilution factor D as follows:

D = 1, when 100 ul. of sample are used.

D = 1.97, when 50 ul. of sample are used.

D = 4.86, when 20 ul. of sample are used.

If D is other than 1, multiply the answer by this dilution factor.

- 5- Calculation example (in International milli-units):

When $A_0 = 0.350$ and $A_5 = 0.470$ at 30°C.

When using 100 ul. of sample (F and D both equal 1):

$$\Delta A \text{ (for 5 minutes)} = 0.470 - 0.350 = 0.120$$

$$0.120 \times 1000 = 120 \text{ mU/ml. at } 30^\circ\text{C.}$$

RESULTS

RESULTS

Table I: Cerebrospinal Fluid Glutamic Oxalacetic Transaminase in Schistosomiasis. (Reitman and Frankel units):

Case No.	Control units/ml	Hepatomegaly units/ml	Hepatosple- nomegaly units/ml	Splenomegaly units/ml	Ascites units/ml
1	10.5	18.0	7.0	11.0	13.0
2	13.0	7.0	15.0	26.0	16.0
3	7.0	11.0	13.0	15.0	18.0
4	7.0	7.0	11.0		7.0
5	7.0	29.0	13.0		15.0
6	13.0				11.0
7	11.0				18.0
8	9.0				
9	7.0				
10	9.0				
Range:	7.0-13.0	7.0-29.0	7.0-15.0	11.0-26.0	7.0-18.0
Mean:	9.35	14.4	11.8	17.33	14.0
S.D.:	2.4272	9.3166	3.0331	7.7674	4.0000
t :	-	1.6617	1.7017	3.0507	2.9936
P :	-	>0.05	>0.05	<0.05	<0.05

Table II: Cerebrospinal Fluid Glutamic Pyruvic Transaminase
in Schistosomiasis. (Reitman and Frankel units):

Case No.	Control units/ml	Hepatomegaly units/ml	Hepatosple- nomegaly units/ml	Splenomegaly units/ml	Ascites units/ml
1	3.0	0	0	3.0	3.0
2	4.0	3.0	3.0	4.0	4.0
3	4.0	4.0	4.0	6.0	8.0
4	4.0	3.0	4.0		3.0
5	4.0	3.0	4.0		0
6	0				6.0
7	3.0				6.0
8	4.0				
9	6.0				
10	3.0				
Range:	0-6.0	0-4.0	0-4.0	3.0-6.0	0-8.0
Mean:	3.50	2.60	3.00	4.33	4.29
S.D.:	1.5092	1.5165	1.7320	1.5275	2.6276
t :	-	1.0871	0.5773	0.8336	0.7889
P :	-	>0.05	>0.05	>0.05	>0.05

Table III: Cerebrospinal Fluid Lactate Dehydrogenase in Schistosomiasis. (Cabaud and Wroblewski units):

Case No.	Control units/ml	Hepatomegaly units/ml	Hepatosple- nomegaly units/ml	Splenomegaly units/ml	Ascites units/ml
1	11.7	30.0	11.7	15.8	15.8
2	15.8	11.7	27.5	30.0	15.8
3	15.8	15.8	20.0	15.8	30.0
4	11.7	8.3	11.7		8.3
5	8.3	27.5	20.0		11.7
6	20.0				11.7
7	15.8				8.3
8	10.0				
9	23.0				
10	4.0				
Range:	4.0-23.0	8.3-30.0	11.7-27.5	15.8-30.0	8.3-30.0
Mean:	13.61	18.66	18.18	20.53	14.51
S.D.:	5.6200	9.6266	6.6608	8.1983	7.4854
t :	-	1.2989	1.4000	1.7039	0.2839
P	-	>0.05	>0.05	>0.05	>0.05

Table IV: Cerebrospinal Fluid Creatine Phosphokinase in Schistosomiasis. (Oliver and Rosalki units):

Case No.	Control mU/ml	Hepatomegaly mU/ml	Hepatosple- nomegaly mU/ml	Splenomegaly mU/ml	Ascites mU/ml
1	21.0	6.0	12.0	7.5	12.0
2	25.5	7.5	6.0	7.5	7.5
3	7.5	6.0	9.0	6.0	15.0
4	43.5	6.0	7.5		12.0
5	31.5	9.0	22.5		7.5
6	7.5				7.5
7	7.5				12.0
8	12.0				
9	24.0				
10	25.0				
Range:	7.5-43.5	6.0-9.0	6.0-22.5	6.0-7.5	7.5-15.0
Mean:	20.5	6.9	11.4	7.0	10.5
S.D.:	11.9582	1.3416	6.5897	0.8660	3.0000
t :	-	2.4885	1.5673	1.8948	2.1461
P :	-	<0.05	>0.05	>0.05	>0.05

Table V: Cerebrospinal Fluid Enzymes in healthy controls.

Case No.	G.O.T. units/ml	G.P.T. units/ml	L.D.H. units/ml	C.P.K. mU/ml
1	10.5	3.0	11.7	21.0
2	13.0	4.0	15.8	25.5
3	7.0	4.0	15.8	7.5
4	7.0	4.0	11.7	43.5
5	7.0	4.0	8.3	31.5
6	13.0	0	20.0	7.5
7	11.0	3.0	15.8	7.5
8	9.0	4.0	10.0	12.0
9	7.0	6.0	23.0	24.0
10	9.0	3.0	4.0	25.0
Mean:	9.35	3.50	13.61	20.5
S.D.:	2.4272	1.5092	5.6200	11.9582

Table VI: Cerebrospinal Fluid Enzymes in Hepatomegaly.

Case No.	G.O.T. units/ml	G.P.T. units/ml	L.D.H. units/ml	C.P.K. mU/ml
1	18.0	0	30.0	6.0
2	7.0	3.0	11.7	7.5
3	11.0	4.0	15.8	6.0
4	7.0	3.0	8.3	6.0
5	29.0	3.0	27.5	9.0
Mean:	14.4	2.6	18.66	6.9
S.D.:	9.3166	1.5165	9.6266	1.3416

Table VII: Cerebrospinal Fluid Enzymes in Hepatosplenomegaly.

Case No.	G.O.T. units/ml	G.P.T. units/ml	L.D.H. units/ml	C.P.K. mU/ml
1	7.0	0	11.7	12.0
2	15.0	3.0	27.5	6.0
3	13.0	4.0	20.0	9.0
4	11.0	4.0	11.7	7.5
5	13.0	4.0	20.0	22.5
Mean:	11.8	3.0	18.18	11.4
S.D.:	3.0331	1.7320	6.6608	6.5897

Table VIII: Cerebrospinal Fluid Enzymes in Splenomegaly.

