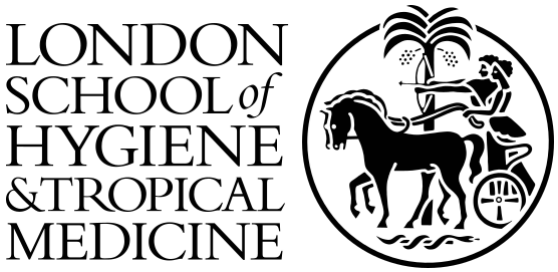

THE CLINICAL AND IMMUNOLOGICAL CHANGES INDUCED IN MAN BY SCHISTOSOMIASIS

Thesis

Submitted to the University of London

for the degree of

DOCTOR

OF

PHILOSOPHY

IN

THE FACULTY OF MEDICINE

BY

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January, 1980

Abstract

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by
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This study commenced with an investigation of the role immunological changes have in the production of the anaemia of schistosomiasis and of the role that complement-mediated mechanisms play in the production of that anaemia. The investigation of these mechanisms necessitated infecting mice experimentally with Schistosoma mansoni. The anaemia which developed in these animals was found to be mainly normochromic, progressive and of a haemolytic nature. A technique for the measurement of complement in the sera of the mice was developed using radioactive materials and employing an immuno-haemolytic mechanism. Anaemia was found to be significantly more related to a fall in complement rather than to splenomegaly. The importance of complement in the production of anaemia led to the investigation of its importance in other pathological processes involving membranes upon which complement could be deposited. Fluorescent techniques were used for this part of the work. Nephropathy caused by complement deposits in the renal glomeruli was particularly investigated.

In view of the evidence existing stat. a similar mechanism might be involved in trypanosomiasis, however, the study was extended to this infection. A study of complement in human infection with schistosomiasis was also carried out and in this study the complement was measured by a radial immuno-diffusion test. A strong positive relationship between haemoglobin and complement was found in infected patients but not in those with schistosomiasis but without anaemia, nor was complement found to be reduced in a series of controls. Treatment with Hycanthone in man was found to be followed six weeks later by a fall in the complement level in the serum and in the serum content of immunoglobulin M (IgM). This part of the work led to the deduction that there is an association between complement and immunoglobulin M in attacking schistosoma worms.

A further disease in which a complement-mediated mechanism was thought to be possible is sprue in which damage to the intestinal mucosa leading to malabsorption could be complement mediated. Some patients with sprue were therefore investigated. Similarities were detected, in so far complement-mediated immune processes are concerned between patients with sprue and those with schistosomiasis.

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ACKNOWLEDGEMENTS

I am deeply indebted to Professor A.W.Woodruff, Professor and Director of the Department of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine and the Hospital for Tropical Diseases, for his supervision, advice, helpful discussion and encouragement throughout this work.

I am sincerely grateful to Mr. L.E.Pettitt, Senior Technical Officer, Department of Clinical Tropical Medicine, for his unceasing co-operation and skilled technical assistance.

My gratitude is due to Dr. C.James of Winches Farm for aiding with the infection of animals and to Dr. S.Y.Salih, Department of Medicine, Faculty of Medicine, University of Khartoum, for his collaboration in providing sera of Sudanese patients.

I wish to thank Mr. T.Marshall and Mr. A.Radalowicz of the Department of Medical Statistics and Epidemiology for their statistical help. I also wish to thank members of the Visual Aid Department and staff of the Library for their kind assistance. I am also grateful to Mrs. P.Foley for typing this thesis.

I am deeply obliged to my wife, Salwa, for her patience and encouragement.

Finally, I would like to thank the Government of Egypt for granting me a scholarship to fulfil this study.

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PART 1

INTRODUCTORY CHAPTERS

CHAPTER 1

INTRODUCTION

The prime question to which an answer was sought by this study was whether complement mediated mechanism plays an important part in the genesis of anaemia in schistosomiasis.

Previous studies have shown that during schistosomal infection, anaemia with a haemolytic component develops. This has been explained by an immunological mechanism whereby complement binding to the red cell membrane subsequently damages it with loss of the cell contents (Woodruff, 1973). Many other mechanisms have also been implicated in the production of anaemia observed in schistosomiasis. These mechanisms include chronic blood loss; hypersplenism; haemodilution; schistosomal toxins and haemolysis. The relative importance of the complement dependent mechanism in comparison with other mechanisms need further appraisal and this was attempted in the study here reported.

There is evidence that the same complement mediated mechanism is of widespread applicability and is responsible for bringing about some other tropical anaemias as those associated with kala-azar (Woodruff et al,1972); trypanosomiasis (Woodruff, 1973) and malaria (Woodruff et al, 1979). It was also suspected that

processes involving complement might proceed on membrane other than that of the red cell and evidence of this had been found in the kidneys of animals infected with schistosomiasis (Mahmoud et al, 1972).

The antigenicity and the cosmopolitan distribution of schistosomiasis render the problem one of great importance. Over 600 million people in 71 countries are thought to be exposed to the infection and over 200 million are infected (WHO, 1975). In Egypt alone, more than 14 million of the 32 million inhabitants (1967) were known to be suffering from schistosomiasis (WHO, 1965 & Farooq et al, 1967). This prevalent condition has been calculated to be responsible for an annual economic loss of about $ 560 million (Farooq et al, 1967). The situation in Egypt could well be applicable to other developing countries where schistosomiasis is known to be endemic and increasing in prevalence as in Sudan (Omer et al, 1976) ; Iraq (Baquir, 1977) and East-Africa (WHO, 1978).

The importance of these health and economic problems increases the need for the pathogenesis of schistosomiasis to be better understood. This particularly necessary in view of the attemps being made to control schistosomiasis (WHO, 1975).

The purpose of the present work, therefore, is to try to find the answers to the following questions :

In animals :

(i) What type of anaemia develops in mice infected with Schistosoma mansoni ? An answer to this was sought by examining the Hb ; PCV ; MCHC and blood films.

(ii) What relationship , if any , exists between such anaemia and serum complement in the same infected mice ? An answer was sought by using a radioactive immuno-haemolytic technique to measure complement.

(iii) What other complement mediated pathology is present ? To answer this, kidneys and livers of infected mice were examined by means of immunofluorescent assay.

(iv) What difference exists between the results obtained from the above procedures in mice and those of others infected with Trypanosoma rhodesiense.

In man :

(i) What are the complement levels in schistosomiasis ? This was studied in a group of Egyptian patients and was related to the degree of anaemia present.

(ii) What is the effect of treatment of schistosomiasis on the complement levels ? This was studied in another group of Sudanese patients.

(iii) Is it possible that the absorptive defects in tropical sprue could result from complement mediated damage to the small

intestine ? To study this, complement levels in patients with tropical sprue were compared with those of patients with schistosomiasis.

The method employed to measure complement in all groups was a radial immunodiffusion test.

CHAPTER 2

REVIEW OF LITERATURE

In this review evidence for the importance of anaemia as a public health problem will first be examined. This will be followed by evidence of current concepts concerning the causation of some common anaemias, particularly those related to schistosomiasis. The need for further study of these fields of knowledge becomes apparent from the literature.

Anaemia of significant degree is widespread throughout the tropical and sub-tropical world and constitutes a considerable world health problem (Woodruff, 1973 & Omer et al, 1976 & Abaza et al, 1978 and Greenham, 1978). These and other surveys have shown that schistosomiasis often play an important aetiological role in the development of tropical anaemias.

Schistosomiasis, although it is an ancient illness having been deep rooted in history for more than 3000 years (Ruffer, 1910), at present is extending (Jordan et al, 1972 & Warren, 1973) and creating still greater problems than it did in the past. It is known to be endemic in 72 countries and islands having a total population of 1.348 million (Wright, 1970) and it could be imported to other countries such as Britain (Woodruff, 1964).

Schistosomiasis is increasingly prevalent in association with agricultural development schemes and artificial water impoundments

such as man made lakes (WHO, 1975). These new irrigation projects that have being rapidly expanding in many countries constitute a considerable hazard especially to the rural population in the regions involved (WHO, 1978).

In Egypt, in addition to the effect of Aswan High Dam (Deschiens, 1972) other factors are contributing to and for increase prevalence of schistosomiasis. These include swimming (Farooq et al, 1966); religious and social habits (Farooq & Mailah, 1966) and the vast rate of the population growth added to the inefficient and inadequate health education and control programmes (Farooq, 1969). These socioeconomic effects were reckoned to cause more than $ 560 annual loss (Farooq et al, 1967) probably as the productivity of infected persons fall by 25 to 50 percent (Sultan, 1976). Meanwhile, the annual cost of protection per person in endemic areas ranges between $ 0.4 to 12 (WHO, 1973). However, there is no doubt that these losses are now greater than these estimates and over the very long existence of schistosomiasis the total loss must amount to a considerable aggregate.

Since the beginning of this century; particularly in the last decades, there has been intensive research into the various aspects of schistosomiasis aimed at its eventual control. However, gaps exist and are widening between research workers and those who actually faced with the task of fighting schistosomiasis in the field (WHO, 1967). The efforts of the governments and WHO to improve the situation (De Maar, 1979) and

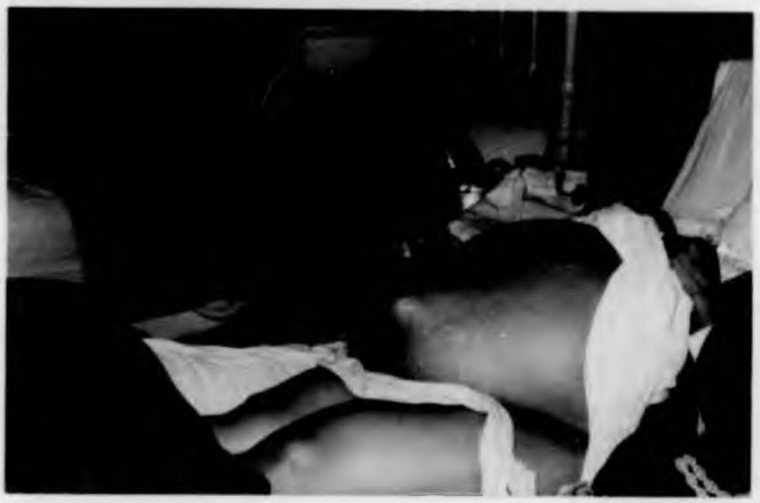
the considerable advances which have been made since 1965 in the techniques and materials available for schistosomiasis control (WHO, 1973) did not, however, solve the problem. These efforts indicated that the current public health measures are inadequate to prevent the spread of schistosomiasis (Philips et al, 1978). Moreover, the lack of reliable data on its prevalence and geographical distribution ; inadequate diagnostic capabilities and unknown aspects of relation between host, agent and environment remained as overburden obstacles (WHO, 1978). These prospects add to the importance of control by immuno-prophylaxis programs achieved through better understanding of the pathological mechanisms schistosomiasis brings about. Accordingly, the present study is devoted to assessment of these mechanisms, particularly those associated with anaemia, splenomegaly and nephropathy.

For long it was difficult to know whether man had become adapted to live with schistosomiasis or not. There is, however, now much evidence that it causes a serious and frequently fatal illness. Close association between infection with S.haematobium and cancer of the urinary bladder in endemic areas has been emphasized by El-Halawani et al (1970). Death from schistosomal polyposis is not uncommon (Abaza et al, 1978). Complete relief of symptoms in patients with hepatic fibrosis and ascites one month after a decongestion operation was reported (Badawi et al, 1976), however, hepatosplenomegaly with ascites is a common and grave consequence of schistosomiasis (Fig. 1).

Figure 1

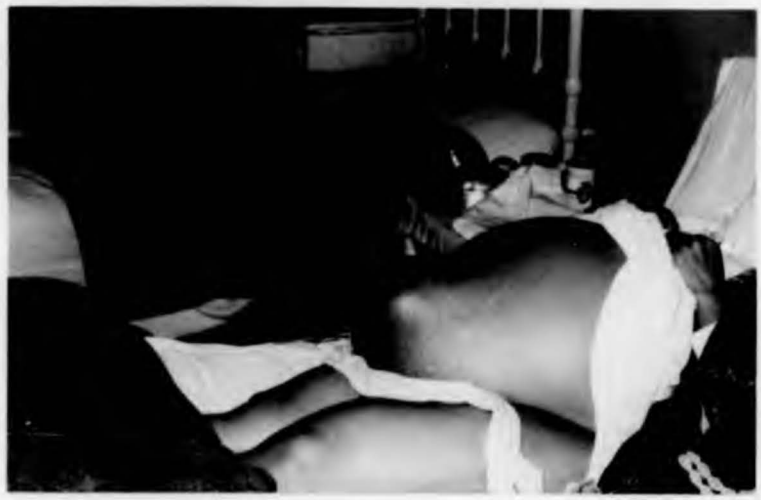
38 years old Egyptian female infected with S.mansoni in terminal stage with hepatosplenomegaly, huge ascites and anaemia

Figure 1



38 years old Egyptian female infected with S.mansoni in terminal stage with hepatosplenomegaly, huge ascites and anaemia

Figure 1



38 years old Egyptian female infected with S. mansoni in terminal stage with hepatosplenomegaly, huge ascites and anaemia

Even 45 years ago, anaemia of schistosomal origin was described as " the insidious enemy of the youth " (Girges, 1934). Schistosomiasis has been implicated as a cause of anaemia in almost every country in which it is known to be endemic. These include Egypt (Day, 1911) ; Tanganyika (Bahr, 1929) ; Japan (Ozawa, 1931) ; Sudan (Archibald, 1933) ; Puerto Rico (Rodriguez, 1936) ; Iraq (Jalili et al, 1952) ; Brazil (Pessoa et al, 1955) ; Uganda (Nelson, 1958) ; Rhodesia (Frii-Hansen, 1961) and many other places.

Despite these records and the intensive research in schistosomiasis, and although the pioneering observation on the blood changes in it was made as early as the beginning of this century (Coles, 1902), the mechanism whereby the schistosomal anaemia occurs is still uncertain. Haemolytic and dyshaemopoietic mechanisms have been suggested. The destruction rate of the red cells in schistosomiasis has been suggested to be three times the normal, while the production rate is decreased (Jamra et al, 1964). The bone marrow has been described as being either normal or hyperplastic but incapable of compensating for the increase in the destruction of the red cells (Sabour et al, 1967). It has, however, also been found that in schistosomiasis there is a marked haemolytic factor and that anaemia is not prelimenary dyshaemopoietic (Woodruff et al, 1966 & Mahmoud et al, 1972 & Woodruff, 1973 and Suad, 1978).

Anaemia in pure human schistosomiasis has been described as being normocytic or microcytic , hypochromic and never macrocytic

(Salah, 1935 and Aweny, 1962). Other workers have suggested that in mice infected with S. mansoni the anaemia is hypochromic (Mahmoud, 1971 and Suad, 1978). It has also been suggested that in patients with schistosomiasis about 40 % have normocytic anaemia and 60 % have microcytic anaemia but a single case of macrocytic anaemia was never found (El-Dewi et al, 1957). Some forms of macrocytosis, however, were found by other workers (Pesignan et al, 1951 and Pessoa et al, 1952).

In the light of these many reports, the causation of anaemia in schistosomiasis must be regarded as deserving of further study. Various working hypotheses for its causation, therefore, have been examined.

1. Chronic blood loss :

Since Day (1911) described the schistosomal anaemia as being secondary to blood lose, other workers have also found that blood loss may be in part responsible for it (Azmy et al, 1934 & Mahmood, 1965 and Farid et al, 1966 ; 1967 and 1968). It has however also been found that such blood loss as occurs in patients infected with S. mansoni is irregular and small, thereby providing neither a handicap to haemopoiesis nor an avenue for significant loss of nutrients and metabolites (Walker et al, 1954). It has also been shown that there is shortening of the red cell life span in patients infected with schistosomiasis who were not losing appreciable amounts of blood

(Woodruff et al, 1966). Moreover, anaemia developed readily in mice infected with irradiated cercariae of S.mansoni (Mahmoud et al, 1972). In such animals, no liver fibrosis develops and there is no blood loss for eggs which bring about both these pathological states are absent. Farid et al,(1967) stated that blood loss in patients with chronic schistosomiasis mansoni is unlikely to be much OR TO lead to overt anaemia. Meanwhile, severe anaemia was only observed in patients suffering from urinary schistosomiasis with obvious haematuria and combined S.mansoni and Ancylostoma duodenale infections (Farid et al, 1968).

It thus seems that except in cases in which there is recurrent bleeding from multiple intestinal polypi or oesophageal varices or severe haematuria, the evidence dose not support blood loss as being the main mechanism for pure schistosomal anaemia.

2. Splenomegaly and associated sequelae :

Gross splenomegaly may produce anaemia through :

a. Pooling of erythrocytes in the enlarged spleen causing relative dilution of the circulating peripheral blood (Reimann et al, 1960).

b. The associated hypervolaemia ; haemodilution and increased plasma volume may result in a form of dilution anaemia (Saif et al, 1966 & Farid et al, 1966 and Saboure et al, 1967).

c. Sequestration of the red cells and other blood elements trapped in the big spleen (Woodruff et al, 1966).

d. The increase in the splenic size itself could cause haemolysis (Holzbach et al, 1964)) and pancytopaenia (Motulsky et al, 1958).

e. The slowing of the blood flow in the big spleen is another factor that might assist red cell haemolysis (Farid et al, 1966).

In support of these proposals, it has been reported that anaemia and leucopaenia of schistosomiasis are ameliorated by splenectomy (Kenawy et al, 1958). Such improvment, however, may only be temporary. Thus, in a patient with anaemia and splenomegaly there has been some improvement of anaemia two months after splenectomy, but two years later, the haemoglobin and the PCV values were 9.5 gm/dl and 32 % respectively (Richmond et al, 1967). In similar conditions, the increase in the plasma volume recurs in the few years subsequent to splenectomy (The Lancet, 1972). Anaemia has also been observed in splenectomised animals infected with S.mansoni (Mahmoud et al, 1972).

Mechanisms such as chronic blood loss and splenomegaly, though they may not be the only causes or the major causes of anaemia in schistosomiasis, could aggravate it once it has occurred.

3. Schistosomal toxins :

It has been suggested that blood loss and toxaemia in the early stages of schistosomiasis of all three human species can cause hypochromic anaemia particularly in children (Foy et al, 1963). Schistosome toxins might also inhibit the bone marrow leading to diminished red cell production in mice infected with S.mansoni

(Nasser et al, 1967). If such toxins are present they would be expected to attack the parenchymal cells of the liver and thus bring about derangement of liver functions sufficient to be detected by standard tests. This would particularly be expected if the hepatosplenic syndrome was present. In fact, it has been observed that mice infected with S.mansoni are able to digest and absorb dietary fats almost as uninfected ones (DeWitt, 1957). It has also been observed that in mice infected with S.mansoni albumin levels ; icteric index and cephalin flocculation tests are not significantly different from those of the control mice (DeWitt et al, 1959).

4. Immune haemolysis :

A haemolytic mechanism has been shown to be present in the anaemia of patients with schistosomiasis (Woodruff et al, 1966) and in mice experimentally infected with S.mansoni (Mahmoud et al, 1972). Evidence was put forward that this haemolytic mechanism was initiated by an immune process in which complement is activated and either with one molecule of immunoglobulin M (IgM) or two molecules of immunoglobulin G (IgG) was bound to the erythrocyte surface and thus led to the cell lysis (Woodruff, 1973). The binding sites on the erythrocyte surface are likely either to be adsorbed antigens produced by parasite or irregularities produced by damage in some way (Bruninga, 1971). Antigens could be adsorbed from the circulating schistosomal antigens that have been detected by many workers (Berrgren et al, 1967 &

Smithers et al, 1973 & Nash et al, 1974 and Medwar et al, 1975).

The mathematical analysis of the immune haemolytic process has been reported several times since von Krough (1916) first described it. Such analysis includes different equations made to represent the average number of damaged sites per cell in proportion to the number of the lysed cells. Thus, Borsos et al, (1961) postulated that one molecule of complement is reacted sequentially to one site on the cell to cause haemolysis. Kolb et al (1974) suggested that up to 6 molecules of complement 9 (C9) fixed to one site on the cell surface are required for the haemolysis. Recently, Kitamura et al (1976) suggested that such haemolysis could be acheived with only 2 molecules of (C9).

Looking at the differences between and the applicability of these hypotheses it seems that the need for clinical evidence for the complement mediated mechanism is rather more important than the study of the mathematical and the immunochemical aspects of such mechanism.

Although haemolytic complement was first reported in the last century (Bordet, 1898), it was only after the initial demonstration of its importance as a cause of haemolysis in parasitic infections (Woodruff et al, 1972) that it attracted attention. Many workers have now found complement responsible for erythrocyte haemolysis (Ruddy et al, 1972 ; Eisen et al, 1973 ; Lint et al, 1976, Santoro et al, 1979). It has been shown that complement may contribute to (IgG)

and (IgM) mediated cell destruction (Frank et al, 1977). With (IgG) or (IgM) or many cold antibodies severe haemolytic anaemia can occur either by direct complement mediated intravascular lysis or by (C3) fixation leading to red cell sequestration (Hoffbrand et al, 1975). The lysis of erythrocytes by complement in the absence of antibodies was also suggested by Götze et al (1970).

The observation that (C3) coated red cells are taken up by the reticuloendothelial system particularly in the liver (Brown et al, 1970) is probably consistent with the decrease in serum complement observed in patients with urinary schistosomiasis, salmonellosis and massive proteinuria (Farid et al, 1972); trypanosomiasis (Assoku et al, 1977) and in some renal diseases (Williams et al, 1972) and in rats infected with T.lewisi (Jarvinen et al, 1976). It would appear, therefore, that complement fixation in vivo is commonly expressed by depression of its serum levels. By contrast, serum complement (C3) was reported to be increased in patients with liver hydatid disease probably as an immune response in which complement consumption appears to be overcompensated by a feed-back like mechanism (Seitanidis et al, 1976).

The fall in (C3) observed in schistosomiasis and salmonellosis (Farid et al, 1972) may result from the immune haemolysis suggested by Woodruff (1973) in which complement is consumed. Complement may also be involved in immunological processes on membranes other than that of the red cells and thus partially removed from the serum.

Hereby, in mice infected with S.mansoni complement (C3) deposits were demonstrated in the kidneys (Mahmoud et al, 1975 and Natali et al, 1976). Moreover, (C3) has also been detected on the tegument of S. mansoni cercariae (Machado et al, 1975) ; schistosomula (Sher, 1976) and the adult females with some of its eggs (Kabil, 1976). Host antigens were also found on the tegument of S.mansoni worms (Goldring et al, 1977). It would appear, therefore, that an immunological process in which complement is actively involved is present on the schistosomes teguments. This is in agreement with the strong suggestion made by Tavares et al (1978) that the complement system is one of the effector mechanisms in concomitant immunity in schistosomiasis.

Complement (C3) deposits have also been demonstrated in the kidneys of patients with the loin pain and haematuria syndrome (Naish et al, 1975) . It has also been shown that patients with schistosomiasis mansoni developed glomerular lesions of immunological origin (Da Silva et al, 1970 and Rocha et al, 1976).

The activity of complement on the erythrocyte surface (Suad et al, 1976) and on the glomerular membrane (Mahmoud et al, 1975) suggest that the important aspects of these multifaceted processes concerns the function of serum complement in mediating damage to cells and tissues. The wider picture of the complement dependent lysis, therefore, may include damage to the kidney and probably the

liver, hence the importance to examine both together with the anaemia.

Taken together these studies suggest that anaemia in schistosomiasis is the result of the interplay of several mechanisms in which the immune haemolytic mechanism plays an important part.

Similar immune haemolytic mechanism could also be responsible for the anaemia of African trypanosomiasis (Woodruff, 1973 and Woodruff et al, 1973). In both schistosomiasis and trypanosomiasis the causative parasite does not invade the red cell. Thus, it was considered possible that they might share some of the mechanisms whereby anaemia is brought about. Some aspects of trypanosomiasis, therefore, were investigated along with schistosomiasis.

Another tropical illness in which the complement is probably mediating damage to cells and tissuses is sprue. The association between malabsorption syndrome in patients with immunoglobulin deficiency is now well established (Brown et al, 1972). However, the pathogenesis of sprue is poorly understood, and many of its clinical and immunological features are incompletely characterized. It was thought, therefore, that probably as in schistosomiasis serum complement may be involved in the genesis of some of these features. Thus, the comparison between serum complement levels in both schistosomiasis and sprue might yield useful information about the actiology of sprue.

From this review, it is concluded that the study of the relationship

between serum complement activities and some of the clinical and immunological aspects of schistosomiasis is of a prime significance for better understanding of the genesis of such aspects thus add to the importance of schistosomiasis control.

PART 2

EXPERIMENTAL SCHISTOSOMIASIS AND HAEMATOLOGICAL METHODS

CHAPTER 1

PARASITOLOGICAL METHODS

The susceptibility and suitability of common laboratory animals as experimental host of S.mansoni enable several aspects of schistosomiasis to be thoroughly investigated. In this work the main object is to study a possible relationship between complement and both anaemia and renal lesions in schistosomiasis and to compare schistosomiasis with trypanosomiasis as far as complement induced pathology is concerned. For these purposes many laboratory animals were needed.

In mice after ten weeks of exposure to 130 cercariae of Puerto-Rican strain of S.mansoni there develops a syndrome similar to human hepato-splenomegaly ; portal hypertension and anaemia(Warren et al, 1958). It was later suggested that these pathological features developed in mice with a worm load of only 1 or 2 pairs per mouse (Andrade et al, 1964). Mice could also easily be infected with human species of trypanosomes ; they survive a reasonable period of time ; are cheap ; easy to handle and can be kept under constant laboratory observation. Moreover, the presence of the classical components of complement in the mice sera was demonstrated by Dorsos et al, (1961). This was further confirmed and extended by Rosenberg et al, (1962) who emphasized that factors other than a lack of components of complement may explain the previous failure of sera from several strains of mice to lyse sensitized erythrocytes as reported by Brown

(1943). Genetic differences between different strains of mice rather than lack of complement were thought to be among the factors responsible for this failure (Rosenberg et al, 1962). Meanwhile, other workers found that within each strain of mice, serum from males contains more haemolytic complement than serum from females (Terry et al, 1964). In view of these studies, an inbred strain of male mice was used for all the work here described and was found to be suitable for the purpose.

Laboratory cycling of schistosomes

This cycling was performed by the passage of schistosomes in inbred Swiss albino Tisilius original strain of mice and Biomphalaria glabratus snails.

1. Extraction of eggs used for infection of snails:

To delay miracidial hatching exposure of eggs to direct light was avoided. Eggs were extracted from the livers of infected mice by macerating the tissues, and sieving it in "No. 60 mesh - 250 microns". The tissue was then washed in physiological saline and this was followed by sedimentation for 20 minutes. This washing and sedimentation was repeated three times till the supernatant was clear; the final wash was in distilled water at 4-5°C. Faeces of infected mice were triturated in saline and sieved in "No. 30 mesh - 500 microns. The filtered deposit was washed and sedimented as before.

In a lighted incubator at 29-30°C hatching was then carried out in petri-dishes containing distilled water.

2. Infection of snails:

Within one hour of hatching, 6 miracidia were picked up from the petri-dishes. A micropipette was used under a dissecting microscope. It was introduced with the snails into chambers of haemagglutination plates containing 1-1.5 ml distilled water per snail "Biomphalaria glabratus, 4-5 mm maximum diameter". The plates were covered with sheets of glass and maintained at 27-29°C for a minimum of 5 hours. The snails were then transferred into clean glass tanks "of 4-7 litres capacity; approximately 10 snails per litre", containing sterilized silver sand, clean aquatic plants e.g. Vallesneria spiralis or Elodea canadensis and conditioned " copper, calcium and chlorine ion free water " of pH higher than 7. For 24 hours per day these tanks were thermostatically controlled at " 26-28°C ", aerated by Hy-Flo pumps and illuminated by warm-white fluorescent tubes. The latter were equipped with a time switch control.

Snails were left for 4-5 weeks as a prepatent period during which their feeding was maintained by special alginated diet.

3. Shedding of cercariae:

Snails were placed in beakers with a small volume of distilled water and exposed to strong illumination at less than 27°C for at

least 2 hours to enhance and help cercariae to emerge.

4. Estimation of number of cercariae:

At the end of the exposure period, the cercarial suspension was carefully agitated and 3 aliquot samples 0.5 ml each were taken on glass slides; the cercariae killed and stained with Lugol's iodine and counted using a dissecting microscope. The mean of the 3 counts was used for evaluation of the number of cercariae present in the total volume of the suspension. The methods used in steps 1 to 4 are described in detail by Webbe et al (1971).

5. Infection of mice:

Three groups each consisting of 30 inbred white males of the Swiss albino TO strain of mice " average 5 weeks old, 18-22 gm in weight " were infected respectively with 50, 100 and 150 cercariae per mouse of the Wellcome Puerto-Rican strain of S.mansoni using the ring method described by Smithers et al (1965).

The mice were anaesthetised by veterinary Nembutal "Abbot Laboratories, 60 mg/ml ". One part of Nembutal was diluted with 10 parts of 10% ethanol in distilled water and injected into the mice intraperitoneally in doses of 0.2 ml per mouse. The fur was clipped from the lower abdomen and the mouse was placed on its back between wooden strips fixed to a baseboard. The abdomen was moistened with water and a nickel-plated brass ring

" 1.3 cm inside diameter, 12 gm in weight and holding about 1.2 ml of water " was placed in position. The cercarial suspension was gently stirred and a volume containing the required number of cercariae was then pipetted into the ring. After an interval of about 10 minutes the ring was removed and the mouse was permitted to recover. Mice usually did so within one hour.

6. Maintenance and feeding of animals:

The infected and control mice were housed in isolated cages and fed a conventional pellet diet (R.G.P. 86 E Dixons & Sons "Ware" Limited) and water ad libitum. Throughout the course of the experiment mice cages were examined periodically to detect any dead animals.

CHAPTER 2

BASIC HAEMATOLOGICAL TECHNIQUES

1. Collection of blood:

After an incubation period of 7 weeks, 2 mice from each group, together with an equivalent number of controls, were collected randomly from the cages at weekly intervals. The weight of each mouse was measured. The mice were then either anaesthetised by ether inhalation or killed directly by cervical prossure and avulsion. The mouse was immediately placed on its back and fixed to a dissecting plate by metal pins. The thoracic cavity was opened and blood was collected either by cardiac puncture or by incising the caudal artery. Blood was drawn into small plastic tubes. A small part was taken immediately for haematological examination and the rest was centrifuged at 3000 r.p.m. for a few minutes.

Sera were sealed and stored in dry ice at -30°C for subsequent examination.

2. Tissue processing:

At the time the animals were sacrificed, all livers and spleens were removed and their weights were measured in grams. Livers were examined for the presence of granulomas and preparations of squashed livers were routinely examined at the time of sacrifice to

make sure that the animal had been infected. Some adult worms were recovered.

Kidney, liver and adult worm samples were collected and dipped in liquid nitrogen, sealed in metal foil and placed in dry ice at (-30°C) for subsequent immunofluorescence studies.

3. Estimation of haemoglobin:

The cyanmethaemoglobin (HiCN) method was used to measure the haemoglobin values as described by Dacie et al (1975). The diluent used had a pH between 7.0-7.4 and contained potassium ferricyanide 200 mg, potassium cyanide 50 mg, potassium dihydrogen phosphate 140 mg, Nonidet P40 1 ml and water to 1 litre. The reagent was always clear and pale yellow in colour. When measured against water as blank in a photoelectric colorimeter at a wavelength of 540 nm, the absorbance was zero. Twenty ul of blood were added to 4 ml of the diluent. The tube containing the solution was stoppered with a rubber bung and inverted several times. After being allowed to stand at room temperature for a sufficient period of time, usually " 10 minutes ", to ensure the completion of the reaction, the solution was compared with the standard (diluent) and a reagent blank (water) in a photoelectric colorimeter at 540 nm (A^{540}). Haemoglobin values were calculated as follows:

Hb gm/dl =

$$\frac{A^{540} \text{ of test sample}}{A^{540} \text{ of standard}} \times \frac{\text{conc std (mg/dl) x dilution factor (e.g. 201)}}{1000}$$

4. Estimation of Packed Cell Volume (PCV):

This was measured by a micro method described by Dacie et al (1975). Blood was drawn into microhaematocrit tubes with a capillary (1 mm)internal diameter , (77 mm)in length. The blood was allowed to enter the tube by capillarity, leaving at least 15 mm unfilled. The tube was then sealed by heating the dry end of the tube rapidly in a fine flame "the pilot light of a Bunsen burner" combined with rotation. The tubes were placed in a special centrifuge at 12,000 r.p.m. for about 5 minutes. The PCV values were then measured using a reading device.

5. Blood films:

A small drop of blood was spread on a glass slide and allowed to dry. The dry film was well covered with Leishman's stain. At the end of 1 minute, double the quantity of distilled water was carefully added and mixed with the stain. At the end of 7 minutes, the mixture was poured off and the film covered with distilled water for 2 minutes. The water was then washed off with fresh distilled water , and the film gently blotted dry with clean blotting paper. The films were mounted with cover slips using Canda balsam and examined under a microscope.

PART 3

ANAEMIA AND SPLENOMEGALY IN SCHISTOSOMIASIS

CHAPTER 1

ANAEMIA IN MICE INFECTED WITH SCHISTOSOMA MANSONI

The last twenty years or so have witnessed an astonishing increase in the knowledge of schistosomiasis. Some of this knowledge, however, has been extensively contradictory and created innumerable problems needing investigations. Thus, it has been suggested that the immunity in schistosomiasis is the result of a granulomatous response to the schistome eggs manifested as a form of a delayed hypersensitivity (Warren et al, 1967 and Warren, 1975). By contrast, other workers suggested that it is a concomitant immunity as schistosomes are covered by the host proteins, and the eggs are probably not important in stimulating the immunity (Smithers et al, 1967 and Smithers, 1972). A third group suggested that heterologous immunity is also important as infection of animals with one species of schistosomes reduce the expected egg load in subsequent challenge with another species (Nelson et al, 1968 and Hussein et al, 1970).

The present knowledge that has been gathered on schistosomiasis has indicated that the problem of anaemia was insufficiently tackled and the hypotheses cited for its causation still need further investigation. This study , therefore , will investigate the main mechanisms whereby the anaemia of schistosomiasis is brought about.

During the course of S.mansoni infection of mice, the Hb; PCV and MCHC were examined weekly. Two mice of each group were killed and their blood values were measured according to Dacie et al, (1975,) in (part 2, p.38-39). No attempt was made to measure the "absolute" values which depend on the accuracy of the red cell count. In practice, unless electronic methods of counting are available, the red cell counts are tedious and inaccurate and seldom necessary (Bomford et al, 1978). Most workers, therefore, regard the MCHC as the true measure of the degree of saturation of the red cell with haemoglobin, and the mean corpuscular haemoglobin (MCH) as of slight value and the mean corpuscular volume (MCV) as of diminished one (Hunter et al, 1965 and Bomford et al, 1978). Blood films stained with Leishman's stain, therefore, were examined at 2 weeks intervals to assess cell diameters, shapes and colours. All the results were compared with those of the control series.

R E S U L T S

The following illustrate the varying degrees of anaemia observed as the infection progressed.

1. The first group: "infected with 50 cercariae".

Moderate degree of anaemia was observed at the end of the incubation period "Hb 10.5 gm/dl & PCV 32.8%" (table 1). Anaemia gradually increased until the 10th week of infection when the haemoglobin was

"7.1 gm/dl and the PCV 23.6%". This was followed by some limited recovery towards the end of the 19th week (fig. 2 a and b).

In 52% of mice the anaemia was normochromic and in the others hypochromic, throughout the course of infection. The mean MCHC of all the infected mice was 30.7% with the standard error (S.E.) of 0.64 and range of 9.8. As this indicates, the hypochromic anaemia in the remaining 48% was of a slight degree (fig. 2 c). Throughout the experiment the Hb; PCV and MCHC values were normal in the control mice. As measured by the two sample comparison t-test (Armitage, 1971), significant statistical differences were observed between all the haematological values of the infected and control mice (table 1).

2. The second group: "infected with 100 cercariae".

Results almost similar to those of the first group were found in the second group (table 2 and fig. 3 a and b). The t-test showed that the differences between the haematological values of the infected mice of both groups were not significant. Meanwhile, the differences between the haemoglobin and the PCV values of the infected and control mice were highly significant ($P < 0.001$), while the difference between the MCHC values was less significant ($P < 0.02$) (table 2). Of the infected mice 33% had hypochromic anaemia and the rest normochromic, but in the infected group the mean MCHC was 32.4% and S.E. 0.89. This suggests that the hypochromic anaemia in this group (fig. 3 c) was less severe than that of the first group (fig. 2 c).

Table 1

Comparison between basic haematological values of mice infected with 50 cercariae of S.mansoni and their controls

Weeks after infection	Hb gm/dl		PVC %		MCHC %	
	Inf.	Con.	Inf.	Con.	Inf.	Con.
0 *	14.6	14.8	41.0	44.8	35.7	33.0
7 **	10.5	14.5	32.8	43.0	32.3	33.8
8	9.8	14.8	31.5	43.3	31.0	34.4
9	7.4	14.3	25.8	41.3	28.4	34.6
10	7.1	15.2	23.5	42.3	30.5	35.9
11	8.5	14.0	29.5	40.0	28.9	35.2
12	8.2	13.9	27.0	40.5	30.5	34.4
13	9.0	15.0	32.3	41.0	27.8	36.8
14	10.0	13.8	34.8	40.3	28.9	34.2
15	9.4	14.1	31.0	41.5	30.2	32.6
16	8.7	14.0	31.3	39.0	27.8	35.8
17	8.9	15.0	29.8	40.8	30.0	34.7
18	8.9	14.2	28.3	40.8	31.4	35.2
19 ✓	7.3	14.6	21.0	40.5	34.8	36.0
Mean	9.2	14.43	29.9	41.56	30.65	34.74
S.E.	0.37	0.12	1.04	0.48	0.46	0.22
P	P < 0.001		P < 0.001		P < 0.001	

* Beginning of incubation period

** End of incubation period

✓ Weekly readings are the mean of 2 mice, except when marked ✓- = 1 mouse

Table 2

Comparison between basic haematological values of mice infected with 100 cercariae of S. mansoni and controls

Weeks after infection	Hb gm/dl		PCV %		MCHC %	
	Inf.	Con.	Inf.	Con.	Inf.	Con.
0 *	14.2	14.4	41.0	40.0	34.7	36.2
7 **	12.2	14.5	38.3	42.0	32.0	34.5
8	11.6	14.7	35.5	41.0	34.6	35.9
9	10.3	14.2	33.5	39.5	30.8	35.9
10	8.0	14.8	26.0	40.5	31.7	36.5
11	6.6	14.4	20.5	42.3	33.3	34.1
12	6.6	14.6	22.5	39.5	30.0	36.9
13	5.8	14.3	19.8	43.0	29.6	33.2
14	7.8	14.0	23.8	41.0	32.8	34.2
15	8.1	15.1	23.0	41.5	36.1	36.6
16 ✓	5.8	14.3	16.0	40.0	36.3	35.8
Mean	8.94	14.45	27.79	40.95	32.44	35.35
S.E.	0.62	0.11	1.89	0.45	0.89	0.38
P Controls	P < 0.001		P < 0.001		P < 0.02	
P Group 1	P n.s.		P n.s.		P n.s.	

* Beginning of incubation period

** End of incubation period

✓ Weekly readings are the mean of 2 mice, except when marked ✓ = 1 mouse

Fig. (2)

Haematological values for mice infected with 50 Cercaria of S.mansoni and Controls

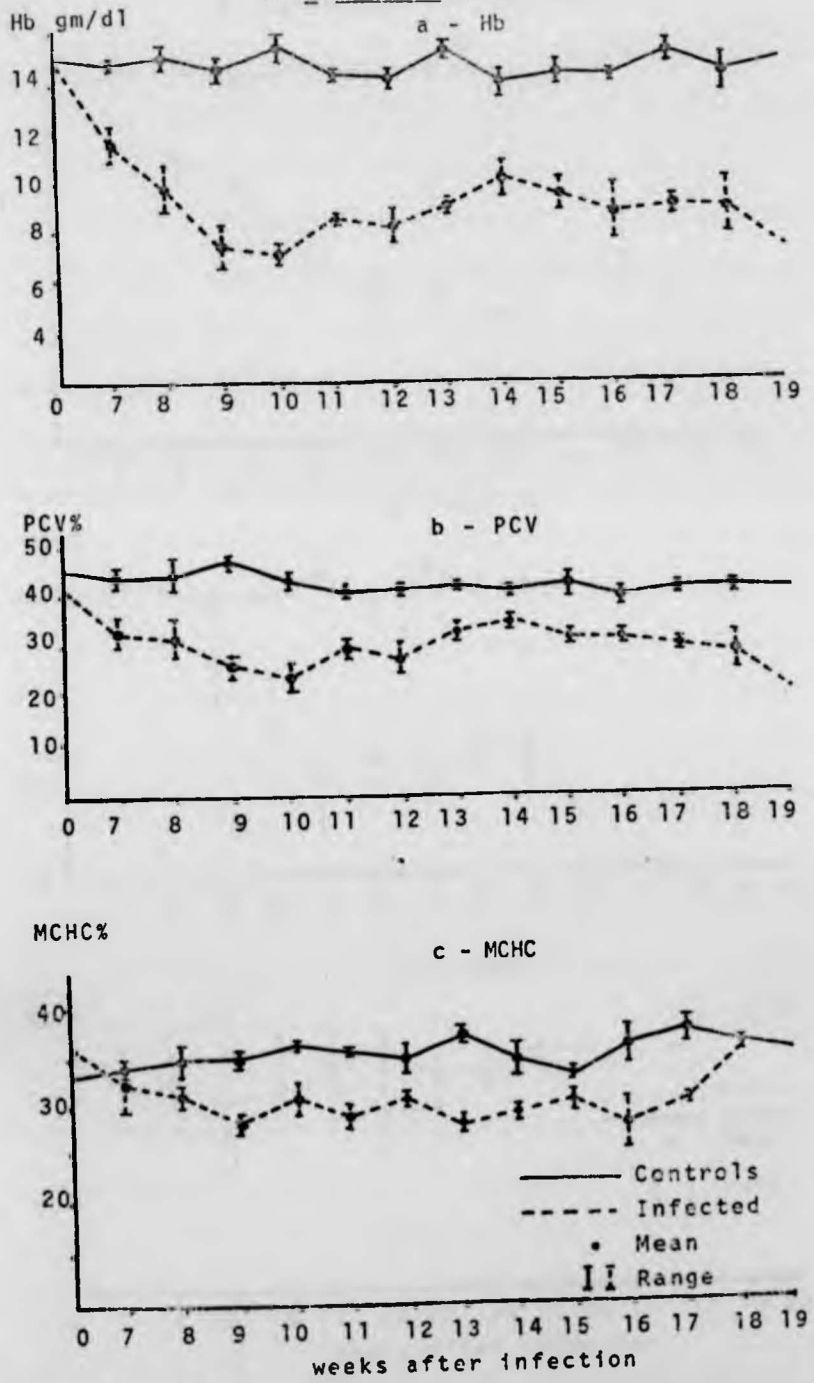
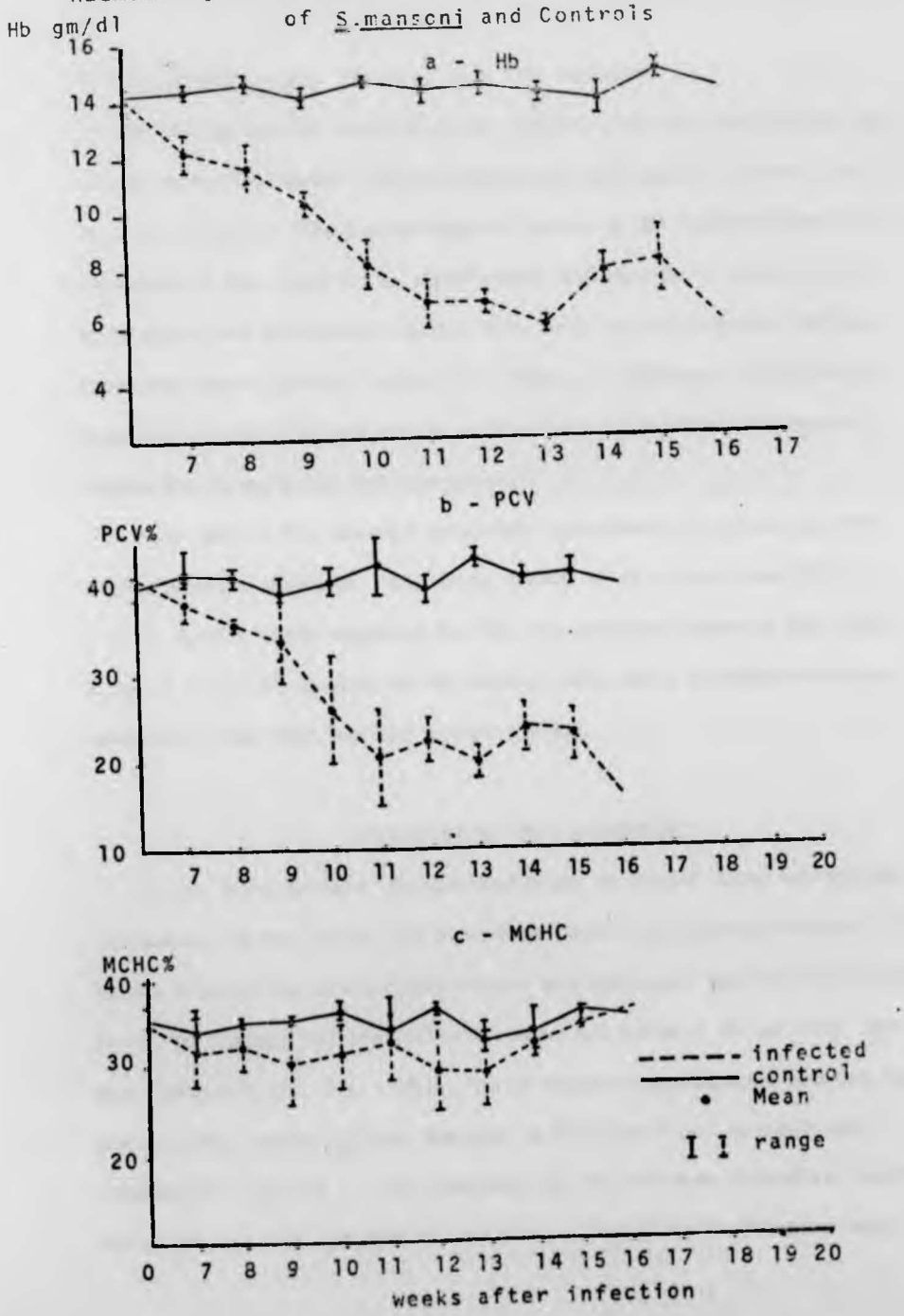


Fig. (3)

Haematological values of mice infected with 100 Cercaria of S.mansoni and Controls



3. The third group: "infected with 150 cercariae"

In this group the onset of severe anaemia was as early as the end of the incubation period "Hb 8.8 gm/dl and PCV 27%" (table 3 and fig. 4 a and b). The haematological values of the infected mice were revealed by the t-test to be significantly different from those of the first group and the control series, but were not significantly different from the second group (table 3). Thus, a significant effect on the intensity of anaemia may occur as the dose of infection is increased within the range 50 to 150 cercariae.