Case No.	G.O.T. units/ml	G.P.T. units/ml	L.D.H. units/ml	C.P.K. mU/ml
1	11.0	3.0	15.8	7.5
2	26.0	4.0	30.0	7.5
3	15.0	6.0	15.8	6.0
Mean:	17.33	4.33	20.53	7.0
S.D.:	7.7674	1.5275	8.1983	0.8660

Table IX: Cerebrospinal Fluid Enzymes in Ascites.

Case No.	G.O.T. units/ml	G.P.T. units/ml	L.D.H. units/ml	C.P.K. mU/ml
1	13.0	3.0	15.8	12.0
2	16.0	4.0	15.8	7.5
3	18.0	8.0	30.0	15.0
4	7.0	3.0	8.3	12.0
5	15.0	0	11.7	7.5
6	11.0	6.0	11.7	7.5
7	18.0	6.0	8.3	12.0
Mean:	14.0	4.29	14.51	10.5
S.D.:	4.0000	2.6276	7.4854	3.0000

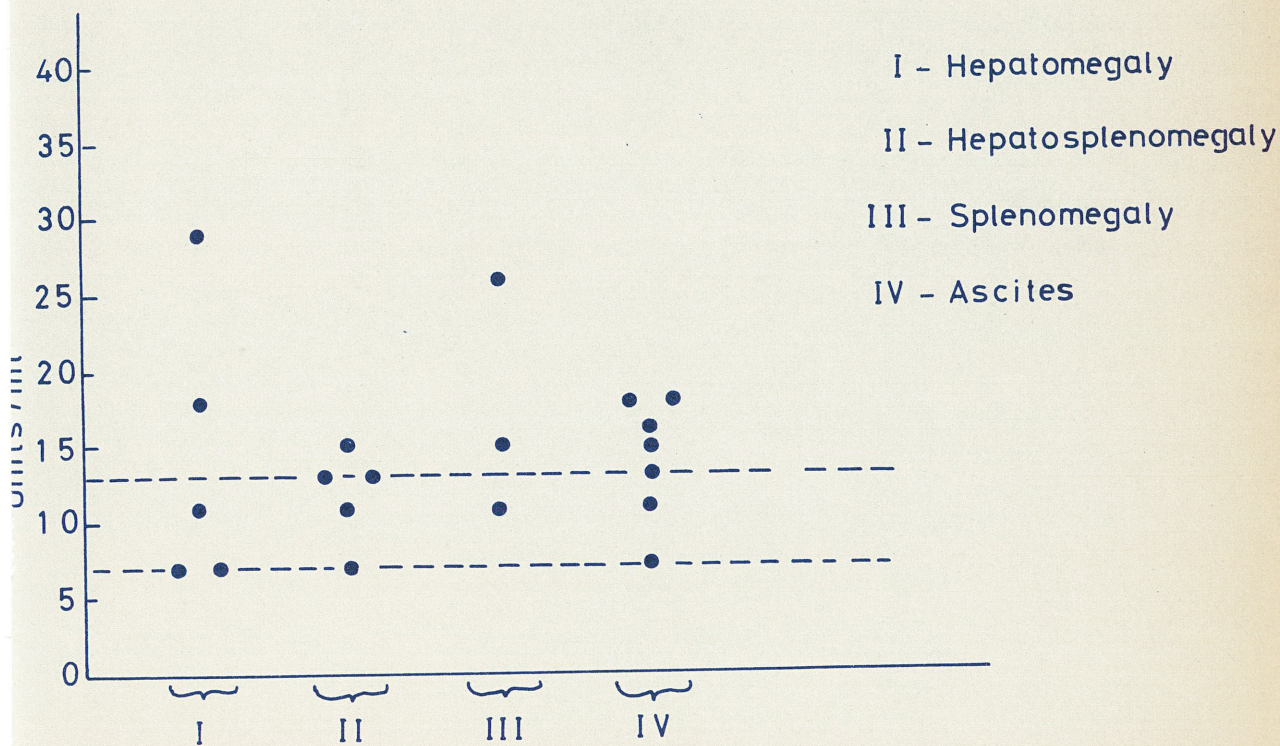
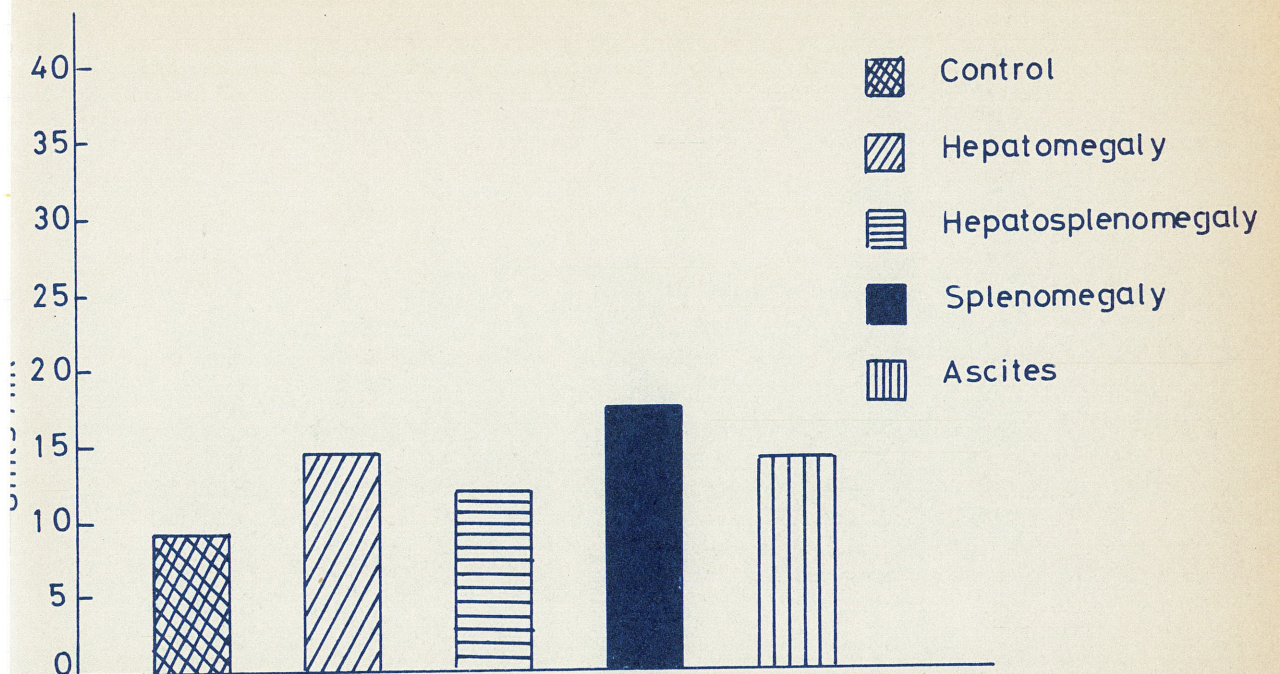