Only 24% of the infected mice had hypochromic anaemia and 76% normochromic anaemia. The mean MCHC in this group was 32.6% (S.E. 0.62) which suggests that the hypochromic anaemia was slight (fig. 4 c). The results in the control mice were analogous to those controls of the first and the second groups.

SUMMATION AND COMMENT

In the three groups, the infected mice developed different degrees of anaemia at the end of the incubation period and perhaps before. The initial drop in the haemoglobin values was followed, four or five weeks later, by limited and insiginficant rises, particularly in the first and third groups (fig. 5). Thus, the correlation coefficients between the haemoglobin values and the duration of infection were consequently significant (table 4). The haemoglobin results were therefore examined using the two way analysis of variance. The doses of infection were

expressed in one way "columns" against the duration after infection in the second "rows" (table 5). Strong evidence of the effect of the duration and the dose of infection on the mean haemoglobin values was observed. There is no evidence to suggest that interaction between the duration and the dose of infection "within the range of doses 50 to 150 cercariae" is significant (table 6). This suggests that both factors, namely the dose and the duration of infection, are interrelated and dependent on each other in producing but not in controlling the degree of anaemia. It was found, however, that the duration of infection affects the intensity of anaemia at a more significant level (0.1%) than did the dose of infection (2.5%) (table 6).

Death of all mice infected with 300 cercariae of S.mansoni after 54 days of infection was reported by Sauod (1965). Longer exposure of mice "84 weeks" to 25 cercariae of S.mansoni was found to cause severe hypochromic anaemia (Suad, 1978). The present study supports these suggestions to the effect that high doses of infection to mice with S.mansoni can cause severe anaemia . in a short period of time . (Sauod, 1965), while longer exposure to a small dose still can cause severe anaemia (Suad, 1978).

Throughout the course of infection, the anaemia was found to be normochromic although in some mice there was some degree of hypochromsia. The MCHC values of all the infected groups of mice fluctuated around the normal level; the correlation coefficients between

the haemoglobin and the PCV values in the three groups of the infected as in the control mice were highly significant ($P < 0.001$) (table 4). Thus, in the infected mice a decrease in haemoglobin was associated with a significant decrease in PCV and vice versa. This observation supports the evidence that the anaemia in schistosomiasis is mainly normochromic.

It has been reported that pathological blood loss such as that of some parasitic infections may cause hypocrhomic anaemia (de. Gruchy, 1973). Such hypochromia is commonly associated with microcytosis and is a characteristic feature of iron deficiency anaemia (MacLeod et al, 1979). In the present study, blood films made from infected anaemic and control mice were examined. Such films did not, however, show evidence of a significant hypochromia or microcytosis. Frequently, there was evidence of polychromasia and fragmentation of red cells and perhaps a slight degree of macrocytosis (fig. 6 and control fig. 7). The presence of the polychromatic cells "diffuse basophilic cells" has the same significance as has reticulocytes i.e. they indicate active blood regeneration, and when present in large numbers result in a slight to moderate degree of macrocytosis (de. Gruchy, 1973). The presence of the fragmented cells suggests that the anaemia is haemolytic in type (de. Gruchy, 1973 and Hoffbrand et al, 1975).

From these studies and the present findings it is suggested that the anaemia in schistosomiasis is mainly normocytic, normochromic with a

slight degree of hyperchromasia. This anaemia could be the result of a haemolytic process rather than the associated blood loss. Such blood loss, however, may be a contributing factor.

Table 3

Comparison between basic haematological values of mice infected with 150 cercariae of S.mansoni and their controls

Weeks after infection		Hb gm/dl		PCV %		MCHC %	
		Inf.	Con.	Inf.	Con.	Inf.	Con.
0 *		14.3	14.7	39.8	40.5	35.4	36.1
7 **		8.8	12.9	27.5	40.5	31.9	31.9
8		7.7	14.5	26.0	40.5	29.5	35.9
9		6.1	14.0	18.0	40.0	34.2	34.9
10		5.9	14.7	17.0	41.0	34.7	35.7
11		7.6	14.3	22.0	42.0	34.5	34.0
12		6.5	13.5	22.5	40.0	28.9	33.7
13		8.8	14.9	24.5	42.5	35.7	34.9
14		7.7	14.8	26.0	42.0	29.6	35.1
15		7.9	14.4	25.0	41.5	31.6	34.6
16		6.9	14.8	19.5	43.0	35.6	34.3
17		7.2	14.0	26.0	39.5	27.9	35.3
18		6.3	14.9	18.0	1.5	34.8	35.8
Mean		7.8	14.3	24.02	41.13	32.61	34.78
S.E.		0.43	0.12	1.2	0.25	0.63	0.3
P	Controls	P < 0.001		P < 0.001		P < 0.02	
	Group 1	P < 0.02		P < 0.001		P < 0.01	
	Group 2	P n.s.		P n.s.		P n.s.	

* Beginning of incubation period

** End of incubation period

Table 4

Comparison between basic haematological values of mice infected with S.mansoni and their controls

Sources of variations		Infected			Controls		
		1	2	3	1	2	3
No. of cercariae		50	100	150	0	0	0
Sample size		27	21	26	27	21	26
Weeks of test		19	16	18	19	16	18
Hb gm/dl	Mean	9.2	8.9	7.8	14.4	14.5	14.3
	S.E.	0.37	0.62	0.43	0.12	0.11	0.77
	Range	8.2	9.1	9.2	2.5	2.0	2.2
	P	1 & 2 n.s.	2 & 3 n.s.	1 & 3 < 0.02	inf. & con. < 0.001		
PCV %	Mean	29.9	27.7	24.0	41.6	41.0	41.1
	S.E.	1.01	1.9	1.2	0.48	0.45	0.24
	Range	21.0	27.0	26.0	8.5	7.5	5.0
	P	1 & 2 n.s.	2 & 3 n.s.	1 & 3 <0.001	inf. & con. < 0.001		
MCHC %	Mean	30.7	32.4	32.6	34.7	35.4	34.8
	S.E.	0.46	0.89	0.63	0.22	0.38	0.3
	Range	9.8	13.2	10.8	5.9	5.8	7.0
	P	1 & 2	2 & 3	1 & 3	inf. & con.		
		<0.05	n.s.	<0.01	<0.001	<0.02	<0.02
Hypochromsia		48%	33%	24%	O	0	3.8%
r.	Hb v PCV	P < 0.001					
	Hb v weeks	P <0.02	P <0.001	P <0.01	P n.s.		

r. Correlation coefficient

Two Way Analysis of Variance:

(a)

Table 5

Means of Hb values "2 mice weekly"

Weeks after infection	Infecting number of cercariae		
	50	100	150
0	14.6	14.2	14.3
7	10.5	12.2	8.8
8	9.8	11.6	7.7
9	7.4	10.3	6.1
10	7.1	8.0	5.9
11	8.5	6.6	7.6
12	8.2	6.6	7.6
13	9.0	5.8	8.8
14	10.0	7.8	7.7
15	9.4	8.1	7.9

(b)

Table 6

Statistical Terms

Source of variation	D.f.	Mean of Squares	Variance Ratio	Significance Level
1. Between weeks	14	19.3424	11.29	$P < 0.001$
2. Between cercariae	2	10.7287	6.26	$P < 0.01$
3. Interaction weeks x cercariae	28	2.102	1.23	P n.s.

Fig.(4)

Haematological Values of Mice infected with 150 Cercaria of S.mansoni and Controls.

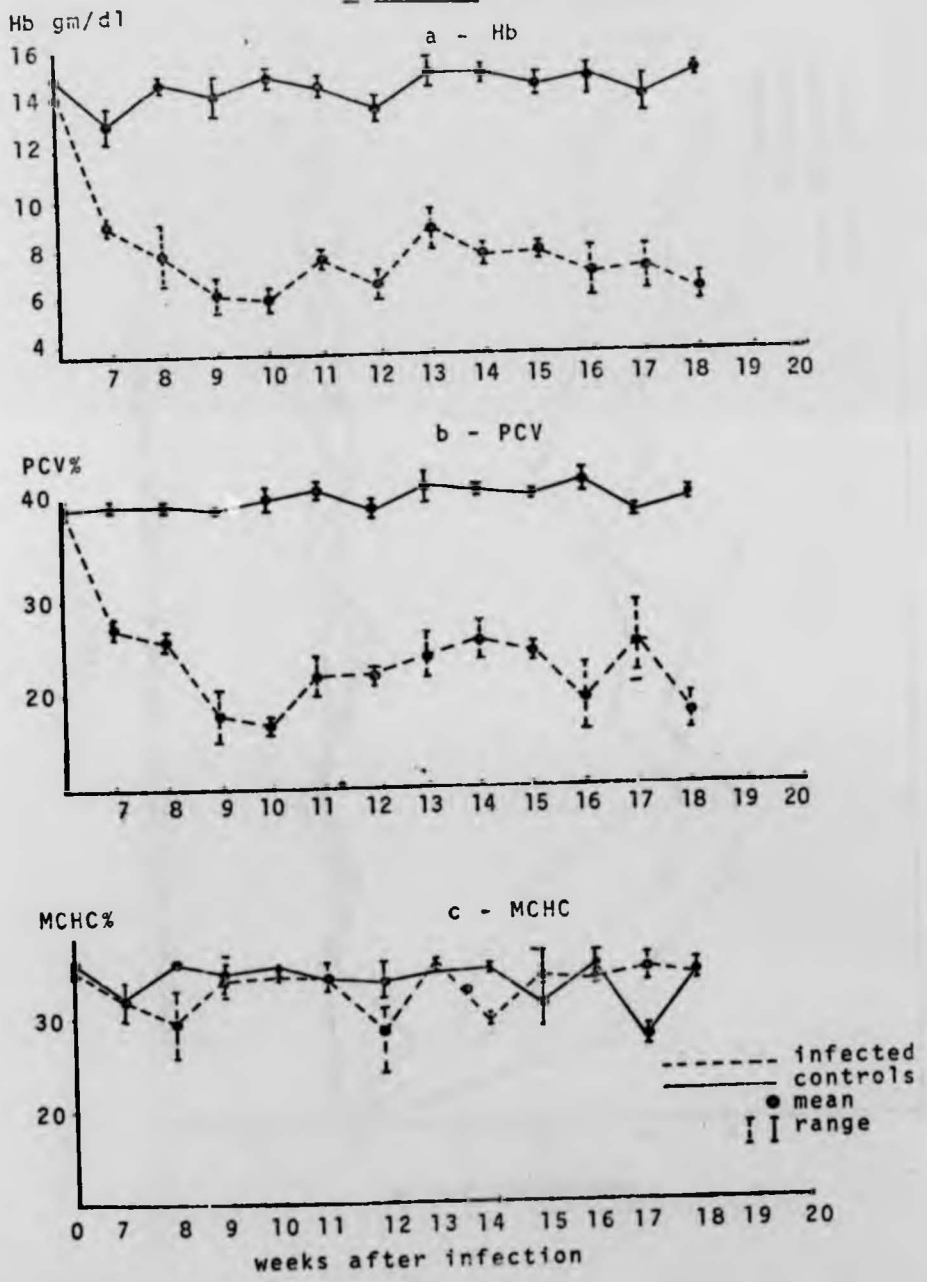


Fig.(5)

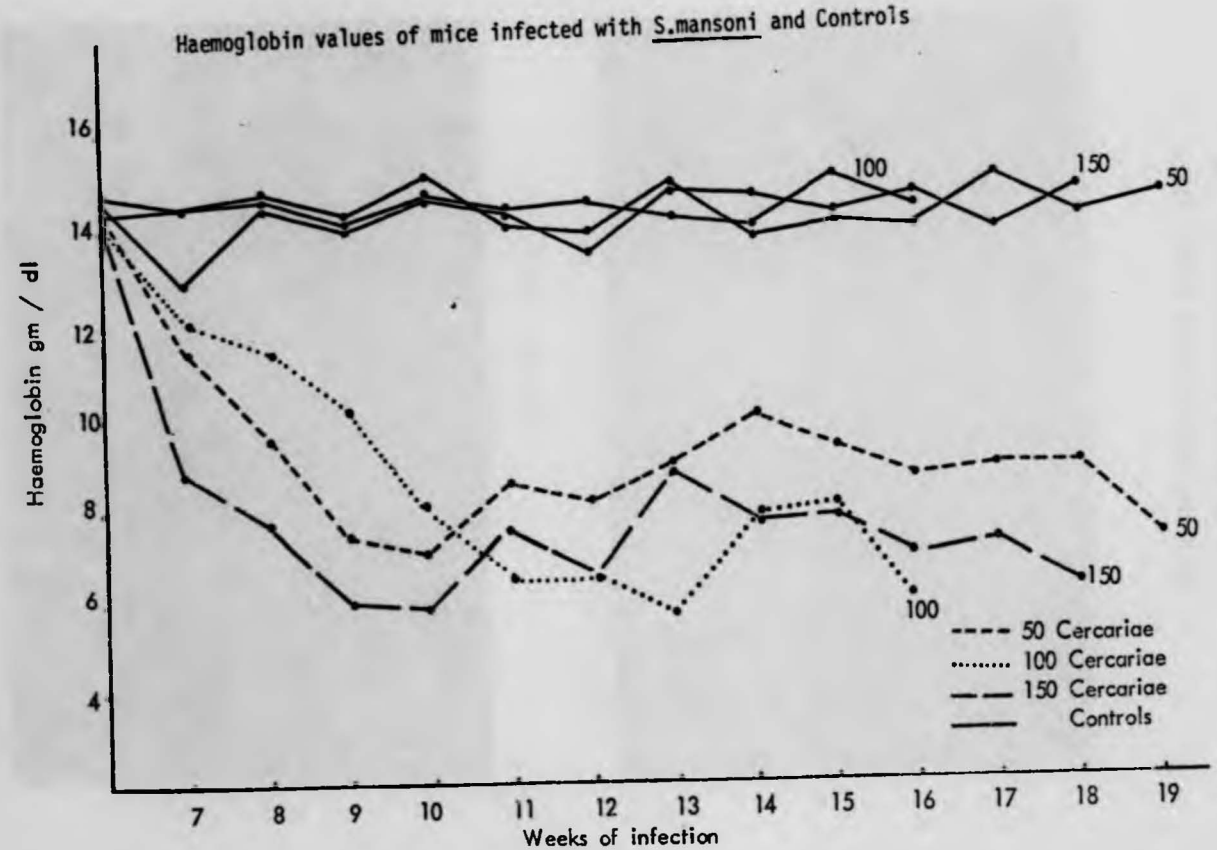

Haemoglobin values of mice infected with S.mansoni and Controls
Haemoglobin gm / dl
16
14
12
10
8
6
4
7
8
9
10
11
12
13
14
15
16
17
18
19
Weeks of infection
100
150
50
50
150
100
50 Cercariae
100 Cercariae
150 Cercariae
Controls

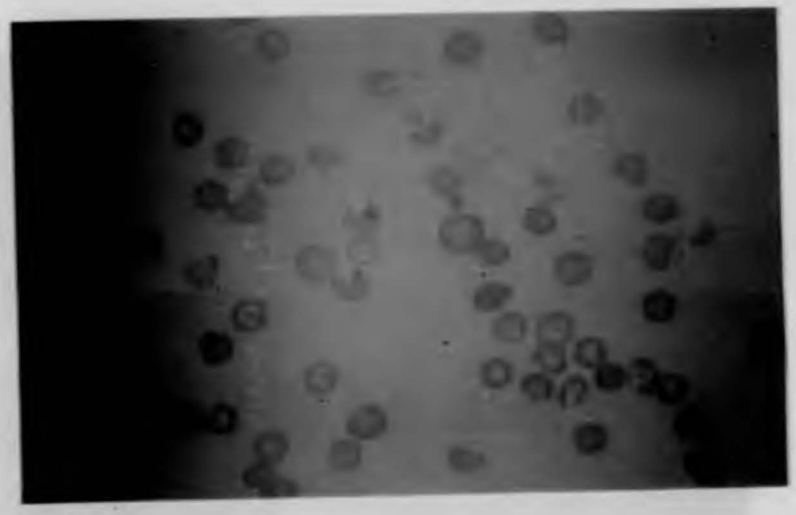
Figure 6

Blood film of mouse infected with 50 cercariae of S.mansoni for 13 weeks showing :

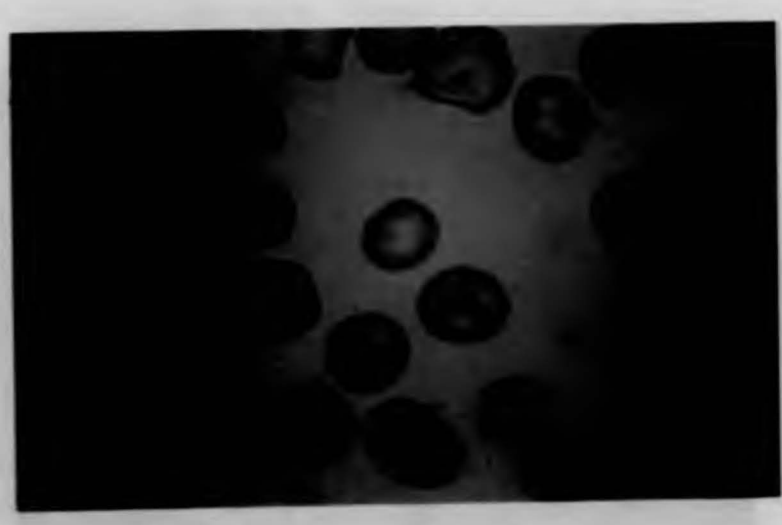
(i) Polychromasia

(ii) Fragmentation of red cells

(iii) Slight degree of macrocytosis



"a" Low Power



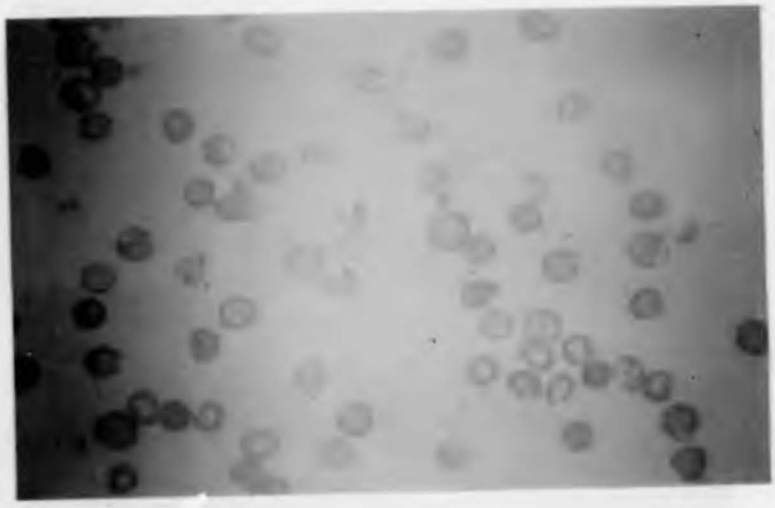
"b" High Power

Figure 6

Blood film of mouse infected with 50 cercariae of S.mansoni for 13 weeks showing : (i) Polychromasia

(ii) Fragmentation of red cells

(iii) Slight degree of macrocytosis



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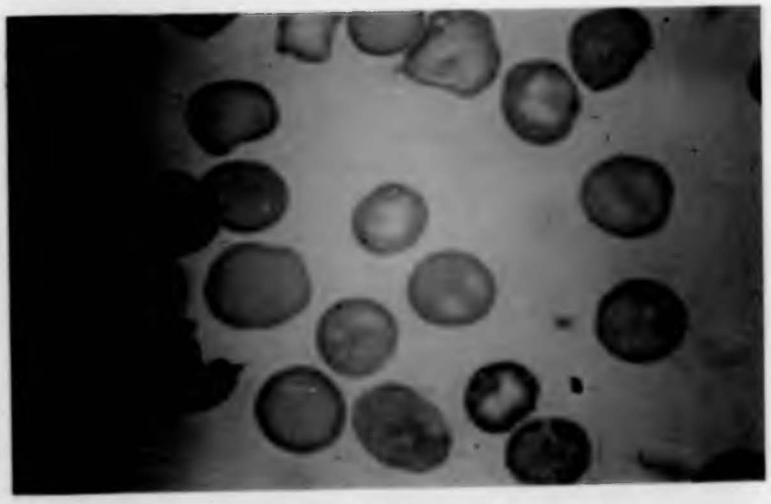