Fig (1) Cerebrospinal-fluid Glutamic Oxalacetic Transaminase
in Schistosomiasis

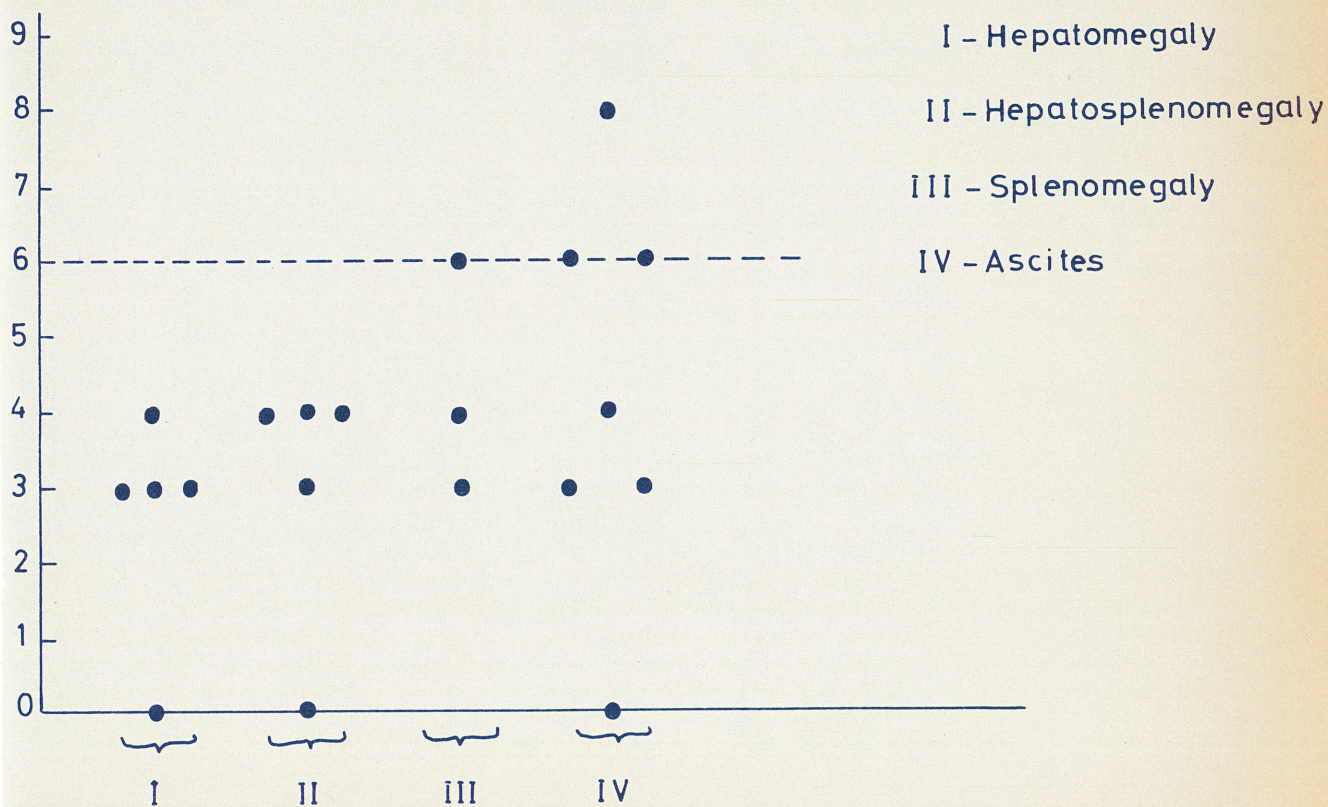
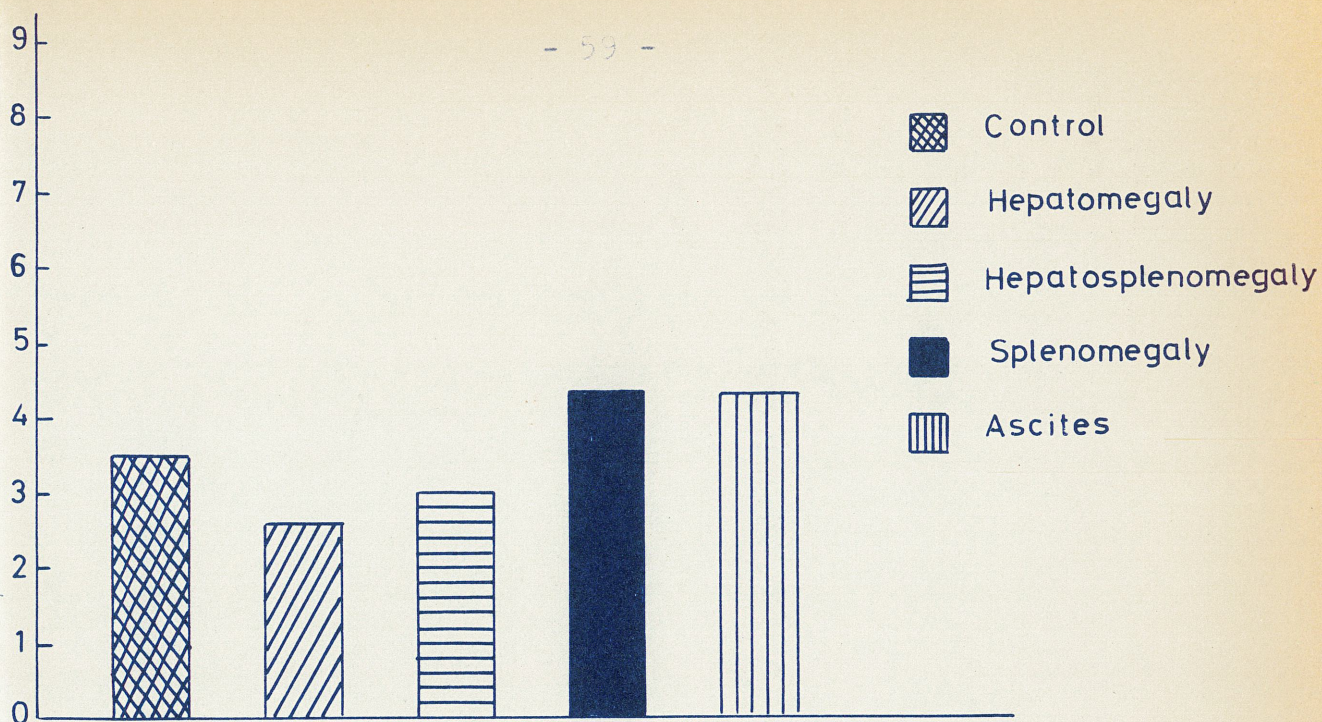


Fig (2) Cerebrospinal -fluid Glutamic Pyruvic Transaminase
in Schistosomiasis

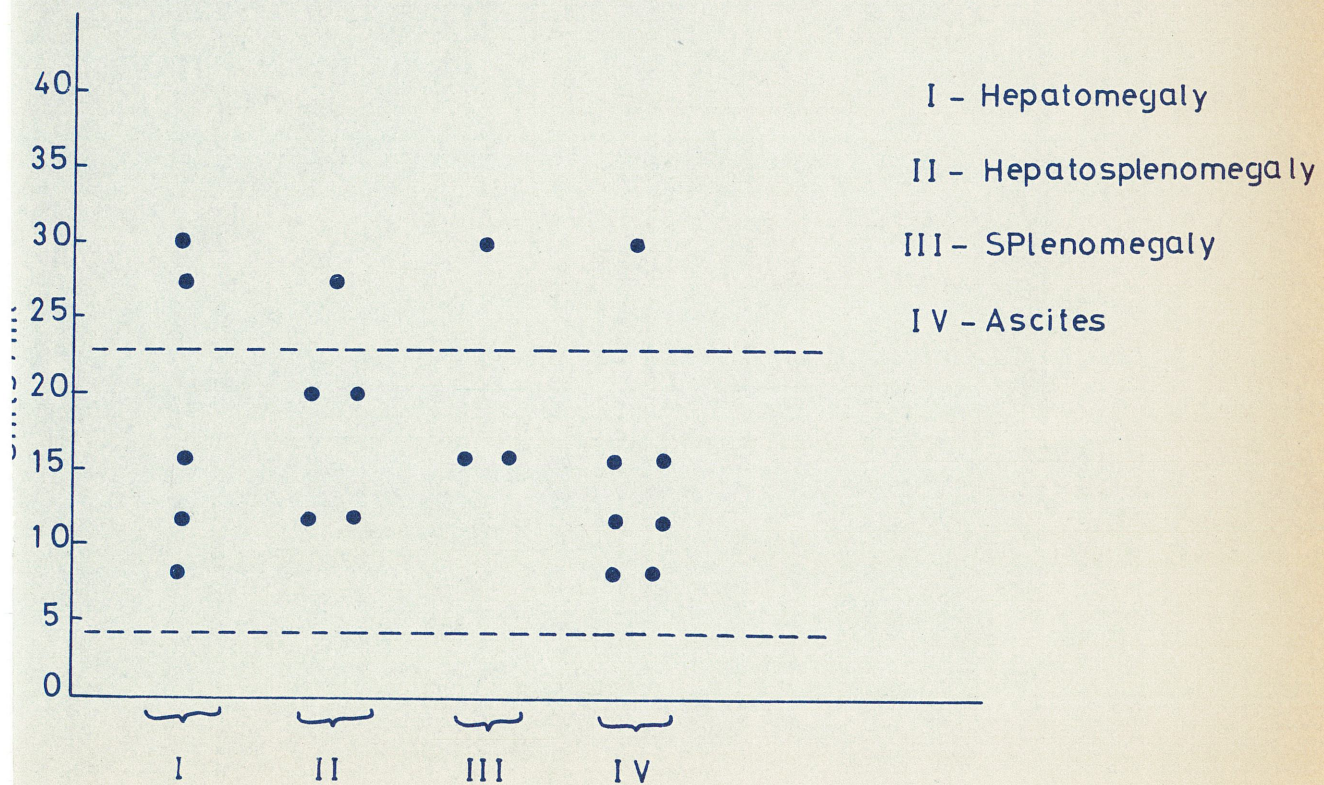
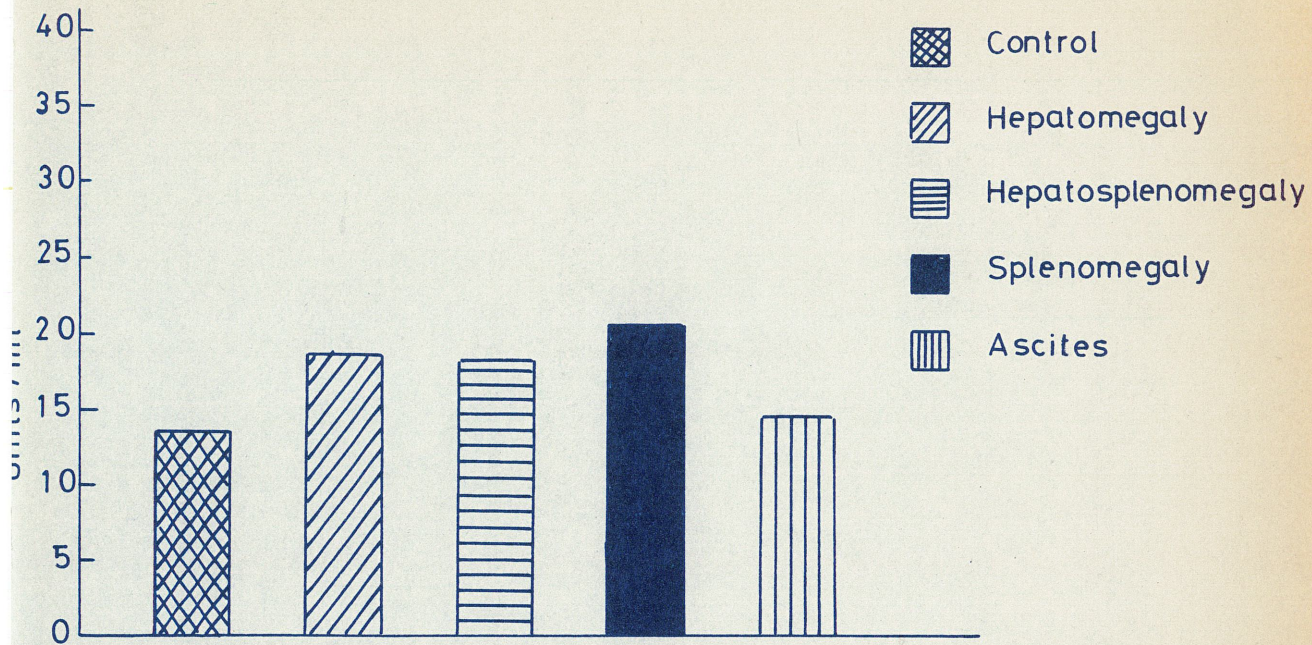