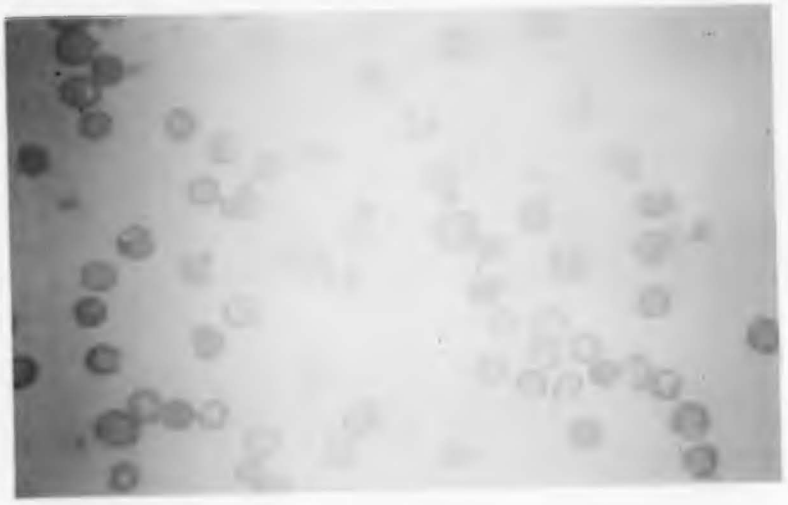
"b" High Power

Figure 6

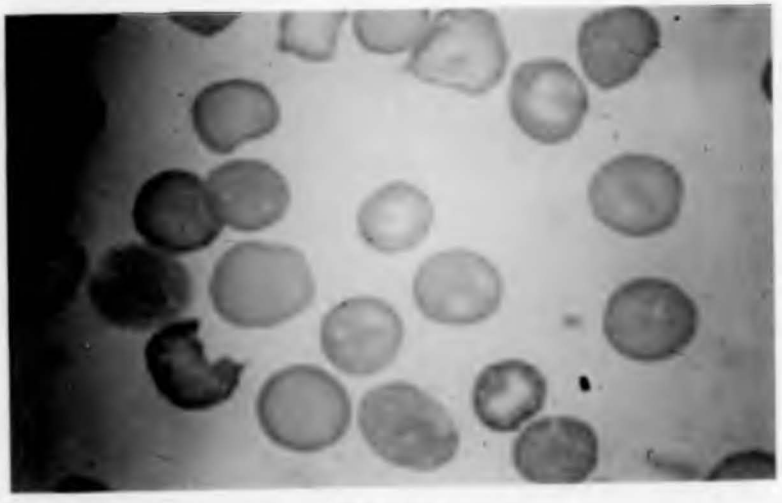
Blood film of mouse infected with 50 cercariae of S.mansoni for 13 weeks showing : (i) Polychromasia

(ii) Fragmentation of red cells

(iii) Slight degree of macrocytosis



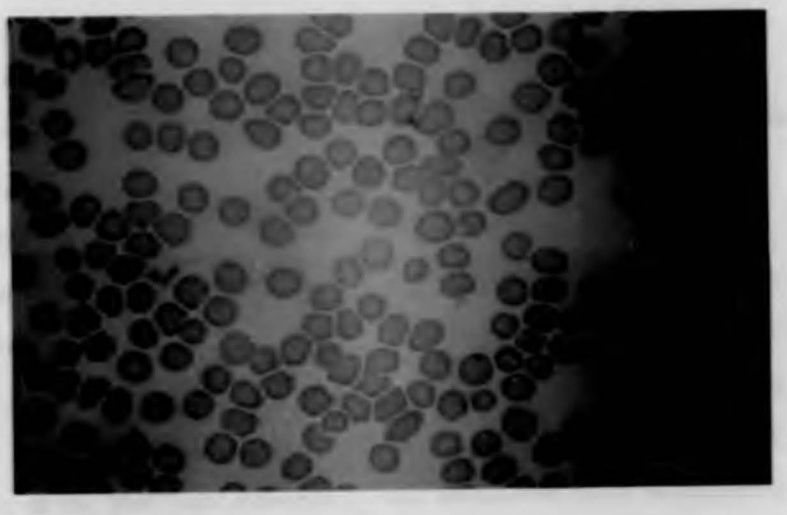
"a" Low Power



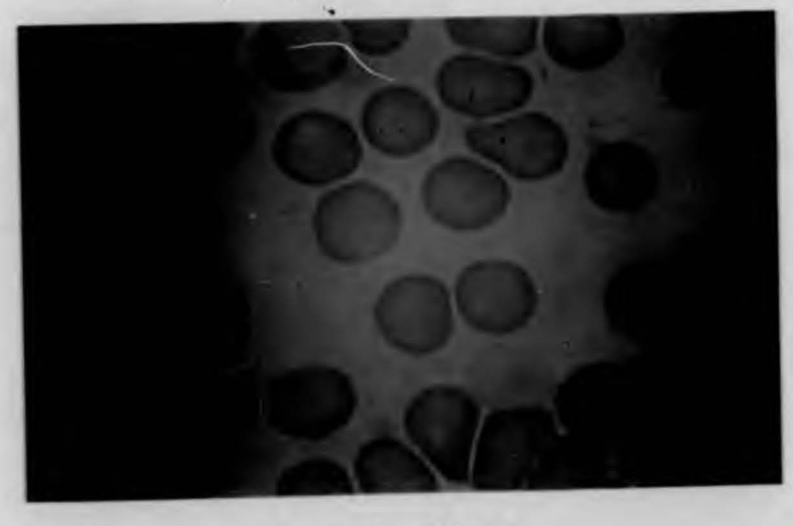
"b" High Power

Figure 7

Blood film of control mouse " week 13 "



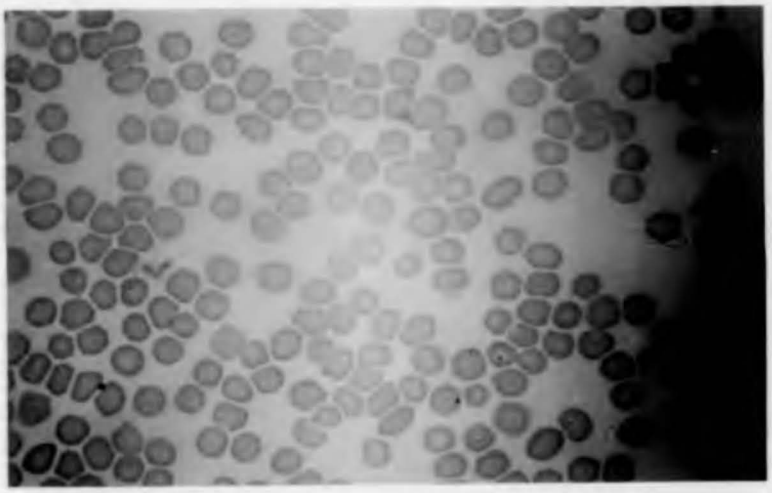
"a" Low Power



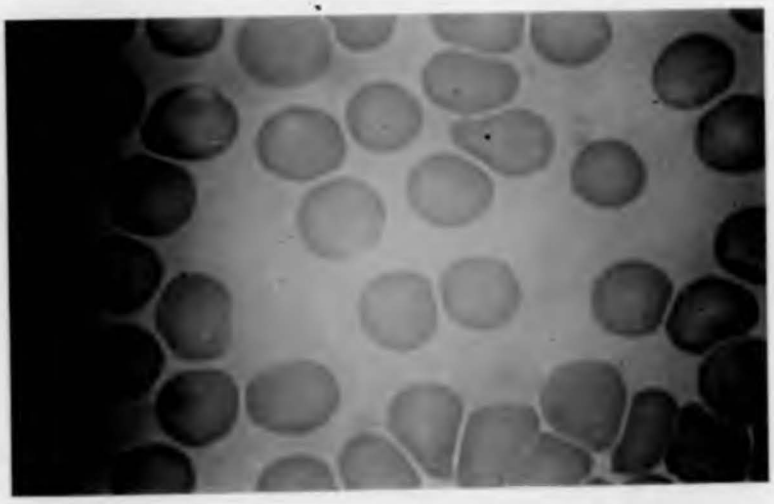
"b" High Power

Figure 7

Blood film of control mouse " week 13 "



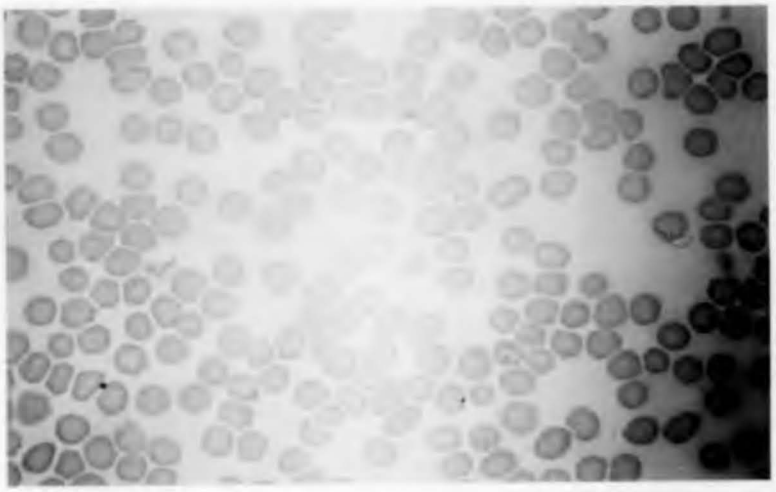
"a" Low Power



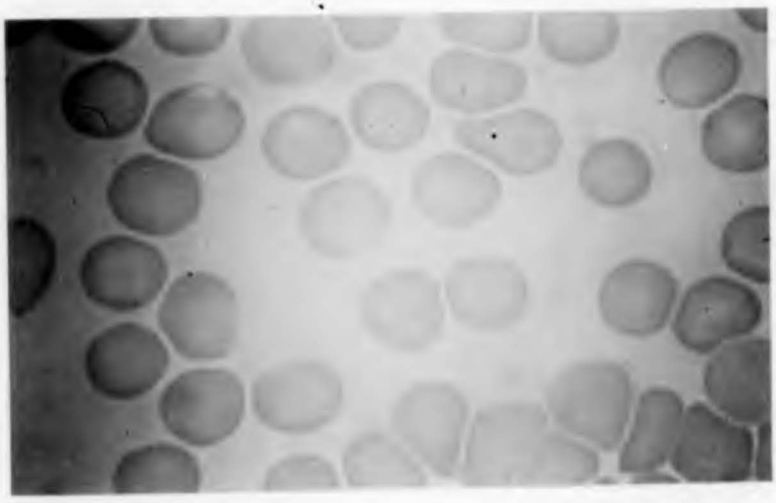
"b" High Power

Figure 7

Blood film of control mouse " week 13 "



"a" Low Power



"b" High Power

CHAPTER 2

TRUE SPLENOMEGALY AND ANAEMIA

Human and animal investigations have shown that the anaemia associated with schistosomiasis can be caused by or aggravated by splenomegaly (Reimann et al, 1960 and Woodruff et al, 1966, Sabourc et al, 1967 and Woodruff, 1973). There has been, however, no previous report on the effect of infection with S. mansoni on the spleen/body weight ratio. There have also been no previous reports of the relationship between the actual increase in the spleen weight and the course of the anaemia in schistosomiasis. In view of the important role some observers consider the spleen to have in schistosomiasis, a study of the kind here referred to was considered to be of importance.

PROCEDURE

The body weights and spleens of all the infected and control mice were measured in grams throughout the experiment at the time the animals were autopsied. The percentage of the actual increase in the spleen weight (true splenomegaly) was calculated as follows:

$$\frac{\text{Spleen weight}}{\text{Body weight}} \times 100 = \%$$

RESULTS

The mean of the spleen/body weight percentages for each group of mice was increased with the increase in the dose of infection (tables 7 and 8 and fig. 8). The difference in true splenomegaly between the groups infected with 50 and 150 cercariae was shown by the t-test to be significant ($P < 0.2$), but neither of these groups were significantly different from the group infected with 100 cercariae (table 8).

Because of the overlapping in the raw data standard curves (figs. 8 and 9 a) and in order to smooth the curves, a non-linear regression fitted model curves "exponent curves", (Geggs et al, 1964) (fig. 9 b) showed clearly that increase in infection (cercarial load) was associated with an increase in splenomegaly.

COMMENT

1. Throughout the experiment, the splenic weights of the control mice varied between 0.07 - 0.68 gm. Such spleen weights in these two extremes represented about 0.47% of their body weights. Measuring the spleen weights by the suggested ratio procedure, therefore, gives no chance for any false impression of splenomegaly. Moreover, it is thought to be more accurate than weighing the spleen in milligrams as reported by Magalhaes et al, (1961).

2. It has been claimed that there is no significant difference among the spleen weights of mice infected with 150 cercariae of S.mansoni from the 3rd to the 79th day after infection, although the variation of the spleen weights within the group is greater than that of the average weight (Magalhaes et al, 1961). In the present study, however, there was progressive increase in the spleen weights of the infected mice from the end of the incubation period; 5 or 6 weeks later, splenomegaly followed more or less a steady course (fig. 8).

Splenomegaly was significantly related to the degree of anaemia (table 8). However, this does not exclude the possibility of other factors still influencing anaemia. Thus, mice with the spleen surgically disconnected from the portal circulation and placed under the skin (marsupialization)where there is no pooling of blood, showed splenomegaly 3 weeks after infection with cercariae of S.mansoni (Andrade, 1964). Andrade (1962) also suggested that in mice infected with S.mansoni, the reticulo-endothelial stimulation is an important factor in the pathogenesis of splenomegaly rather than the chronic passive congestion of blood due to portal hypertension. Moreover, anaemia can be developed in splenectomised mice when infected with S.mansoni (Mahmoud et al, 1972).

Table 7

True splenomegaly "Spleen/Body weight %" in mice infected with *S. mansoni* and their controls

Weeks after infection	Group 1		Group 2		Group 3	
	Inf.	Con.	Inf.	Con.	Inf.	Con.
0	0.49	0.47	0.41	0.44	0.48	0.42
7	0.94	0.47	1.12	0.49	1.11	0.44
8	1.09	0.43	1.4	0.5	1.63	0.49
9	0.99	0.42	1.55	0.53	1.53	0.38
10	1.2	0.43	1.75	0.53	2.18	0.37
11	1.6	0.5	1.65	0.5	1.78	0.47
12	1.6	0.47	1.85	0.47	1.8	0.34
13	1.48	0.48	1.8	0.54	1.54	0.52
14	1.79	0.42	1.6	0.47	1.8	0.54
15	1.83	0.45	1.6	0.52	1.9	0.5
16	1.58	0.52	1.6	0.54	1.9	0.46
17	1.79	0.48	-	-	1.7	0.44
18	1.83	0.44	-	-	1.9	0.5
19	1.8	0.48	-	-	-	-
Mean	1.39	0.46	1.47	0.49	1.7	0.45
S.E.	0.36	0.07	0.34	0.09	0.31	0.12

Table 8

Statistical analysis of some aspects of splenomegaly and its relation to anaemia in schistosomiasis

Doses of infection	t - test		Corr. Coef.	
	Infected	Controls	Hb gm/dl	PCV %
Group 1 50 cercariae	Group 2 P n.s.	P < 0.001	P < 0.01	P < 0.05
Group 2 100 cercariae	Group 3 P n.s.	P < 0.001	P < 0.001	P < 0.001
Group 3 150 cercariae	Group 1 P < 0.02	P < 0.001	P < 0.001	P < 0.001

Fig. (8)

True splenomegaly in mice infected with S.mansoni and their Controls

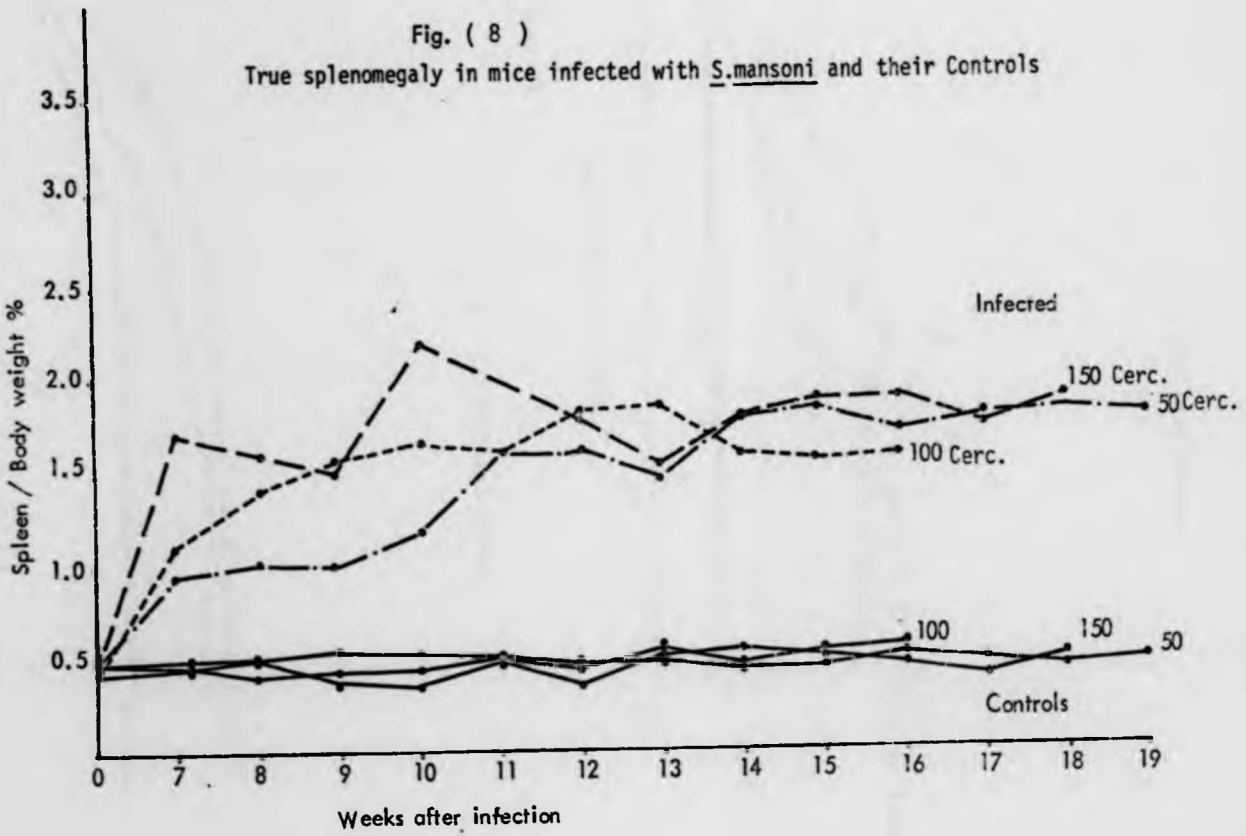
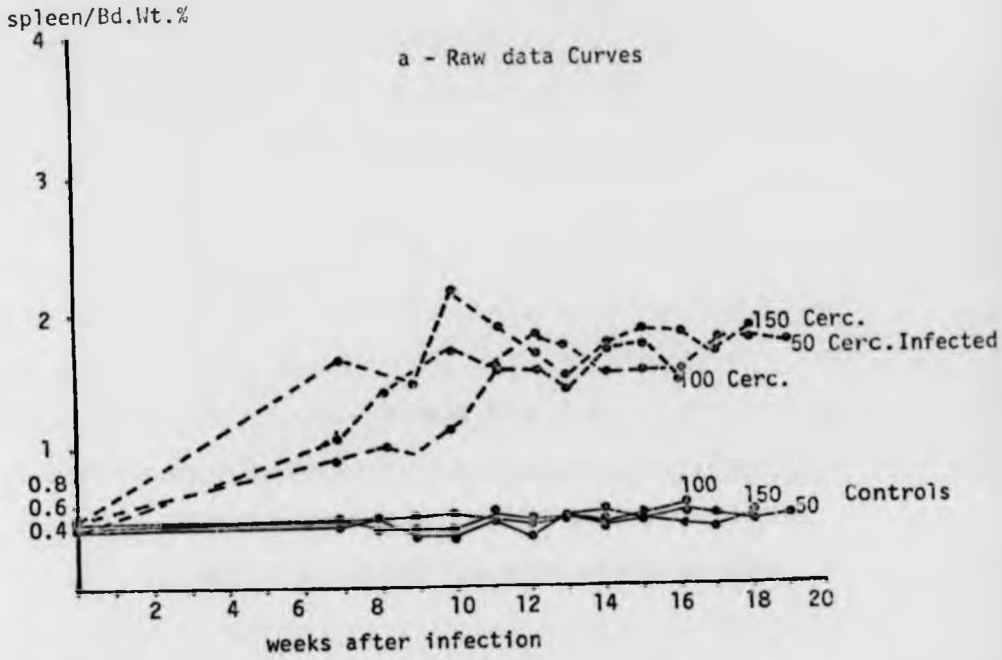
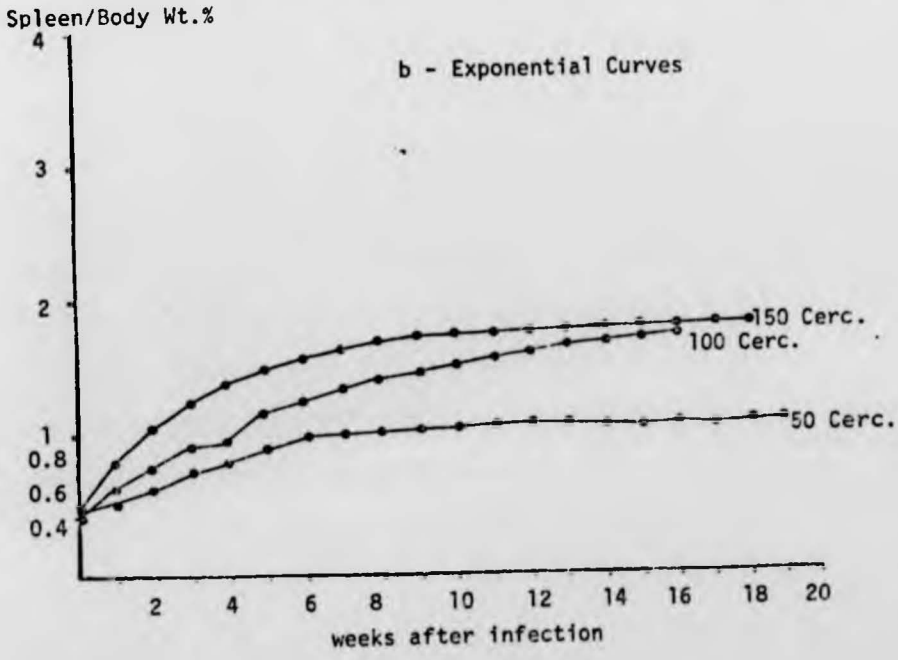


Fig. (9)

True Splenomegaly in mice infected with S. mansoni and Controls.





PART 4

COMPLEMENT ACTIVITY IN ASSOCIATION WITH ANAEMIA IN SCHISTOSOMIASIS

CHAPTER 1

ESTIMATION OF COMPLEMENT ACTIVITY BY MODIFICATION OF A RADIOACTIVE TECHNIQUE

The ability of certain complement components to cause lysis is of particular interest as it may enable some pathological and clinical aspects of disease to be understood. The complement system appears to be present in all higher forms of life (Forsdyke, 1973). Under certain conditions, complement can participate in a variety of inflammatory and membranolytic processes (Glovsky et al, 1974). In this study attention has been concentrated on the process whereby complement is involved in damage to the erythrocyte membrane.

In practice the measurement of complement activity is not easy. Lachmann et al, (1973), reported that the investigation of serum complement tends to be restricted to relatively few laboratories largely because of the difficulties in setting up the relevant techniques. In this study a radioactive immunohaemolytic test (Rosenberg et al, 1962) was found to be appropriate for measuring the haemolytic activity of complement in the mouse sera. The chief advantage offered by this radiometric technique over the other colourimetric methods for detecting haemolysis is the availability of an accurate assay of complement in which any haemolysis of mouse red cells offers no obstacle. As a result of pilot experiments, however, modification of some

aspects of the test was found to be necessary.