Fig (3) Cerebrospinal-fluid Lactate Dehydrogenase in Schistosomiasis

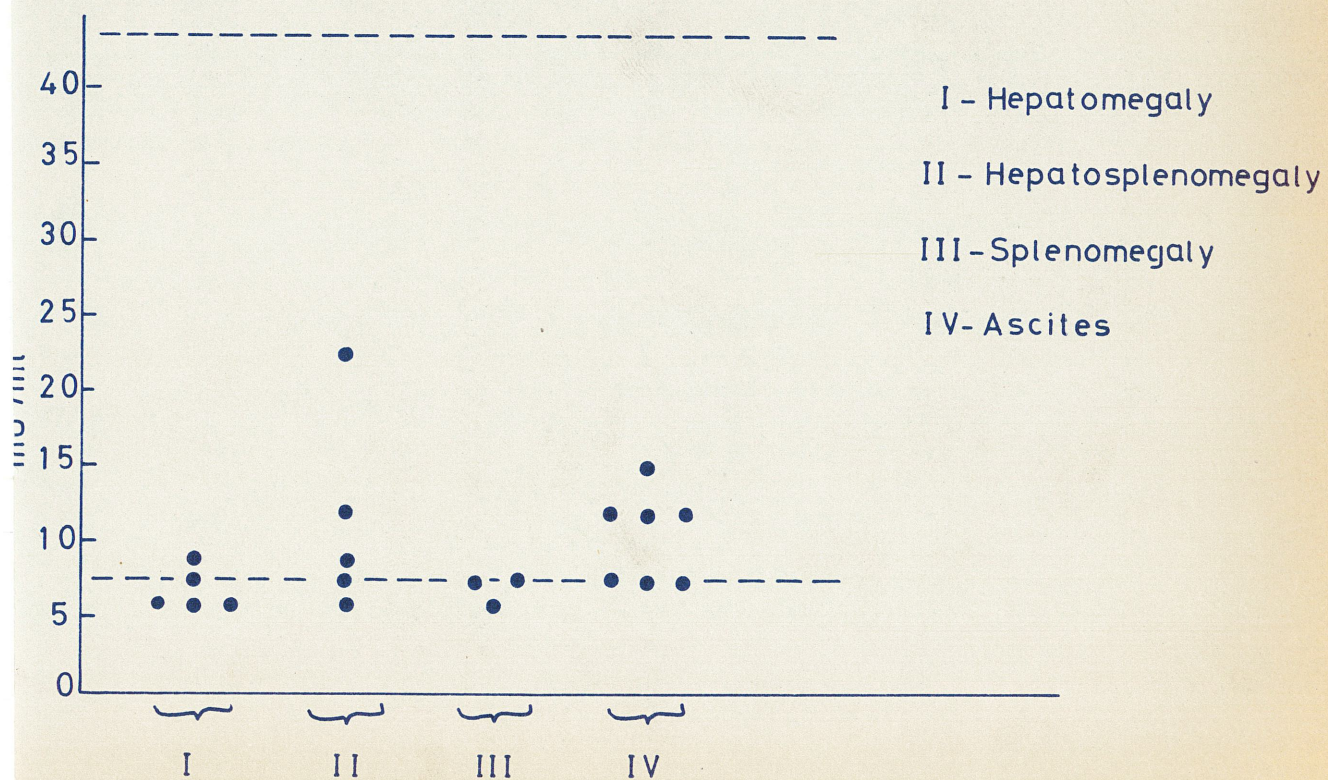
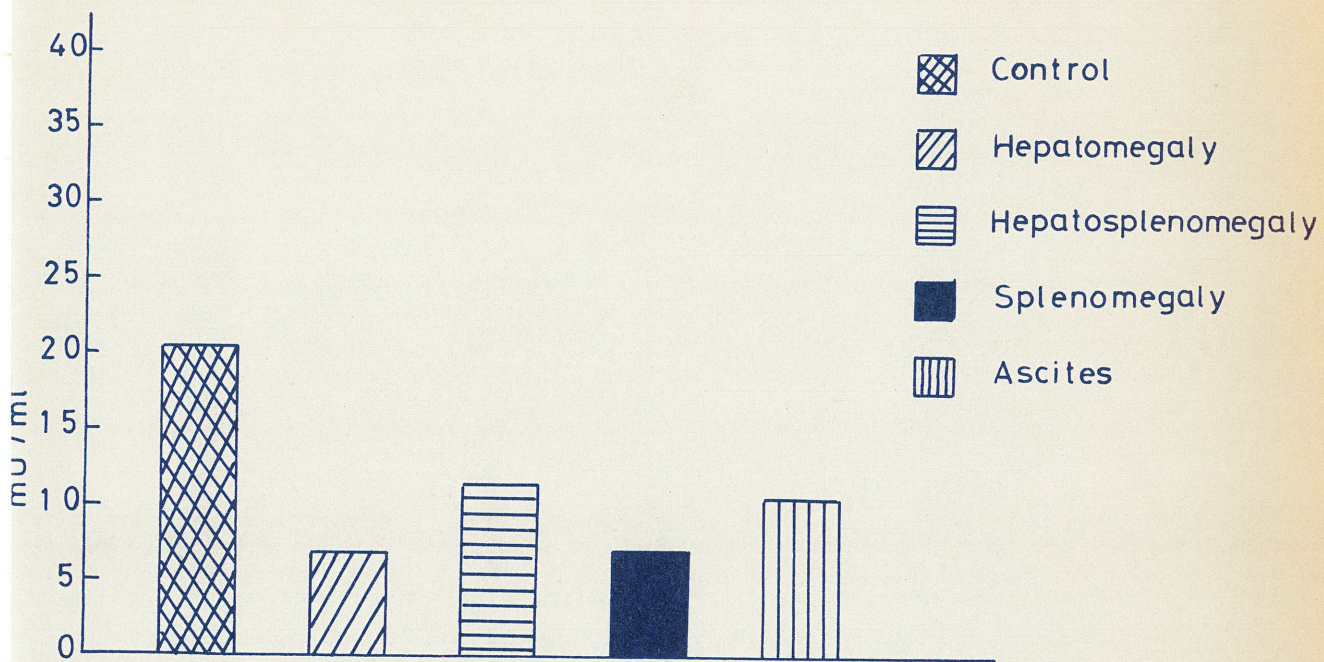


Fig (4) Cerebrospinal-fluid Creatine Phosphokinase in Schistosomiasis

1- Cerebrospinal fluid glutamic oxalacetic transaminase

(Table I, Fig. 1):

The C.S.F. G.O.T. of normal control ranged from 7.0 to 13.0 units per milliliter with a mean of 9.35 units per milliliter (S.D.= 2.4272).

In hepatomegaly the mean is 14.4 units per milliliter with a range 7.0 to 29.0 units per milliliter (S.D.= 9.3166). This mean is not significantly different from the normal.

In hepatosplenomegaly, the mean is 11.8 units per milliliter with a range 7.0 to 15.0 units per milliliter (S.D.=3.0331). This mean is not significantly different from the normal.

In splenomegaly the mean is 17.33 units per milliliter with a range 11.0 to 26.0 units per milliliter (S.D.= 7.7674). The mean value of patients with splenomegaly is significantly higher than the normal ($P < 0.05$).

In ascites the mean is 14.0 units per milliliter with a range 7.0 to 18.0 units per milliliter (S.D.= 4.0000). The mean value of patients with ascites is significantly higher than the normal ($P < 0.05$).

2- Cerebrospinal fluid glutamic pyruvic transaminase

(Table II, Fig. 2):

The C.S.F. G.P.T. of normal control ranged from 0 to 6.0 units per milliliter with a mean of 3.5 units per milliliter (S.D.= 1.5092).

In hepatomegaly the mean is 2.6 units per milliliter with a range 0 to 4.0 units per milliliter (S.D.= 1.5165). This mean is not significantly different from the normal.

In hepatosplenomegaly the mean is 3.0 units per milliliter with a range 0 to 4.0 units per milliliter (S.D.= 1.7320). This mean is not significantly different from the normal.

In splenomegaly the mean is 4.33 units per milliliter with a range 3.0 to 6.0 units per milliliter (S.D.= 1.5275). This mean is not significantly different from the normal.

In ascites the mean is 4.29 units per milliliter with a range 0 to 8.0 units per milliliter (S.D.= 2.6276). This mean is not significantly different from the normal.

3- Cerebrospinal fluid lactate dehydrogenase (Table III, Fig. 3):

The C.S.F. L.D.H. of normal control ranged from 4.0 to 23.0 units per milliliter with a mean of 13.61 units per milliliter (S.D.= 5.6200).

In hepatomegaly the mean is 18.66 units per milliliter with a range 8.3 to 30.0 units per milliliter (S.D.= 9.6266). This mean is not significantly different from the normal.

In hepatosplenomegaly the mean is 18.18 units per milliliter with a range 11.7 to 27.5 units per milliliter (S.D. = 6.6608). This mean is not significantly different from the normal.

In splenomegaly the mean is 20.53 units per milliliter with a range 15.8 to 30.0 units per milliliter (S.D.= 8.1983). This mean is not significantly different from the normal.

In ascites the mean is 14.51 units per milliliter with a range 8.3 to 30.0 units per milliliter (S.D.= 7.4854). This mean is not significantly different from the normal.

4- Cerebrospinal fluid creatine phosphokinase (Table IV, Fig. 4):

The C.S.F. C.P.K. of normal control ranged from 7.5 to 43.5 International milli units per milliliter with a mean of 20.5 International milli units per milliliter (S.D.= 11.9582).

In hepatomegaly the mean is 6.9 International milli units per milliliter with a range 6.0 to 9.0 International milli units per milliliter (S.D.= 1.3416). The mean value of patients with hepatomegaly is significantly lower than the normal ($P < 0.05$).

In hepatosplenomegaly the mean is 11.4 International milli units per milliliter with a range 6.0 to 22.5 International milli units per milliliter (S.D.= 6.5897). This mean is not significantly different from the normal.

In splenomegaly the mean is 7.0 International milli units per milliliter with a range 6.0 to 7.5 International milli units per milliliter (S.D.= 0.8660). This mean is not significantly different from the normal.

In ascites the mean is 10.5 International milli units per milliliter with a range 7.5 to 15.0 International milli units per milliliter (S.D.= 3.0000). This mean is not significantly different from the normal.

DISCUSSION

DISCUSSION

CEREBROSPINAL FLUID TRANSAMINASES:

Glutamic oxalacetic and pyruvic transaminases (Table I & II, and Fig. 1 & 2):

In normal controls C.S.F. glutamic oxalacetic transaminase ranged from 7 to 13 Reitman and Frankel units with a mean of 9.35 R & F units. In patients with schistosomiasis, in the hepatomegalic stage, the mean value was 14.4 R & F units while in hepatosplenomegaly it was 11.8 R & F units. These values were statistically insignificant compared to controls. A significant increase ($P < 0.05$) of C.S.F. glutamic oxalacetic transaminase was noticed in splenomegalic and late ascitic stages with mean of 17.33 and 14.0 R & F units respectively.

Cerebrospinal fluid glutamic pyruvic transaminase of normal controls ranged between 0 to 6 Reitman and Frankel units with a mean of 3.5 R & F units. A non significant change was found in the various stages of hepatic schistosomiasis.

In patient with hepatic schistosomal involvement several authors reported elevation of serum transaminases (Saif et al., 1964; Khattab et al., 1967; Ghanem et al., 1970 and Salah et al., 1976). Various mechanisms may underly such an elevation. Active hepatic cell damage and an increased cell membrane permeability

(Khattab et al., 1967), associated viral affection, malnutrition, antischistosomal therapy (Ghanem et al., 1970) were reported. An extrahepatic factor which might contribute to the higher elevation of serum transaminases is skeletal muscle damage and wasting associated with schistosomiasis (Mansour et al., 1965; Ghanem et al., 1970).

In the present study the elevation of G.O.T. in the C.S.F. was more manifest in late stages of the disease. Such a finding parallels the previously reported rise in serum enzymes in such patients.

Several factors may be of aetiologic importance in the rise of C.S.F. G.O.T. The simple dialysis of the enzyme from the blood to C.S.F. cannot be suggested as the enzyme is usually protein bound. Furthermore the lower levels in C.S.F. than serum stands against such possibility.

Transfer of already elevated serum transaminases to C.S.F. through blood brain barrier may be an operating cause. The presence of the blood brain barrier with respect to G.O.T. was mentioned in a report by La Due and Wroblewski (1955).

Wakin and Fleisher (1956) showed a marked rise of spinal fluid transaminases in experimental cerebral infarction but not after acute hepatic poisoning and demonstrated only minimal

transfer of the enzyme between the vascular and sub-arachnoid spaces after transaminase concentrates were injected intravenously or intracisternally.

The noticed drop of G.O.T. level in serum of patients with ascites may denote escape of some of the already elevated serum enzyme into ascitic fluid (Ghanem et al., 1970).

Ghanem et al., (1973) reported transaminases level in ascitic fluid with a ratio of 0.85 between ascitic fluid and serum, and stressed the importance of presence of peritoneal barrier.

Glutamic oxalacetic transaminase is present in high concentration in nervous tissue (Davies - Jones, 1969). Although the possibility exists of nervous origin of C.S.F. transaminases, yet no clinical evidence of schistosomal affection was found.