MATERIALS AND METHODS

I - Reagents:

1. Sodium chromate (Cr^{51}), a sterile aqueous isotonic solution of radioactive chomium (injection B.P., 1 mCi) was obtained from (The Radiochemical Centre, Amersham, U.K.).
2. Sheep blood in Alsever's solution, and rabbit anti-sheep haemolysin as an amboceptor were obtained from (Wellcome Reagents Limited).
3. Veronal buffered saline was prepared according to the method of Mayer (1961). Preparation of $\frac{1}{100}$ molar EDTA in 0.85% NaCL (PH 7.2) was effected by the addition of 0.1% gelatin to the buffer. This has been found virtually to eliminate loss of radioactive chromium (Rosenberg et al, 1962).

II - Haemolytic test:

1st Pilot experiment

1. Sheep blood was centrifuged at 2000 r.p.m. for 3 minutes and the supernatant was removed. To 0.1 ml of packed cells, 1 ml of Cr^{51} (approximately 150 μCi) was added. While being gently agitated, the mixture was incubated

for 1 hour in a water bath at 37°C. It was then refrigerated at 4°C overnight. After this the labelled cells were washed three to five times with veronal buffered saline and made up to 2.5% suspension (approximately 10^8 red cells/ml).

2. The amboceptor was made to 1:50 dilution. The sheep red blood cells were sensitised at ice bath temperature for 5 minutes at the most.

3. The test was carried out in small 10 x 77 mm sterile plastic tubes (No. 64268 Travenol Laboratories Ltd., Thetford, Norfolk).

In order to obtain reproducibility (± 5%) of results in replicate titrations, extreme cleanliness in every step was essential.

Using a micropipette, the reagents were added in the following order:-

a. 0.01 ml of the diluted haemolysin.

b. 0.05 ml of 2.5% Cr^{51} labelled sheep RBCs.

c. 0.01 to 0.10 ml of infected mice sera (after thawing and agitation).

d. Veronal buffer saline to give a total volume of 0.25 ml.

N.B. At this step marked haemolysis was noticed in all tubes.

4. All relevant controls were included with each set of tests:

   i. Sera from control mice for step (3-c).

   ii. Absence of either step (3-a) or (3-c).

   iii. Mouse sera heated to 56°C for 30 minutes for step (3-c).

5. The reactants were mixed, placed in a (37°C) water bath and mixed at 15 minute intervals. The reaction was stopped by the addition of 2 ml cold veronal buffered saline. The tubes were rapidly centrifuged at 3000 r.p.m. for 3 minutes, the supernatant was poured into separate tubes.

   N.B. At this point complete haemolysis was noticed in all tubes.

6. Both the supernatants and the sedimented cells were placed in a well-type scintillation counter. Samples were counted for 40 seconds, and total counts of between 30,000 - 40,000 were obtained.

7. Correct counts were measured by subtraction of the background count from both the count for the supernatant and that for the sedimented cells. The remaining counts were identified as correct counts. The counts attributable to the non-specific chromium leakage from the cells were determined by measurements of the counts from the

supernatants of the controls (step 4, ii).

The percentage of lysis was calculated as follows:-

$$\% \text{ of lysis} = \frac{S-a}{T-a} \times 100$$

S = counts in supernatant minus background.

T = total counts of supernatant and cells minus background.

a = counts attributable to non-specific leakage of chromium.

It was impossible to use the above formula to determine the percentage of haemolysis for due to almost complete haemolysis in all tubes, the count in (a) was more or less the same as in the other supernatants (S). It was essential, therefore, to review the steps of the test in order to obtain a reasonable haemolysis. Thus, the dilution of the amboceptor and of the radioactive chromium were increased. Sterile normal saline, glass tubes and sheep blood from other laboratories were also used.

The final suitable alternatives were as follows:-

1. Increase the dilution of amboceptor to 1 : 100.
2. Decrease the dose of (Cr^{51}) to 100 µCi/ml.
3. Use of sterile istonic phosphate buffered saline PBS (PH 7.2 with 0.1% gelatine) instead of the veronal buffered saline in all steps of the test.

Using the above modifications, the test was performed again and

sensible results were obtained (table 9).

2nd Pilot experiment

The amount of serum available from a single mouse was almost not enough to be used in step (3-c) "0.01 - 0.10 = 0.56 ml of serum". Thus, according to the availability of serum from an individual mouse, either amounts were used in step (3-c) as follows:-

x. 0.01, 0.03, 0.05, 0.07 and 0.1 = 0.26 ml of serum.

or

xx. 0.02, 0.04, 0.06, 0.08 and 0.1 = 0.3 ml of serum.

The amounts in step (x) were made first and if serum was still available, 0.01 ml of it was added to the first 4 tubes, thus turning step (x) into step (xx). The idea was to measure complement activity in each mouse, and to use all the serum available from such mouse.

In order to test the sensitivity of this variation of the method, pooled sera from two infected and two control mice were used. The differences between the percentage of haemolysis between the odd (x) and even (xx) amounts of sera were found to be not significant (table 10). This means that either amounts of sera (x or xx) could be used to detect haemolysis. Indeed, if all the serum obtained from one mouse i.e. 0.26 - 0.3 ml was used in one step, the degree of haemolysis will be increased and may exceed 100%. This required

re-adjustment of the test and dilution of the reagents. Although this may have seemed more easy than titration of the serum, its main disadvantage is the lack of sensitivity due to the inability to observe the proportional increase in haemolysis with the increase in the amount of sera (table 9 and Fig. 10). However, the one step method may be useful for the purpose of screening mice for the presence of complement (Rosenberg et al, 1962).

Bearing in mind that the amount of any mouse sera used in the test is in fact the whole amount of serum obtainable from the mouse's circulation; the mean of the percentages of haemolysis in an individual mouse serum, therefore, is an accurate measure of the haemolytic activity in that serum. This was expressed as the percentage of haemolytic units per mouse serum.

Using the previous modifications, the test was successfully used to measure the haemolytic activity (complement) in the sera of the infected and control mice.

The test was also used to detect the haemolytic activity in human and monkey sera. In both conditions, the percentage of haemolysis, even with the smallest amount of serum (0.01 ml), was almost 100%. Dilution of the amboceptor may be necessary for the test to be suitable for such sera.

RESULTS

At the end of the incubation period, the three infected groups of

mice showed marked decrease in their complement levels as compared with the control series. There was no more noticeable fall in the complement levels 3 to 5 weeks later, and through to the end of the experiment (figs. 11 and 12). This probably resulted from an increasing immunity against the infection.

In the two groups of mice infected with 50 and 150 cercariae, the complement levels were, as shown by the t-test, significantly different from each other, but not from the group infected with 100 cercariae (table 11). This suggests that an increase in the cercarial load can cause a fall in the complement levels. In the control mice there was no significant difference between the complement levels in all groups (table 11).

In all the three groups of the infected mice there was a significant relationship between either the haemoglobin (fig. 15) or the PCV values and serum complement. A fall in serum complement was associated with a significant increase in the degree of anaemia "$P < 0.001$" (table 13). In the control series there was no such relationship (fig. 15).

Table 9

Titration of an individual serum of mouse infected with 50 cercariae of S. mansoni

Sera in ml	Counts in		Lysis %		
	Deposit	Supernatant	Per ml	Mean	S.E.
0.01	22757	3131	4.5		
0.03	19203	4190	10.0		
0.05	15031	6274	21.9	18.84	0.46
0.07	13854	7248	27.2		
0.1	13514	8010	30.6		
0.0 "No sera"	19713	2095	0		
0.05 "Heated Sera"	20625	1864	0	0	0
0.05 "No ambo-ceptor	19536	1696	0		
Background	189				

Table 10

Effect of usage of different amounts of pooled serum of infected and control mice

Amount of serum in nl	% haemolytic units/mouse serum	
	Infected	Controls
x odd amounts	18.8	38.7
xx even amounts	22.9	43.5
P	1. between infected : P n.s. 2. between controls : P n.s. 3. between infected x : P < 0.05 and controls xx : P < 0.05	

Table 11

Serum complement levels " means of every week reading" in mice infected with S. mansoni and their controls

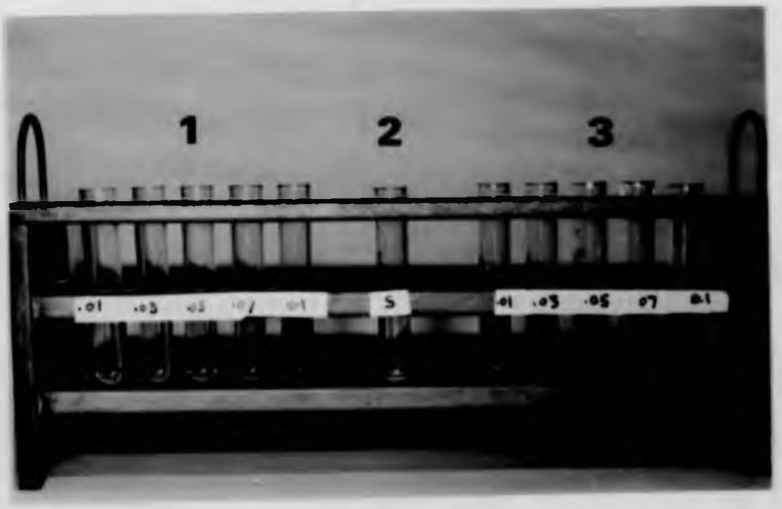
Weeks after infection	Complement " % haemolytic units/mouse sera"					
	Group 1		Group 2		Group 3	
	Inf.	Con.	Inf.	Con.	Inf.	Con.
0	34.2	41.2	35.8	31.5	28.5	33.7
7	22.1	53.5	15.6	27.7	9.0	34.0
8	13.7	34.2	7.6	48.1	7.1	42.6
9	8.6	50.0	11.1	36.0	5.3	34.8
10	10.0	37.3	6.0	48.2	5.0	37.7
11	8.9	34.4	4.8	50.0	9.5	32.2
12	15.0	40.5	5.9	39.0	4.8	46.5
13	17.6	55.0	8.2	38.9	6.4	41.2
14	14.5	40.6	9.7	45.8	7.3	54.6
15	11.2	47.4	10.7	48.6	9.6	45.3
16	13.8	37.9	6.8	49.3	5.5	52.3
17	13.6	64.5	-	-	8.9	37.5
18	9.2	40.6	-	-	9.8	42.3
19 ✓	10.4	47.8	-	-	-	-
Mean	14.6	44.6	11.3	42.1	8.9	41.1
S.E.	1.4	1.7	1.9	1.5	1.3	1.7
P	1&2	1&2	2&3	2&3	3&1	3&1
	n.s.	n.s.	n.s	n.s	0.05	n.s.
	< 0.001		< 0.001		< 0.001	

✓ Readings of a single mouse

Figure 10

Supernatant reactions from

1. Infected Serum
2. No Serum
3. Control Serum



Note increased haemolysis of sheep red cells " density of colour " with the increase in the amount of serum added to the reactants.

Figure 10

Supernatant reactions from

1. Infected Serum
2. No Serum
3. Control Serum

Note increased haemolysis of sheep red cells " density of colour " with the increase in the amount of serum added to the reactants.

Figure 10

Supernatant reactions from

1. Infected Serum
2. No Serum
3. Control Serum

Note increased haemolysis of sheep red cells " density of colour " with the increase in the amount of serum added to the reactants.

Fig.(11)

Complement levels in mice infected with S.mansoni and their Controls

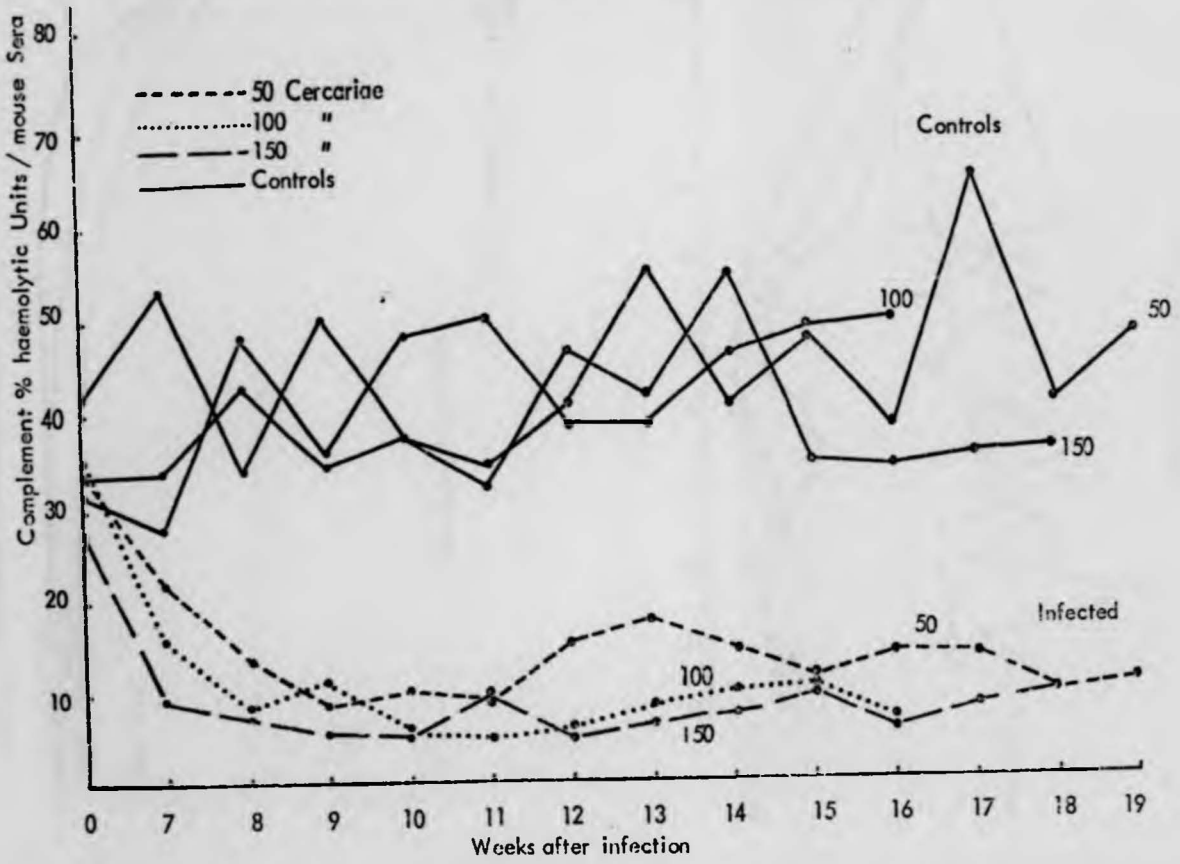


Fig. (12)

Complement levels in mice infected with S. mansoni and Controls

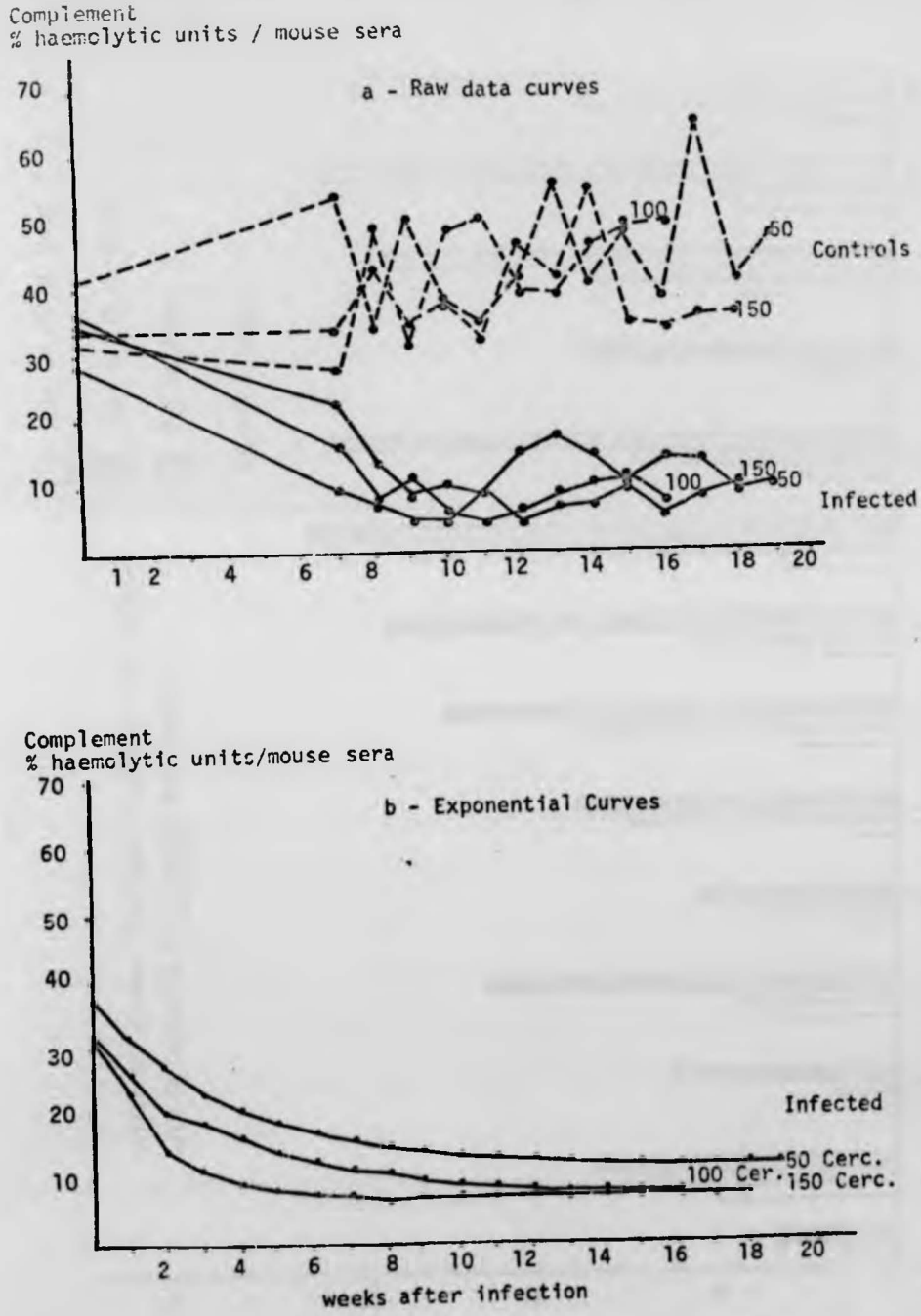
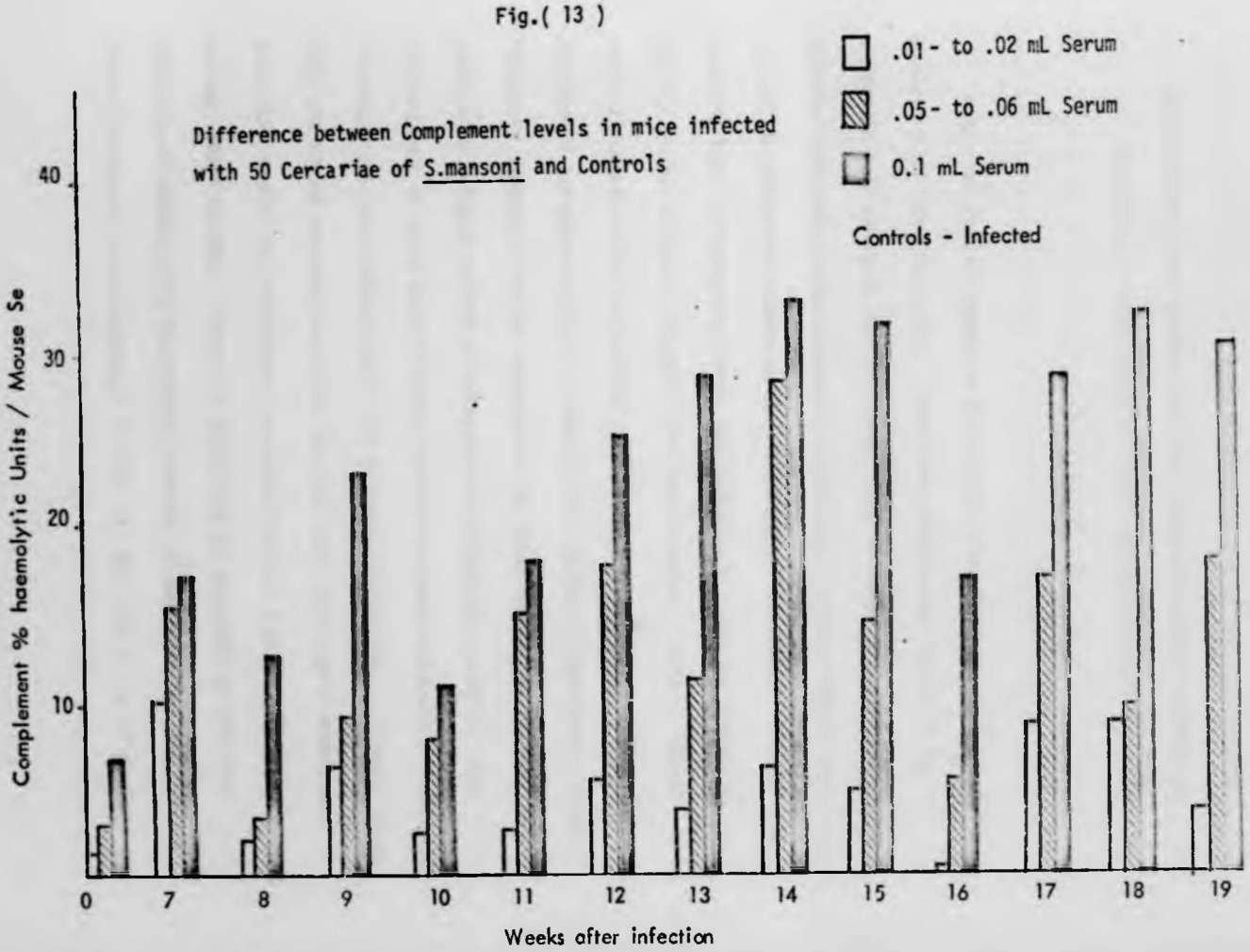


Fig.(13)


.01- to .02 mL Serum
.05- to .06 mL Serum
0.1 mL Serum
Difference between Complement levels in mice infected with 50 Cercariae of S.mansoni and Controls
Controls - Infected
Complement % haemolytic Units / Mouse Se
40
30
20
10
0
7
8
9
10
11
12
13
14
15
16
17
18
19
Weeks after infection

CHAPTER 2

DISCUSSION AND STUDY OF THE RELATIONSHIP BETWEEN ANAEMIA, COMPLEMENT AND SPLENOMEGALY

After the initial phases of this study, complement was detectable in almost all the mice sera. With the modification made in the materials and methods, the test was found suitable and probably more reliable than other colourimeteric techniques. In this study, the method developed to calculate the complement haemolytic activity was found to have an advantage over that commonly used and known as the CH 50/ml method (Mayer, 1961 and Fischer, 1967). Their method measures the haemolytic activity in normal sera, either by dilution of complement over a range where partial haemolysis occurs "Mayer's technique" or by measuring the time needed to achieve 50% lysis with a fixed amount of complement (Fischer, 1967). The percentages of lysis were determined spectrophotometrically; a sigmoid standard curve is plotted and used to read 20-80% lysis of other sera. The CH 50/ml method would use normal mice sera as a reference sera and would not, therefore, measure the low haemolytic activity of the mice weekly. Thus, it would not be possible to test the differences between the haemolytic activity of the infected and control mice throughout the experiment (table 11, fig. 13). It is particularly

important to do this in view of the great variation in complement there is between individual mice sera. It was for this reason that the CH 50/ml method was not used.

One observation worth mentioning here, is that the sera of the infected mice was much paler in colour (containing less haemolysed red cells) than that of the control mice (fig. 14). This may be related to the strength of the haemolytic power of such sera. This observation, therefore, adds to the value of using the radioactive techniques for measuring complement rather than the colourimeteric methods. This is because the colour of the sera would not affect the results obtained from the former method, but could do so in the latter.

In order to study the relationship between anaemia and complement and to compare that relationship with the relationship between anaemia and splenomegaly in all the infected mice, a whole data summary is given in (table 12) and a whole correlation matrix is given in (table 13 and fig. 14). From these two tables, it is obvious that complement and splenomegaly are both significantly related to the haemoglobin and PCV in the three groups. However, although the haemoglobin, PCV, complement and splenomegaly in group 1 and 3 are significantly different from each other (tables 4, 8 and 11), the relationship between either the haemoglobin or the PCV and complement in both groups does not differ to a significant level (table 13). The

relationship between either of the haemoglobin or PCV and splenomegaly in the same groups, however, is significantly different from each other at the 5% level (table 13). This suggests that with the increases in the dose of and/or the duration of infection, the relationship between anaemia and complement remains the same, but the relationship between anaemia and splenomegaly varies. In other words, in schistosomiasis, the degree of anaemia is more associated with a fall in the complement levels than an increase in the spleen weight. The complement dependent immunological mechanism, therefore, is more likely to be the prime cause for anaemia in schistosomiasis. This was suggested by Woodruff (1973), Mahmoud et al (1973) and Suad (1978) and confirmed by this study.

Table 12

Data summary of the infected mice

Variables	No. of cerc.	Sample size	Mean	S.E.	Range
a. Group 1					
1. Hb			9.21	0.37	8.20
2. PCV	50	27	29.90	1.04	21.00
3. Complement			14.62	1.42	28.10
4. Sp/BW			1.41	0.08	1.53
b. Group 2					
1. Hb			8.94	0.82	9.10
2. PCV	100	21	27.78	1.89	27.00
3. Complement			11.29	1.97	36.80
4. Sp/BW			1.47	0.09	1.53
c. Group 3					
1. Hb			7.80	0.43	9.20
2. PCV	150	26	24.01	1.23	20.00
3. Complement			8.90	1.27	28.30
4. Sp/BW			1.70	0.08	1.88

Table 13

Correlation matrix for mice infected with S. mansoni

		Group 1 "infected with 50 cercariae"		
1.	Hb			
2.	.873	PCV		
3.*	.742	.704	Complement	
4.*	-.523	-.385	-.598	Sp/Bwt
	1.	2.	3.	4.
		Group 2 "infected with 100 cercariae"		
1.	Hb			
2.	.911	PCV		
3.	.762	.610	Complement	
4.	-.822	-.740	-.874	Sp/Bwt
	1.	2.	3.	4.
		Group 3 "infected with 150 cercariae"		
1.	Hb			
2.	.930	PCV		
3.*	.864	.760	Complement	
4.*	-.775	-.710	-.703	Sp/Bwt
	1.	2.	3.	4.

All values of (r) are significant at the 5% level or less

Difference between 3* P n.s.

Difference between 4* P <0.05

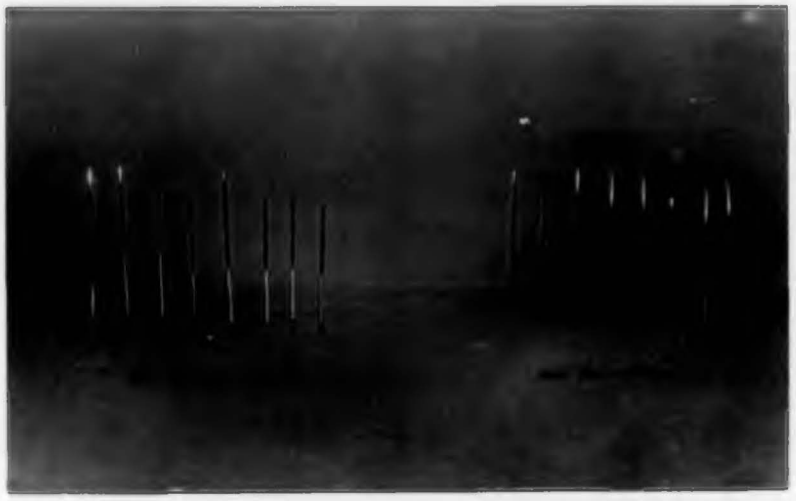
Figure 14

Sera of some infected and control mice showing that the former is paler than the latter

Figure 14

Sera of some infected and control mice showing that the former is paler than the latter

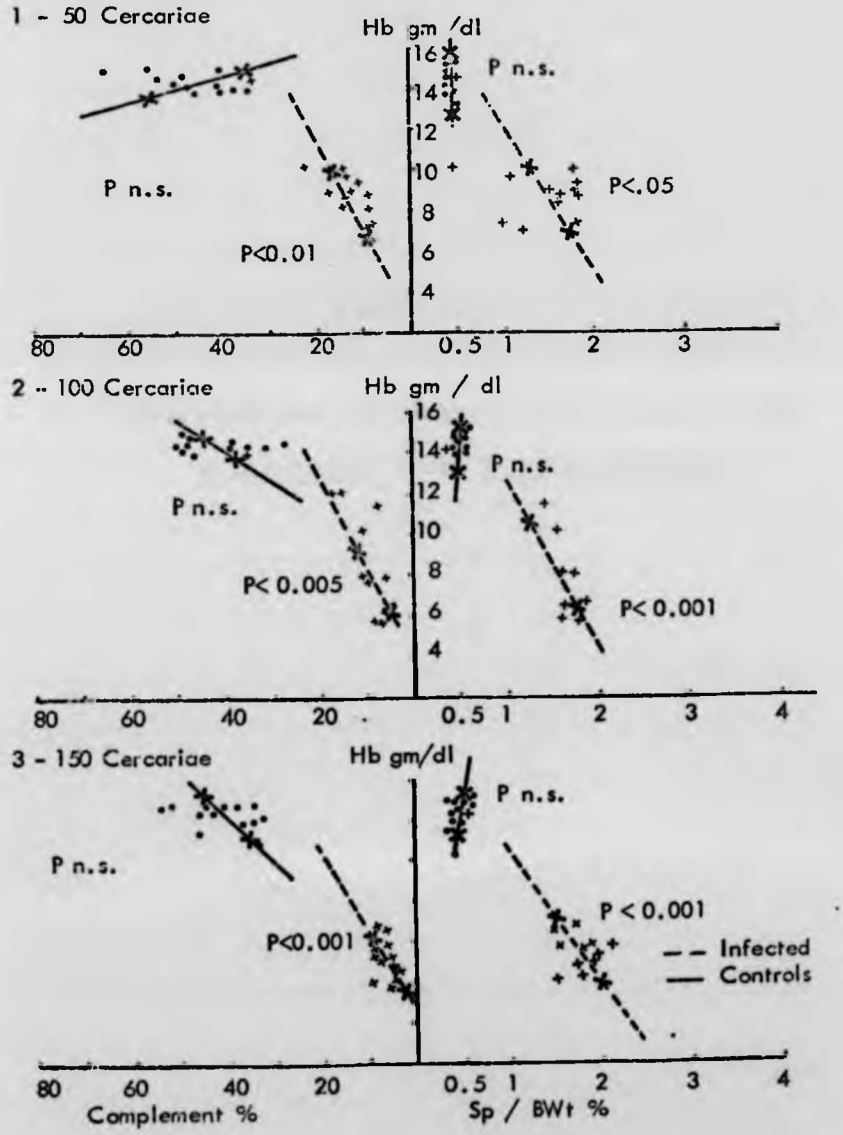
Figure 14



Sera of some infected and control mice showing that the former is paler than the latter

Fig(15)

Correlation matrix between haemoglobin and both Serum Complement and true Splenomegaly in mice infected with S.mansoni and Controls



PART 5

COMPARISON BETWEEN INFECTION WITH *S. MANSONI* AND *T. RHODESIENSE*

CHAPTER 1

EXPERIMENTAL TRYPANOSOMIASIS

In the study reported here, mice infected with Trypanosoma rhodesiense were examined for anaemia, splenomegaly and complement levels and the inter-relationship between these three factors. The results were compared with those of mice infected with S.mansoni.

T. rhodesiense was first described in 1910 from a patient in the Zambesi region where the infection may have existed in endemic form, and whence it gradually spread northwards (Wilcocks et al, 1973). The infection now is mainly confined to Central and Eastern Africa (De Raadt, 1976), though it still exists in "Gambia" West Africa (Lumsden et al, 1979).

The reasons why trypanosomiasis was selected for comparison with schistosomiasis were discussed in the review of literature.

MATERIALS AND METHODS

1. Experimental animals:-

   Two groups of 20 inbred T.O. strain male white mice (the same as that used in infection with S.mansoni) and equivalent number of control mice were used in this experiment.

2. Parasites and infection:-

Two strains of T. rhodesiense were used to infect the mice:

i. Animal strain : "group 1".

Number (D2/11/180 BIA), was kindly obtained from Dr. W.E. Ormerod, Protozoology Department, London School of Hygiene and Tropical Medicine.

ii. Human strain : "group 2".

Isolated from an African patient at the Hospital for Tropical Diseases in November, 1977. The parasites were separated from the blood of the donor patient by gradual centrifugation.

3. Infection:-

The number of trypanosomes in a suspension in sterile 5% dextrose - 0.9% NaCl was counted and adjusted to the desired concentration (1000 trypanosomes/0.1 ml). Each mouse was infected intraperitoneally with 0.2 ml of the T.rhodesiense suspension; controls received an equivalent volume of sterile diluent.

4. Course of infection:-

Mice were kept in isolated cages under conditions similar to that made for mice infected with S.mansoni.

Two mice from each group were killed weekly from the time of infection. Parasitaemias were followed by obtaining blood samples at the death of the mice with the aid of a haemocytometer counting chamber. For the haemocytometer counts, the blood samples were

diluted in erythrocyte-diluting pipettes with 1% formalin - 0.9 NaCl containing 10% Giemsa stain.

Haemoglobin, PCV, MCHC, complement and true splenomegaly were all measured as in schistosoma infected mice.

During the course of infection, 7 mice from the first group and 9 mice from the second group died.

RESULTS

After one week of infection a slight degree of normochromic anaemia was observed in the two infected groups of mice. With the progress of infection, anaemia gradually increased (figs. 16 and 17), but remained normochromic (table 14). The spleen/body weight (fig. 18) and the number of trypanosomes/ml of blood (fig. 19) also increased (table 15). Meanwhile, a drastic reduction of serum complement occured and remained so to the end of the experiment (table 16 and fig. 20). In the blood films stained with Giemsa no sign of hypochromasia was present but many polychromatic cells and some fragmented cells were found (fig. 21).

From these tables and figures, the results in the two groups of mice are seen to be very similar although one group was infected by a strain of trypanosome isolated from a patient's blood and the other by a strain of trypanosome kept in the laboratory for several years. The t-test revealed that the difference between all the results in the two

groups was not significant. It was, therefore, decided to add together the results obtained each week and use the mean as one group (table 17). This was particularly useful for comparison with the results obtained in schistosomal infected mice.

The relationship between anaemia, complement, splenomegaly in trypanosomiasis:

The relationship between the haemoglobin and the PCV was almost perfectly direct and highly significant "P < 0.001" (table 18) as a consequence of the development of normochromic anaemia during the course of the infection.

The relationships between anaemia and either serum complement, splenomegaly or the number of trypanosomes/ml of blood were all significant at the 5% level or less (correlation matrix, table 18). The decrease of serum complement was not significantly related to either the increase in the spleen weights or the increase in the number of trypanosomes/ml of blood and there could be other reasons for the drastic fall of serum complement observed in the infection. These reasons are discussed in the following chapter. Likewise, the almost perfect correlation between the haemoglobin and the PCV (r. 0.93), the relationship between the spleen/body weight % and the number of trypanosomes/ml of blood was highly significant (r. 0.94) (table 18).

Table 14

Basic haematological values of mice infected with T.rhodesiense and their controls

Sources of variations	Duration of infection								P
	1 day	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	
Group 1									
a.Hb Inf.	14.5	10.7	10.0	10.2	10.0	9.7	11.5	-	P < 0.001
Con.	14.5	15.0	15.2	14.5	15.2	14.5	14.9	-	
b.PCV Inf.	41.0	30.5	28.0	28.3	28.7	27.5	32.0	-	P < 0.001
Con.	40.5	41.0	42.5	40.5	42.5	41.3	41.5	-	
c. MCHC								-	P n.s.
Inf.	35.3	35.0	35.8	36.1	35.8	35.3	35.6	-	P n.s.
Con.	35.8	36.6	35.8	35.9	35.9	35.3	35.6	-	
Group 2									
a.Hb Inf.	14.2	12.9	11.4	11.4	8.2	9.1	7.9	9.5	P < 0.001
Con.	14.7	13.7	13.3	15.3	15.5	14.6	14.6	14.5	
b.PCV Inf.	39.0	36.0	31.3	31.8	22.8	25.5	22.3	26.5	P < 0.001
Con.	41.5	38.0	37.8	42.0	43.3	41.0	40.2	40.0	
c. MCHC									
Inf.	36.3	34.9	36.3	35.8	35.8	35.7	35.7	35.8	P < 0.001
Con.	35.3	35.9	36.1	35.8	35.8	35.8	35.9	36.3	

Table 15

Spleen/body weight % "true splenomegaly" and the numbers of Trypanosomes/ml blood in mice infected with T.rhodesiense and their controls

Sources of variations	Duration of infection								P
	1 day	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	
Group 1									
a. Sp/Bwt %									
Infected	0.57	3.23	3.5	3.55	2.54	5.78	4.72	-	P < 0.001
Controls	0.44	0.53	0.51	0.5	0.55	0.55	0.52		
b. Tryps/ml ÷ 1000	000	256	96.5	215	257	352	138	-	-
Group 2									
a. Sp/Bwt %									
Infected	0.57	1.74	1.95	3.75	4.72	5.14	6.23	7.0	P < 0.001
Controls	0.5	0.6	0.43	0.54	0.38	0.35	0.45	0.33	
b. Tryps/ml ÷ 1000	000	37.5	22.0	250	385	510	520	580	-

Table 16

Serum complement levels (% haemolytic units/mouse sera) in mice infected with T.rhodesiense and their controls

Duration of infection	1st Group		2nd Group	
	Infected	Controls	Infected	Controls
1 day	30.5	48.9	44.15	38.1
1 week	3.54	36.9	1.97	35.0
2 weeks	2.7	40.25	0.6	53.9
3 weeks	3.51	33.0	4.1	42.6
4 weeks	1.93	37.2	3.5	36.8
5 weeks	0.72	30.8	0.58	49.0
6 weeks	3.0	24.2	1.05	38.3
7 weeks	-	-	0.8	66.3
P	P < 0.001		P < 0.001	
	P n.s.			

Table 17

The results of all mice infected with T.rhodesiense and their controls

Sources of variations	Weeks after infection								Mean	S.E.	P
	0	1	2	3	4	5	6	7			
No. of mice: Inf.	4	3	4	4	4	4	4	1			-
Con.	4	3	4	4	4	4	4	1			
Hb gm/dl : Inf.	14.3	11.8	10.7	10.8	9.0	7.2	9.7	9.5	10.4	0.74	<0.001
Con.	14.6	14.1	14.3	14.9	15.4	14.6	14.7	14.5	14.6	0.11	
PCV % : Inf.	40.0	34.2	29.6	30.0	25.8	26.5	27.1	26.5	29.9	2.9	<0.001
Con.	41.0	39.0	40.1	41.3	42.9	41.1	41.1	40.0	40.8	0.4	
MCHC % : Inf.	35.9	35.8	36.0	35.9	35.3	35.5	35.7	35.8	35.8	0.08	n.s.
Con.	35.5	35.6	35.5	36.0	35.8	35.5	35.8	36.3	35.7	0.1	
Complement % :											
Inf.	37.3	2.8	1.7	3.9	2.7	2.2	2.6	0.8	6.8	4.4	<0.001
Con.	43.5	36.5	47.1	37.8	37.0	39.9	31.2	66.3	42.4	3.8	
Sp/Bw % : Inf.	0.57	2.23	2.76	3.59	3.43	5.46	5.47	7.0	3.8	0.73	<0.001
Con.	0.5	0.58	0.47	0.52	0.46	0.45	0.38	0.33	0.46	0.03	
Tryps/ml ÷ 1000	000	110	59.3	233	321	426	329	580			-

Fig(16)

Haemoglobin values in mice infected with T.rhodesiense and their Controls

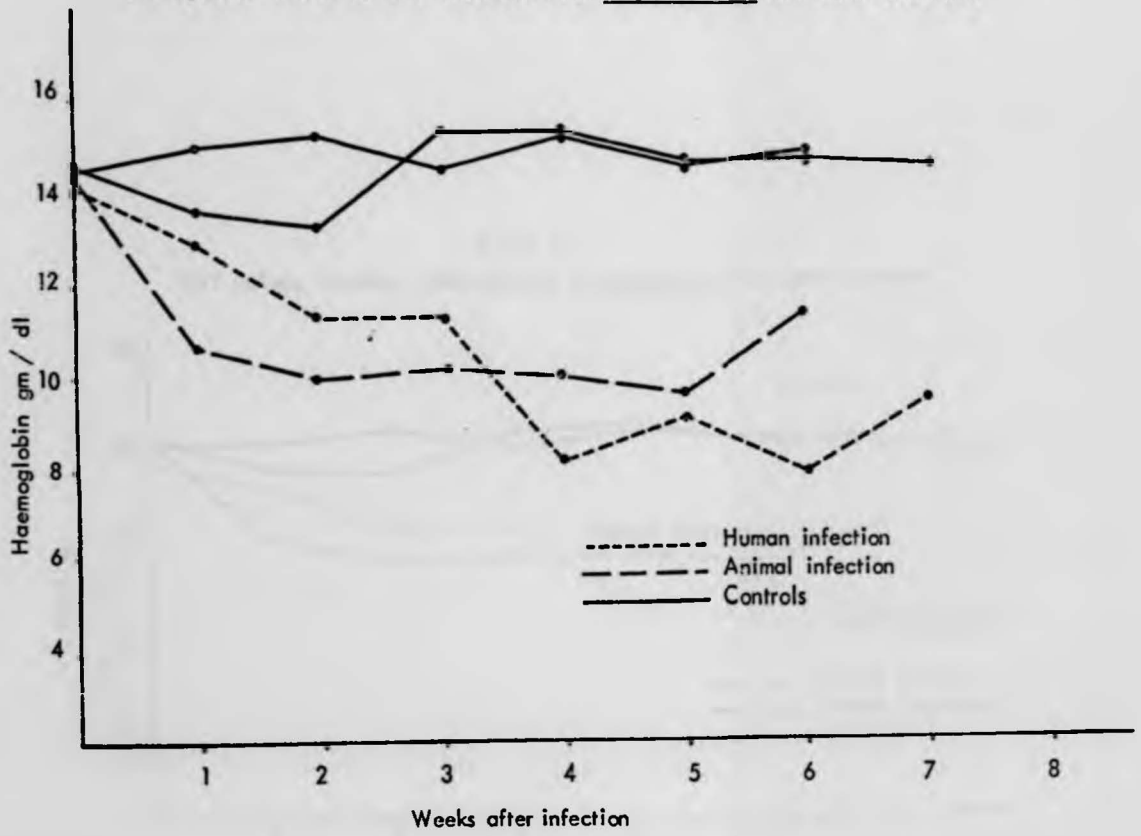


Fig.(17)

PCV% values in mice infected with T.rhodesiense and their Controls

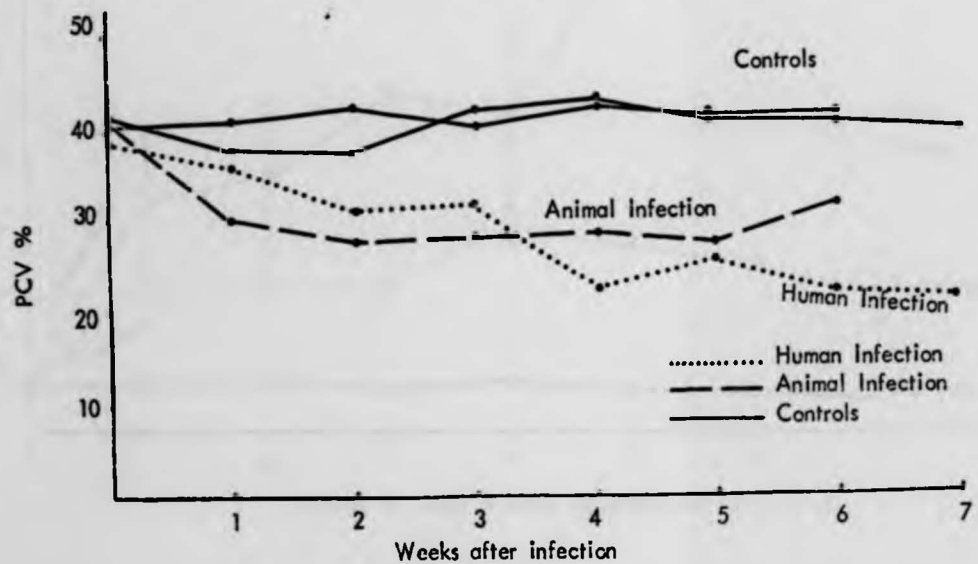


Fig.(18)

True Splenomegaly in mice infected with 150 Cercariae of S.mansoni; mice infected with T.rhodesiense and Controls.