Analysis of C.S.F. showed no remarkable increase of red cells in C.S.F. or haemolysis as haemolysed samples may lead to elevation of the enzymes. Moreover the increase occurred in G.O.T. alone.

Again it must be stressed that no patients were under any medicament or drug therapy that may lead to alteration of the transaminases.

The lack of any demonstrable change of G.P.T. C.S.F. in hepatic schistosomiasis may be due to absence of any cerebrospinal fluid transfer mechanism for such enzyme and also to its higher molecular weight.

CEREBROSPINAL FLUID LACTATE DEHYDROGENASE (Table III and Fig. 3):

Cerebrospinal fluid of normal controls showed a mean of 13.61 Cabaud and Wroblewski units with a range of 4 to 23 C & W units (S.D. = 5.62). In schistosomal patients a rise of lactate dehydrogenase amounts to 18.66, 18.18, 20.53 and 14.51 C & W units were found in hepatomegaly, hepatosplenomegaly, splenomegaly and ascitic groups respectively. These values were statistically insignificant compared to that of controls.

Estimation of lactate dehydrogenase in serum was found to be of considerable value in the diagnosis of acute liver disease. It is usually raised in virus hepatitis, toxic hepatitis and infectious mononucleosis. In chronic liver disease, such as cirrhosis, normal or slightly elevated levels are invariably found (Wu and Sung, 1962).

Ghanem et al., (1970) reported values with normal levels in serum of hepatic bilharziasis. In the presence of ascites the mean values was slightly increased. They found much higher

levels with appearance of jaundice and in presence of malignant transformation. They reported activity of L.D.H. in ascitic fluid to be 25.8 per cent of the serum value.

Salah et al., (1976) demonstrated normal serum total L.D.H. activity in hepatic schistosomiasis with increase of L.D.H₄.

Lactate dehydrogenase is a glycolytic enzyme which catalyses the inter-conversion of lactate and pyruvate. Various mechanisms may underly the mild increase of L.D.H. in schistosomiasis. Increased rate of release from the liver cells in the advanced and late stages of the disease may be of importance. Although the pathological lesion in hepatic bilharziasis is mainly mesenchymal, yet, minor degenerative changes do occur in the hepatic cells in the territory of the fibrosed portal tracts (Erfan, et al., 1957). Altered cell memberane permeability may be of significance (Ghanem et al., 1970). An extrahepatic factor which might contribute to elevate of serum LDH is the skeletal muscle. This postulation is based on the clinical observation of striking muscle wasting in these cases and on the finding of Mansour et al., (1965) that myopathy occurs in mice infected with schistosoma mansoni.

The C.S.F. activity of L.D.H. may originate from cells in C.S.F., from central nervous system or passage through blood brain barrier.

Although higher values of L.D.H. in C.S.F. were reported with malignant tumours and infection of nervous system (Fleisher et al., 1959) yet our cases showed no clinical manifestation of associated nervous diseases.

Wroblewski and Wroblewski (1958) gave the earliest report about the greater L.D.H. activity of serous effusions containing or bathing malignant cells. Fleisher et al., (1959) related the increased level in malignant effusion to leakage from tumour cells in which glycolysis supplies most of the energy. This is helped by the associated condition of anoxia and of a limited supply of glucose in these cases. Wroblewski (1961) added that necrotic and destroyed leukocytes and erythrocytes contribute to the increased activity of L.D.H. in these effusions. It must be pointed out that our samples of C.S.F. had no gross increase of leukocytes or red cells which may account for presence of increased L.D.H. activity.

Altered blood brain barrier have been suggested by Beaty et al., (1968), but this altered barrier is not possible working factor since the serum L.D.H. in our cases have not been elevated to higher values.

CEREBROSPINAL FLUID ACID AND ALKALINE PHOSPHATASES:

In the present study samples of C.S.F. showed no acid and alkaline phosphatase activity. Although patients with hepatic bilharzial involvement are known to be associated with increase serum alkaline phosphatase and normal acid phosphatase (Ghanem et al., 1970) yet, their C.S.F. has no enzyme activity.

Such demonstrable finding may point to absence of any transfer through C.S.F. barrier and or to presence of specific inhibitor to these enzymes.

CEREBROSPINAL FLUID CREATINE PHOSPHOKINASE (Table IV and Fig. 4):

In normal control C.S.F. creatine phosphokinase ranged from 7.5 to 43.5 Oliver and Rosalki units with a mean of 20.5 O & R units (S.D. = 11.9582). Significant lowering ($p < 0.05$) occurs in the stage of hepatomegaly in which a mean value of 6.9 O & R units was found. A non significant diminution was found in hepatosplenomegaly, splenomegaly, and in patients with ascites.

Creatine phosphokinase catalyses the reversible formation of adenosine triphosphate and creatine from adenosine diphosphate and creatine phosphate.

Lowering of creatine phosphokinase may originate from several mechanisms. Diminished transfer of this enzyme through C.S.F. blood barrier may be of importance.

Many patients with hepatic schistosomiasis are usually emaciated and associated with diminished muscle mass. Mansour et al., (1965) pointed out the presence of myopathy in bilharziasis and this in turn may lead to diminished muscle function and lowered creatine output.

Mueller et al., (1975) found lowered serum creatine phosphokinase in patients with high alkaline phosphatase activity. Elevated serum alkaline phosphatase levels were found in hepatic schistosomiasis (Ghanem et al., 1970).

In patients with hepatic bilharziasis serum alkaline phosphatase are known to show slight increase in various stages of the disease (Ghanem et al., 1970).

In hepatic disease Nissen et al., (1965) demonstrated normal creatine phosphokinase in the serum and considered to be more specific than G.O.T. for hepatic damage due to congestive heart failure.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The effect of active schistosomiasis on the activity of some cerebrospinal fluid enzymes of hepatic origin has been studied in 20 patients with hepatic schistosomiasis and 10 healthy controls. Patients were classified into four groups according to the stage of hepatic involvement:

Group 1: Schistosomal Hepatomegalic stage.

Group 2: Schistosomal Hepatosplenomegalic stage.

Group 3: Shrunken liver and Splenomegaly.

Group 4: Late Ascitic stage.

These enzymes included transaminases, lactate dehydrogenase, alkaline phosphatase, acid phosphatase and creatine phosphokinase.

The studies revealed the following results.

I- Cerebrospinal fluid transaminases:

Cerebrospinal fluid glutamic oxalacetic transaminase of controls shows a mean of 9.35 Reitman and Frankel units. In hepatomegaly, hepatosplenomegaly, splenomegaly and late ascitic stages, the mean values were 14.4, 11.8, 17.33 and 14.0 R & F units respectively. A significant increase ($P < 0.05$) of cerebrospinal fluid glutamic oxalacetic transaminase was noticed in splenomegalic and late ascitic stages.