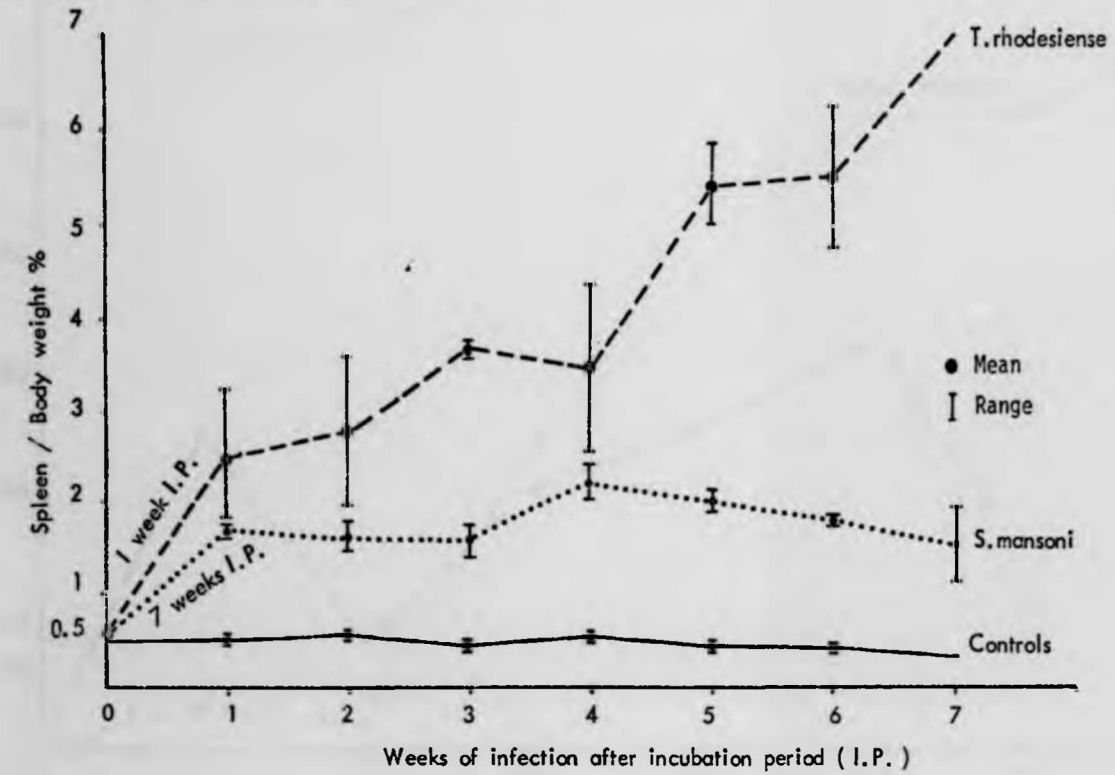


Fig.(19)

Progress of Parasitaemia in mice infected with T.rhodesiense

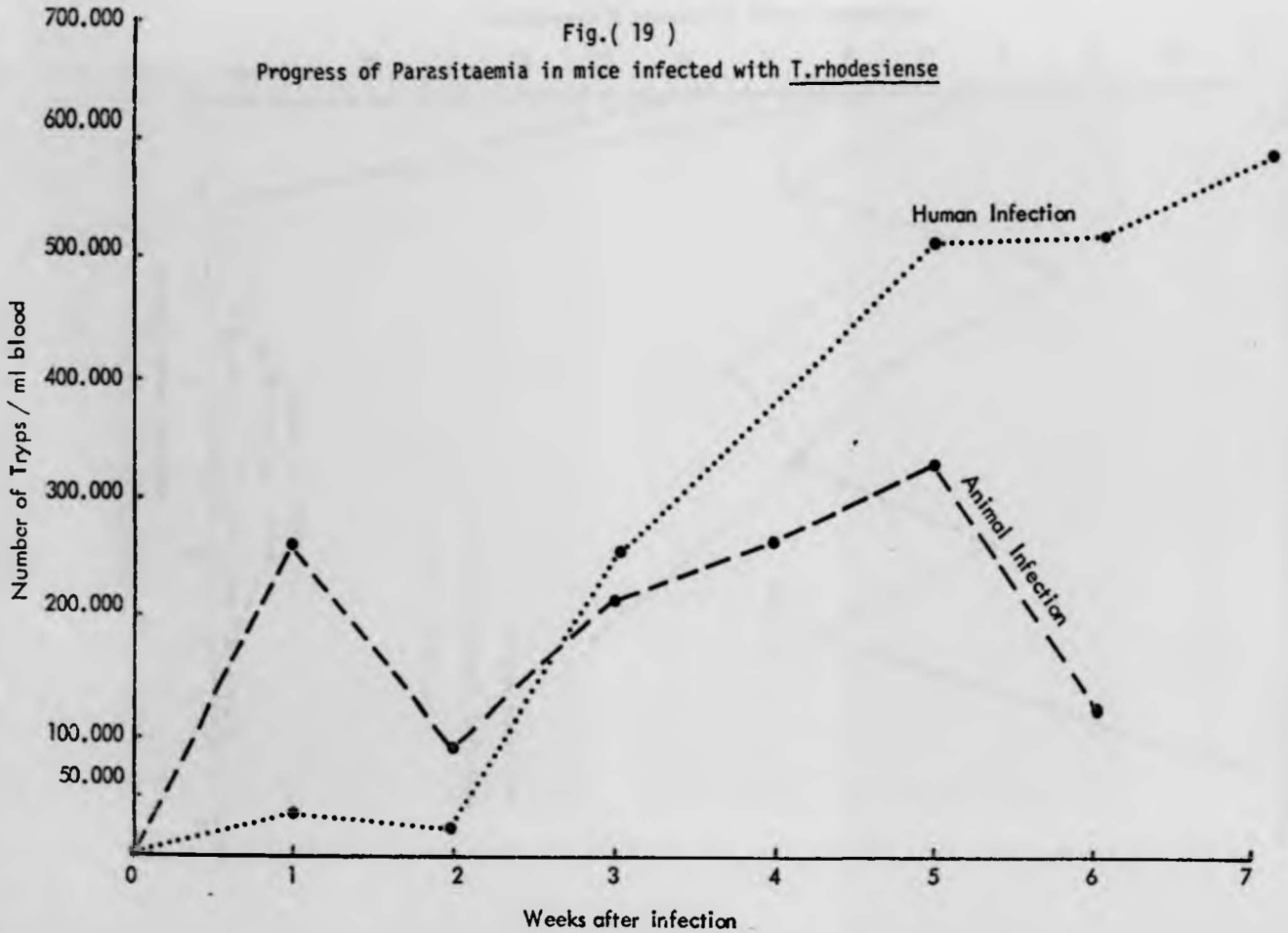


Fig.(20)

Fall in serum Complement in mice infected with T.rhodesiense.

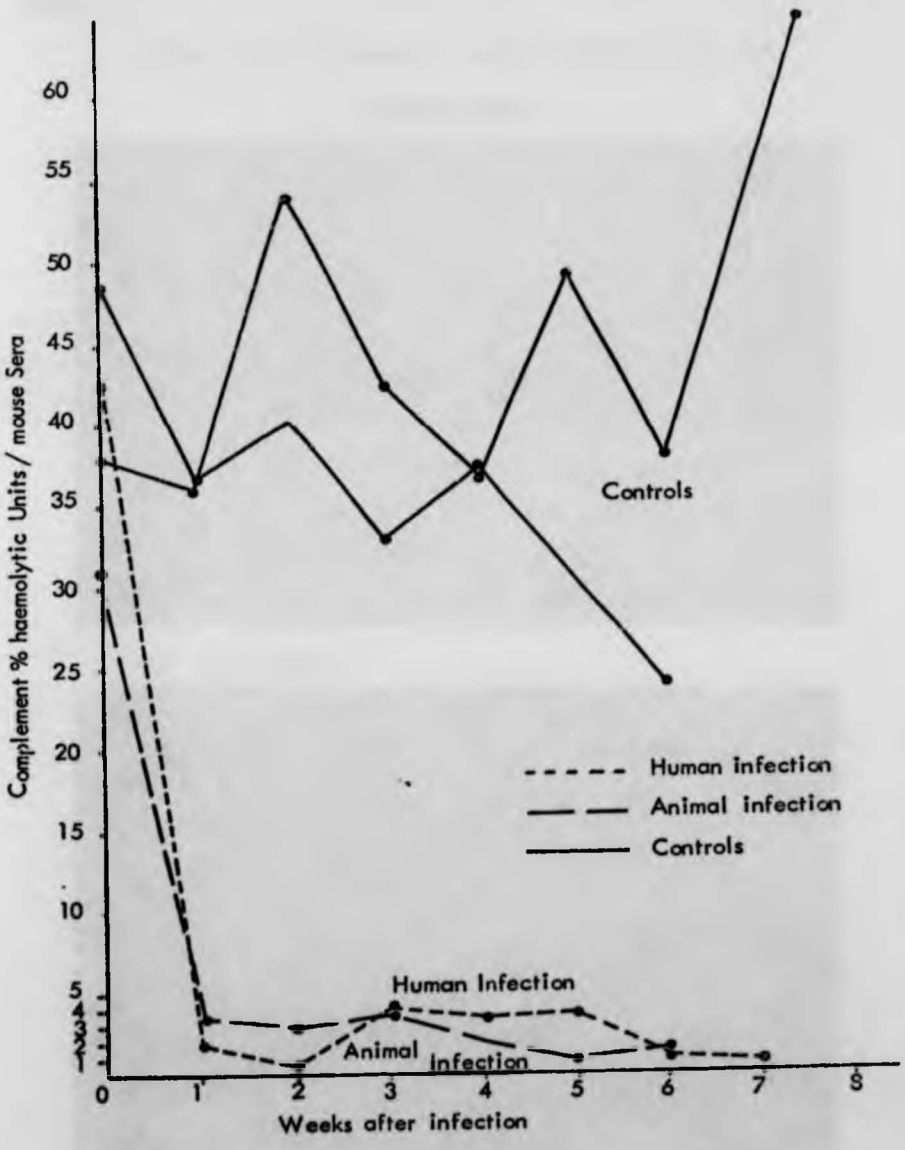
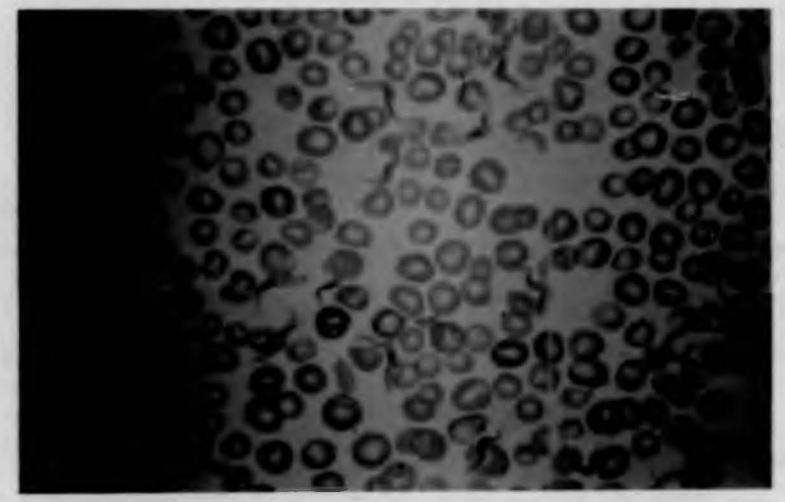


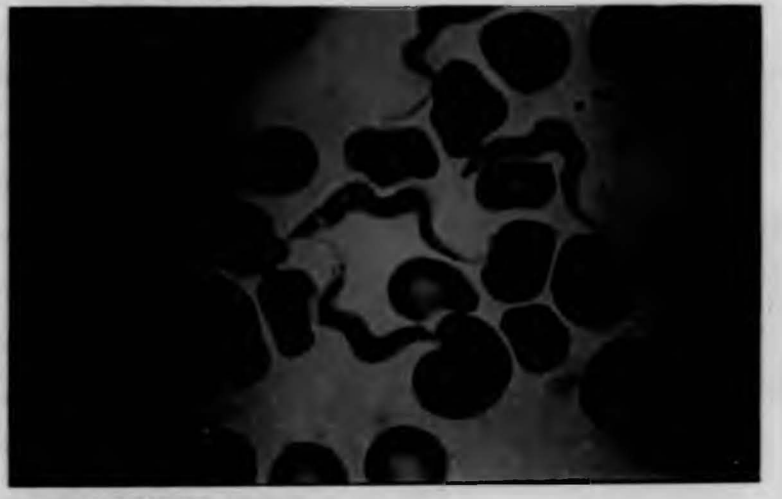
Figure 21

Blood film of a mouse 3 weeks after infection with

T.rhodesiense



"a" Low power

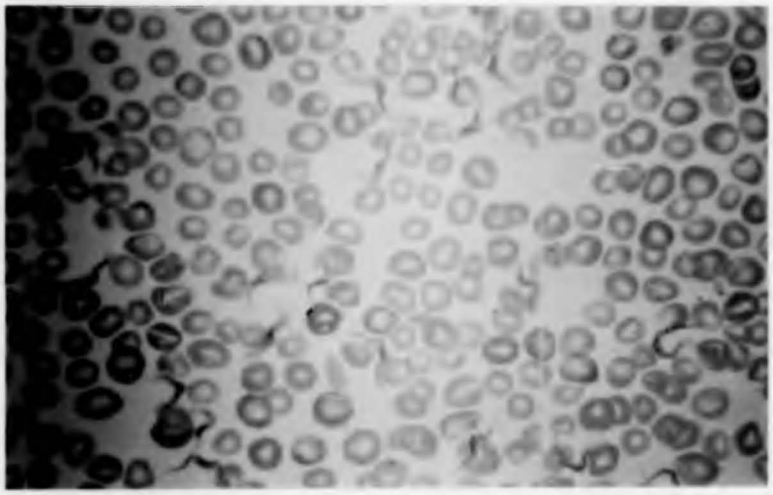


"b" High power

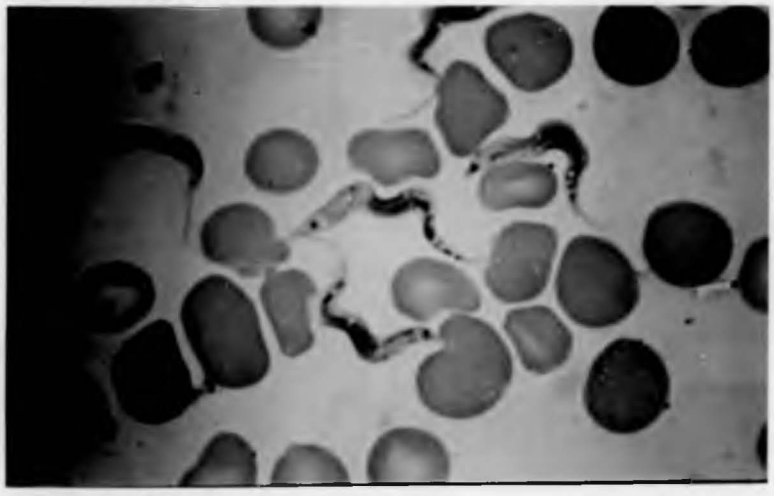
Figure 21

Blood film of a mouse 3 weeks after infection with

T.rhodesiense



"a" Low power

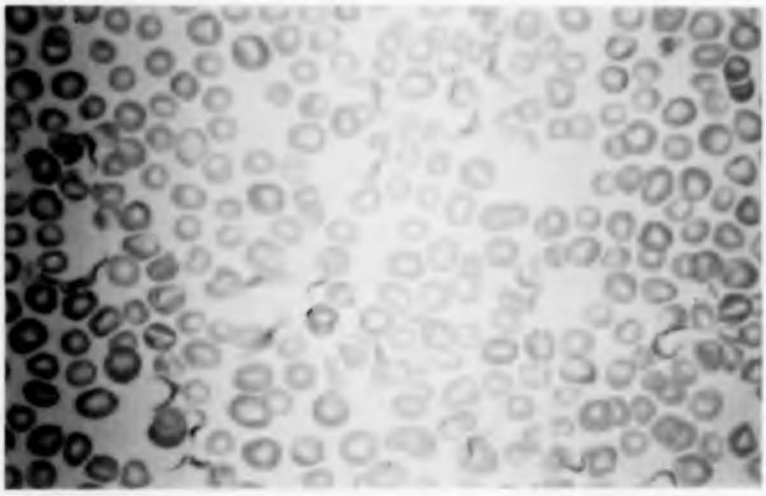


"b" High power

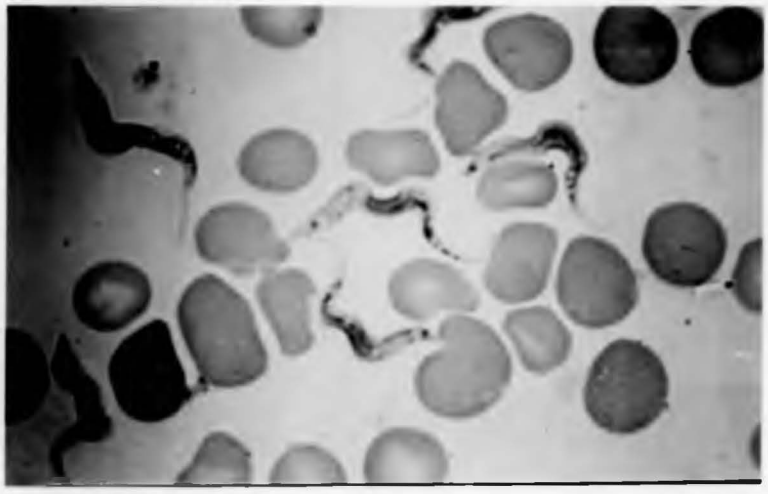
Figure 21

Blood film of a mouse 3 weeks after infection with

T.rhodesiense



"a" Low power



"b" High power

CHAPTER 2

DISCUSSION AND COMPARISON WITH SCHISTOSOMIASIS

In the experiment reported here, mice infected with two strains of T.rhodesiense (human and animal) had no significant differences between their anaemia, splenomegaly and complement levels as shown by the t-test. This may suggest that the virulence of T.rhodesiense is not lost or reduced if the trypanosomes are kept in the laboratory for several years.

Anaemia was reported to be common in African trypanosomiasis (Woodruff et al, 1966 and Assoku et al, 1977). Woodruff et al (1966 and 1976) also suggested that a haemolytic factor is present and associated with the anaemia in patients with African trypanosomiasis. Murray et al (1973) reported that some rats infected with T.brucei underwent a major haemolytic crisis, with haemoglobinaemia and haemoglobinuria before they died. Herbet et al (1973) also suggested that in mice infected with T.brucei the erythrocytes may play a part in the immune response by adsorbing antigen on their surface; this may provide a clue to the cause of anaemia. It has also been suggested that calves infected with T.congolense develop a complement mediated haemolytic anaemia (Assoku et al, 1977).

In 6 patients suffering from infection with T.rhodesiense the haemoglobin ranged from 4.8 to 11.9 gm/dl (Woodruff et al, 1966). However, the black African patient from whom the trypanosomes were isolated for infection of the 2nd group of mice had a moderate degree of anaemia (haemoglobin 13.7 gm/dl and PCV 36.5%) on admission to the Hospital for Tropical Diseases. A moderate degree of anaemia has also been observed in mice infected with T.lowisi (El On et al, 1976) and in the present study. As in schistosomiasis, the anaemia was normochromic (fig. 22), however, in schistosomiasis there was some degree of hypochromasia; the anaemia was more severe, and rather more haemolytic in nature. It thus seems that the anaemia associated with African trypanosomiasis is of a moderate degree; normochromic picture and haemolytic nature.

The t-test showed that the haemoglobin and the PCV values of mice infected with T.rhodesiense differed at the 2% and 5% levels from those values in mice infected with 150 cercariae of S.mansoni, but did not differ from those values in mice infected with 50 or 100 cercariae. In the mice infected with 150 cercariae of S.mansoni, the correlation coefficient between low haemoglobin and low serum complement was highly significant (r 0.93, Fig. 23). Although we must be careful when drawing conclusions about the "cause and effect" of this almost perfect correlation. We can be almost certain that the fall in serum complement results from its adsorption onto erythrocytes that are

subsequently destroyed and removed from the circulation.

In trypanosomiasis, the correlation between haemoglobin and serum complement was also significant (r. 0.74, fig. 23), but the dramatic fall in serum complement and the moderate degree of anaemia observed in the infection is unlikely to be a coincidence. However, the moderate degree of anaemia which developed did not exclude or minimise the role played by complement in its development. Clearly, the differences between the complement levels and splenomegaly in both infections did require statistical comparison (figs.23;24;25&27).

A comparison of the slopes of the regression coefficients of the haemoglobin and either or serum complement (fig. 23) or splenomegaly (fig. 27) was made between both infections. Good evidence was obtained suggesting that either an increase in the splenic weights or decrease in the complement levels is associated with a decrease in the haemoglobin values. This decrease in the haemoglobin values is less in mice infected with T.rhodesiense than in mice infected with S.mansoni (table 19). Evidence, confirmed statistically, shows, therefore, that the decline in haemoglobin is more associated with the fall in complement levels and the increase in splenic weights in schistosomiasis than in trypanosomiasis. Moreover, although mice infected with 150 S.mansoni cercariae had significantly larger spleens and lower complement levels than mice infected with only 50 cercariae, the maximal difference between these slopes was between mice infected

with T.rhodesiense and those infected with 150 S.mansoni cercariae (table 19). This difference between the slopes and the values they represent probably excludes the possibility that splenomegaly is the main cause for anaemia in both infections. This is because mice infected with T.rhodesiense were less anaemic and had splenomegaly more than twice as great as in mice infected with 150 cercariae of S.mansoni. Thus, if splenomegaly is the main mechanism for anaemia in both infections, mice infected with trypanosomiasis would be expected to be more anaemic than those infected with schistosomiasis. In trypanosomiasis a high positive correlation (r. 0.94, $P < 0.001$) was found between splenomegaly and the number of trypanosomes/ml of blood and this may explain the huge splenomegaly observed in the infection (fig. 26).

A drastic reduction of serum complement was found in rats infected with T.lewisi (Nagle et al, 1974 and Jarvinen et al, 1976); almost total loss of the complement activity during the same infection was reported by Jarvinen et al (1976). In the present study a similar roduction of serum complement was found in mice infected with T.rhodesiense. This reduction was not related to either the increase in the splenic weight or the increase in the number of trypanosomes/ ml of blood and was not associated with severe anaemia. It would appear, therefore, that in trypanosomiasis, complement is consumed in some immunological processes along with the one producing anaemia. This process may be in the central nervous system or elsewhere. This

suggestion compares favourably with the suggestion of Kobayakawa et al (1979) that polyclonal antibody plays an immuno-pathological role in the development of lesions associated with African trypanosomiasis, including those in the brain. However, the massive deposition of complement (C3) in the renal glomeruli in mice infected with T.rhodesiense, (Kabil et al, 1979), may also help to explain the marked fall of serum complement observed in the infection. This fall in serum complement precedes its appearance in the kidneys by at least one week (table 21). Thus, after complement has diminished perceptibly in the serum, time may be required before complement or complement containing immune-complexes accumulates in sufficient amounts to become detectable in the renal glomeruli.

In trypanosomiasis, there is no external blood loss, and splenomegaly is thought to have minimal effect on anaemia, and the circulating trypanosomal antigens have already been detected in both human and experimental trypanosomiasis (Fruit et al, 1977). It would appear, therefore, that the complement-mediated haemolytic mechanism suggested by Woodruff (1973) and Woodruff et al (1973) is the main mechanism for anaemia in trypanosomiasis as in schistosomiasis.