In normal controls, the mean value of cerebrospinal fluid glutamic pyruvic transaminase was 3.5 Reitman and Frankel units. The mean values of hepatomegaly, hepatosplenomegaly, splenomegaly and late ascitic stages were 2.6, 3.0, 4.33 and 4.29 R & F units. Glutamic pyruvic transaminase was not significantly different in the various stages of hepatic schistosomiasis.

II- Cerebrospinal fluid lactate dehydrogenase:

The mean value of control of cerebrospinal fluid lactate dehydrogenase was 13.61 Cabaud and Wroblewski units. A rise of lactate dehydrogenase in schistosomal patients with a mean of 18.66, 18.18, 20.53 and 14.51 C & W units in the hepatomegaly, hepatosplenomegaly, splenomegaly and in late ascites, compared to controls was not significantly different.

III- Cerebrospinal fluid acid and alkaline phosphatase:

In the present study, no activity of acid and alkaline phosphatase was demonstrated in the cerebrospinal fluid of controls and in the hepatic schistosomal involvement.

IV- Cerebrospinal fluid creatine phosphokinase:

In normal controls, the cerebrospinal fluid creatine phosphokinase showed a mean of 20.5 Oliver and Rosalki units. A significant lowering occurs in the hepatomegalic stage which

showed a mean of 6.9 O & R units. In the stages of hepatosplenomegaly, splenomegaly and late ascites, the mean values were 11.4, 7.0 and 10.5 O & R units. A non significant diminution was noticed in these stages as compared to controls.

Increased cell memberane permeability, skeletal muscle affection and altered blood brain barrier were considered underlying factors for changes in transaminases and creatine phosphokinase activities in cerebrospinal fluid.

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ملخص الرسالة

فى هذا البحث تم دراسة تأثير البلهارسيا على بعض الانزيمات التى تفرز من الكبد وموجودة بالسائل النخاعى وذلك على عشرين مريضا مصابا ببلهارسيا الكبد وكذلك على عشرة اشخاص اصحاء للمقارنة . تم تقسيم هذه الحالات الى اربعة مجاميع اخذت فى الاعتبار مقدار اصابة الكبد، بالبلهارسيا :

- ١- المجموعة الاولى : وتشمل حالات تضخم الكبد .
- ٢- المجموعة الثانية : وتشمل حالات تضخم الكبد والطحال .
- ٣- المجموعة الثالثة : وتشمل حالات تليف الكبد وتضخم الطحال .
- ٤- المجموعة الرابعة : وتشمل الحالات المتقدمة من بلهارسيا الكبد مع وجود استسقاء (رشح بريتونى) .

والانزيمات التى تم دراستها هى انزيم الترآنزامينيز ، لاكتيت ديهيدروجينييز ، الكلايين فوسفاتيز ، اسيد فوسفاتيز وكريتين فوسفوكينييز . واستنبط من البحث ما يأتى : -

١- بالنسبة لانزيم الترآنزامينيز بالسائل النخاعى : -

بالنسبة لحالات المقارنة وجد ان متوسط نسبة انزيم الجلوتامك اوكسالاستيك

ترآنزامينيز بالسائل النخاعى كان ١٣٥ وحدة . اما فى حالات بلهارسيا الكبد

بانواعها الاربعة فقد وجد ان هذه النسبة كانت ١٤٤ ، ١١٨ ، ١٧٣ و ١٤ وحدة

على التوالى . ويتضح من ذلك وجود زيادة ذات قيمة فى نسبة انزيم الجلوتامك اوكسالاستيك

ترانزامينيز في حالات تليف الكبد وتضخم الطحال والحالات المتقدمة من تليف الكبد والاستسقاء (المرحلة الثالثة والرابعة) .

اما بالنسبة لانزيم الجلوتامك بيروفيك ترانزامينيز بالسائل النخاعي فقد وجد ان متوسط نسبته في حالات المقارنة هي ٣٥ وحدة بينما في الحالات المختلفة من بلهارسيا الكبد فان متوسط نسبته كانت ٢٦٥ ± ٣٥ و ٤٣٣ ± ٤٢٩ وحدة في مراحله الاربع على التوالي . ويتضح احصائيا ان الاختلاف في هذه النسب الاخيرة بالنسبة لحالات المقارنة غير ذي قيمة .

٢- بالنسبة لانزيم لاكتات ديهيدروجينيز بالسائل النخاعي وجد ان متوسط نسبته في حالات المقارنة هي ١٣٦١ وحدة بينما في الحالات المختلفة من بلهارسيا الكبد فان متوسط نسبته كانت ١٨٦٦ ± ١٨٨٨ و ٢٠٥٣ ± ١٤٥١ وحدة في مراحله الاربع على التوالي . ويتضح احصائيا ان الزيادة في هذه النسب الاخيرة غير ذات قيمة .

٣- بالنسبة لانزيم الاسيد والاكلالين فوسفاتيز في السائل النخاعي فقد تبين عدم وجودهما في حالات بلهارسيا الكبد وحالات المقارنة .

٤- بالنسبة لانزيم الكرياتين فوسفوكينيز بالسائل النخاعي فقد وجد ان متوسط نسبته في حالات المقارنة هي ٢٠ وحدة . وبالمقارنة فقد وجد ان انزيم

الكريتين فوسفوكينيز في حالات تضخم الكبد (المرحلة الاولى) قد نقص وان هذا
النقص وجد انه ذات قيمة بالنسبة لحالات المقارنة وان متوسط نسبته هي ٦٩ وحدة .
اما في الحالات الاخرى فان نسبته هي ١١٤ ، ١١٠ ، ١٠٠ وحدة في مراحل الثلاثة
الاخيرة (المرحلة الثانية والثالثة والرابعة) ويتضح احصائيا ان الاختلاف في
هذه النسب الاخيرة بالنسبة لحالات المقارنة غير ذي قيمة .

وقد عزيت هذه التغيرات في انزيمات الترانزأمينيز في المرحلة الثالثة والرابعة
والكريتين فوسفوكينيز في المرحلة الاولى لتغير في خاصية جدران خلايا الكبد للنفاذ
وكذلك لظهور في عضلات مرضى البلهارسيا ووجود حائل بين الدم والنخاع عنده
هو "المرضى" .

دراسة الانزيمات فى السائل الشغافى فى مريض البامارسيا

رسالة مقدمة

من

حسين عبد الله خلفان

للحصول على

درجة الماجستير فى الكيمياء الحيوية

كلية الطب

جامعة الاسكندرية

١٩٧٧