Finally, the donor patient from whom the trypanosomes were isolated had a complement level of 135 mg/dl and a control had a complement level of 156 mg/dl; both levels are normal. Nevertheless, all the infected animals, after the incubation period, had marked reduction of their complement levels. This difference between man and animal in their complement levels may be attributed to

different tolerance or duration of the infection. Perhaps if the mice lived long enough, their complement levels would again have risen.

Table 18

Correlation matrix for mice infected with T.rhodesiense

1	Hb				
2	.933.	PCV			
3	.742	.839	Complement		
4	-.794	-.826	(-.662)	Sp/Bwt	
5	-.783	-.788	(-.556)	.944	Tryps
	1	2	3	4	5

All values of (r) are significant at the 5% level or less, except when between brackets (P n.s.).

Table 19

Comparison between the slopes of the regression coefficients of mice infected with S.mansoni and T.rhodesiense

Sources of variations	Slopes	D.F.	t	P
Hb V Complement				
1. S.mansoni : 50 cercariae	0.1945	25	1.77	n.s.
100 "	0.2395	19	2.59	< 0.02
150 "	0.1083	24	4.50	< 0.001
2. T.rhodesiense	0.1083	26		
Hb V Sp/Bwt				
1. S.mansoni : 50 cercariae	-2.4275	25	2.13	< 0.05
100 "	-5.6080	19	5.73	< 0.001
150 "	-3.9608	24	6.88	< 0.001
2. T.rhodesiense	-0.8388	26		

Fig.(22)

Normochromic anaemia in mice infected with 150 Cercariae of S.mansoni and mice infected with T.rhodesiense.

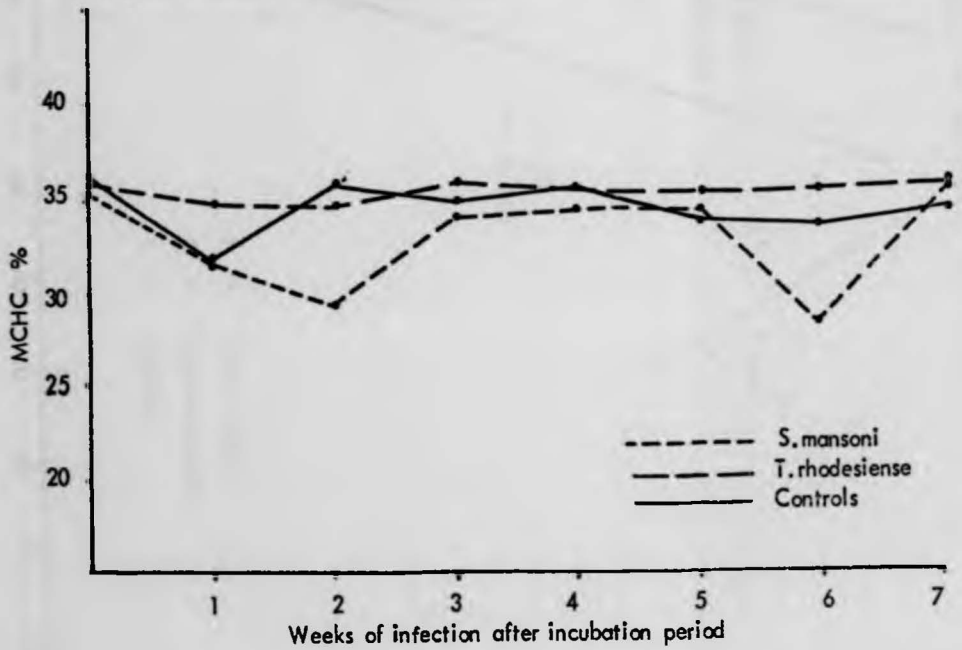


Fig. (23)

Regression slopes of haemoglobin and Complement in mice infected with =50 Cercariae of S.mansoni; and mice infected with T.rhodesiense and Controls.

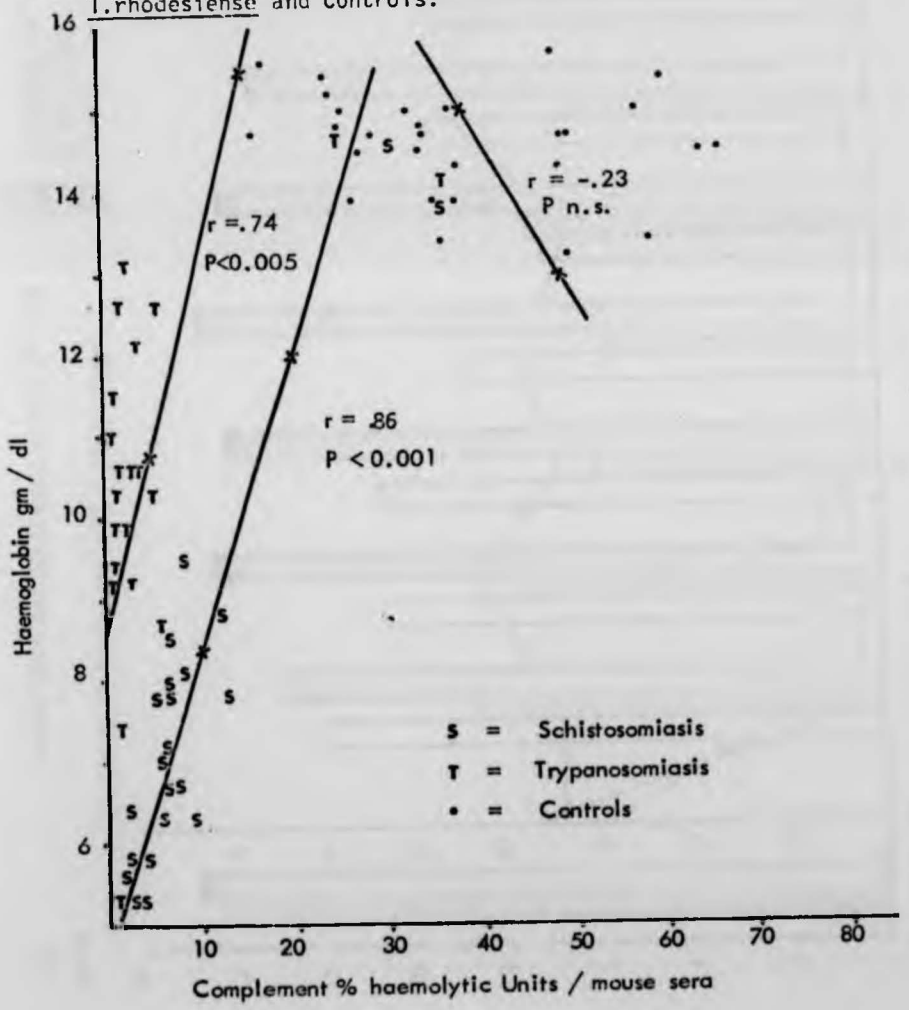


Fig.(24)

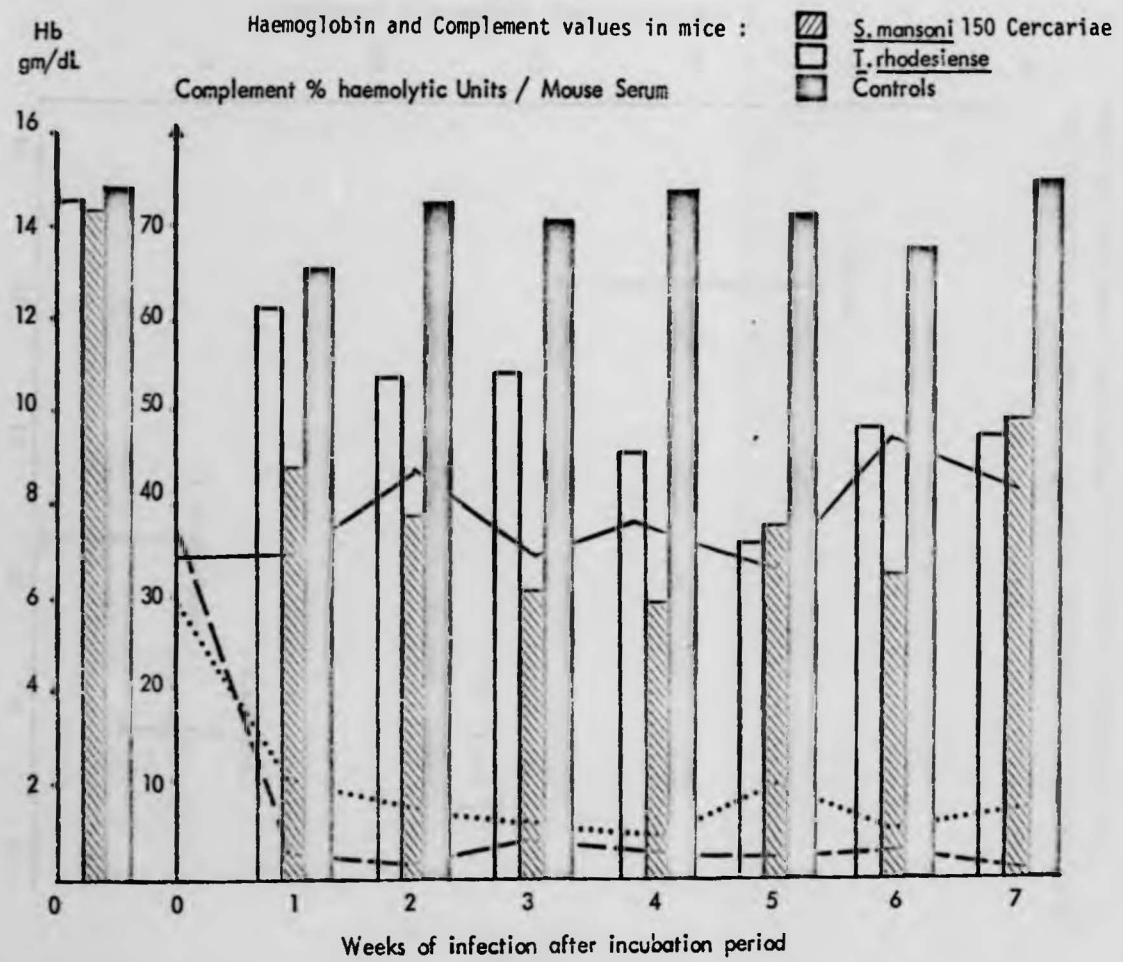

Haemoglobin and Complement values in mice :
S. mansoni 150 Cercariae
T. rhodesiense
Controls
Hb
gm/dL
Complement % haemolytic Units / Mouse Serum
16
14
12
10
8
6
4
2
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Weeks of infection after incubation period

Fig.(25)

Fall of Complement and haemoglobin in mice infected with 150 Cercariae of S.mansoni and mice infected with T.rhodesiense.

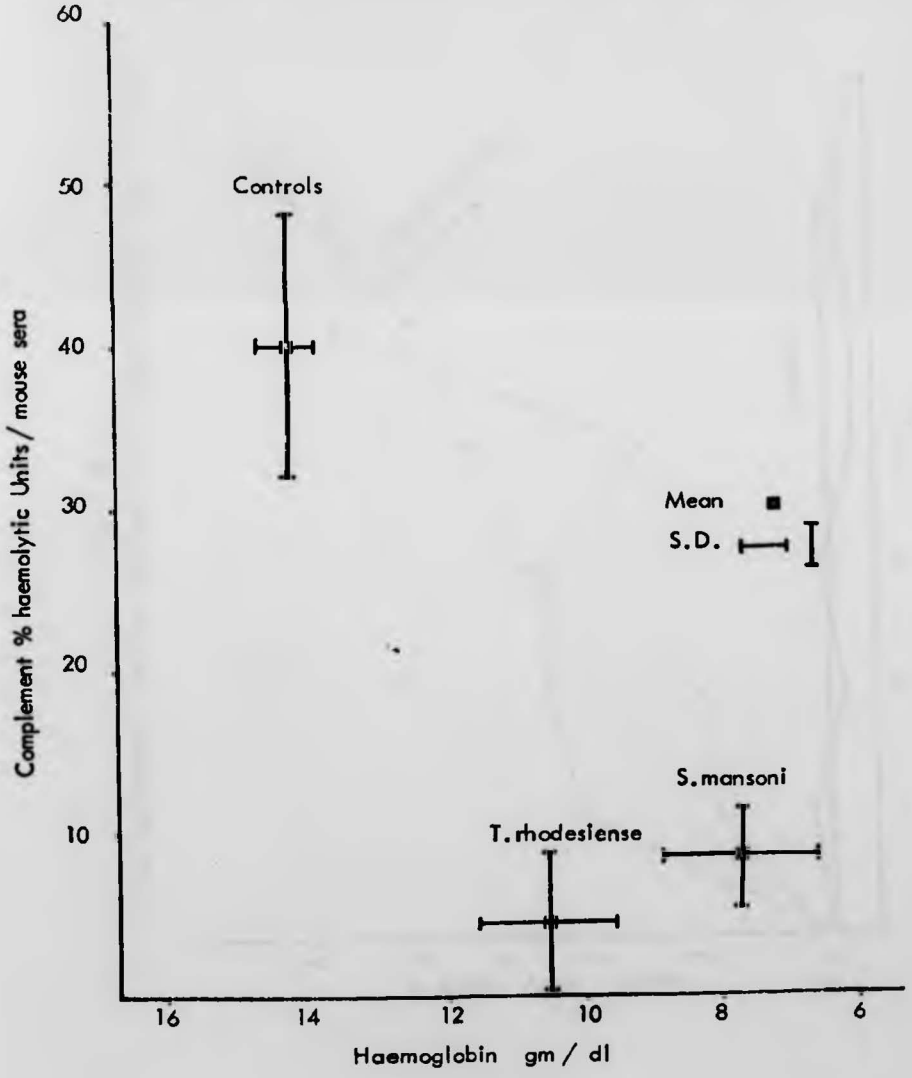


Fig. (26)

True Splenomegaly in mice infected with T.rhodesiense and their Controls

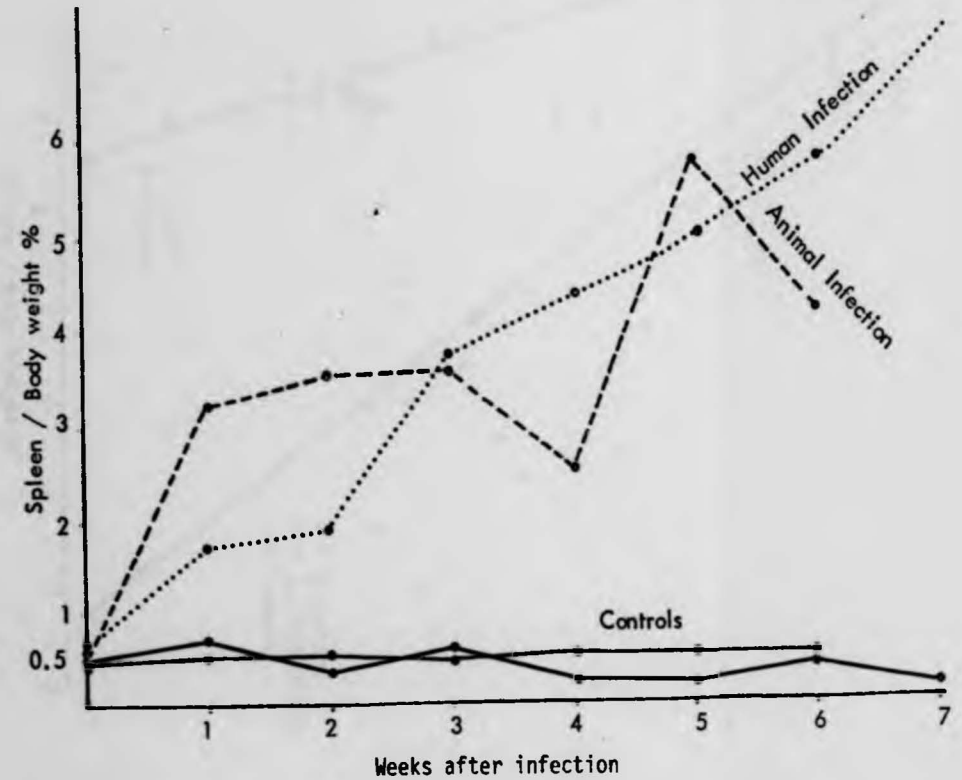


Fig. (26)

True Splenomegaly in mice infected with T.rhodesiense and their Controls

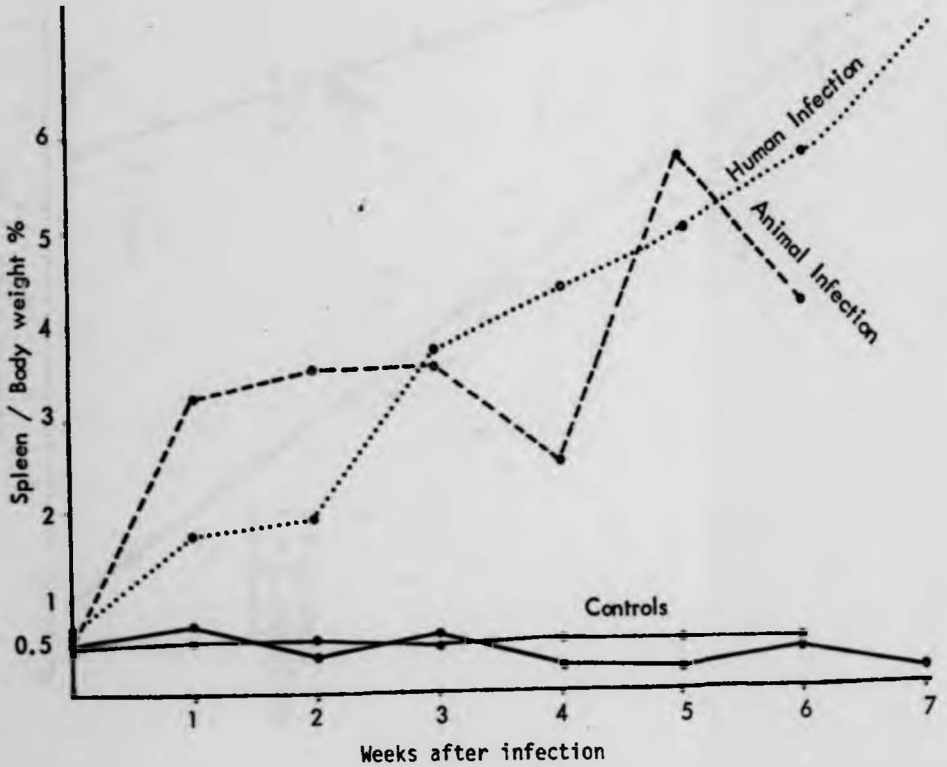
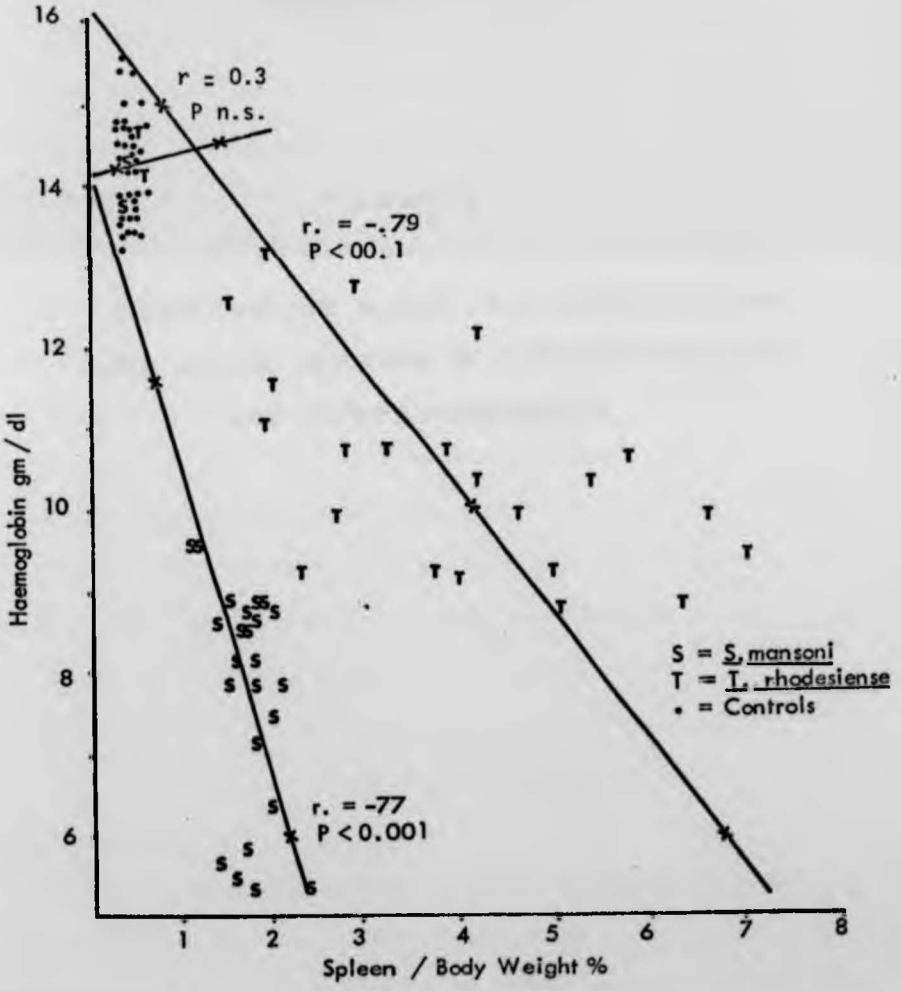


Fig.(27)

Regression slopes of haemoglobin and true Splenomegaly in mice infected with 150 Cercariae of S.mansoni and mice infected with T.rhodesiense and Controls.



PART 6

COMPLEMENT MEDIATED NEPHROPATHY AND LIVER LESIONS IN SCHISTOSOMIASIS AND TRYPANOSOMIASIS

CHAPTER 1

IMMUNOFLUORESCENT TECHNIQUE

Complement (C3) in kidneys of patients (Hoshino - Shimizu et al, 1976) and of mice (Mahmoud et al, 1975 and Natali et al, 1976) infected with S. mansoni is known to be deposited and in this study earlier observations were extended. Serum complement levels were found to be decreased in mice infected with S. mansoni and dramatically reduced in mice infected with T. rhodesiense.

The purpose of this work was, therefore, to determine:-

1. Whether complement containing immune complexes are deposited in the renal glomeruli during infection with trypanosomiasis as in schistosomiasis.
2. Whether such deposition, in schistosomiasis and if any in trypanosomiasis, is associated with or responsible for the hypocomplementaemia observed in vivo.

MATERIALS AND METHODS

Pilot experiment

As a control for the fluorescein staining technique, a pilot experiment was performed. Adult schistosomes, either whole or in pieces, were used in order to save the kidney and liver tissues. The

worms were put into the wells of a microtiter plate at 37°C and stained for 40 minutes with either:

1. Fluorescein conjugated anti-mouse C3 sera.
2. Fluorescein conjugated anti-human immunoglobulin.
3. Plain fluorescein solution.

All were diluted to 1 in 40 with PBS.

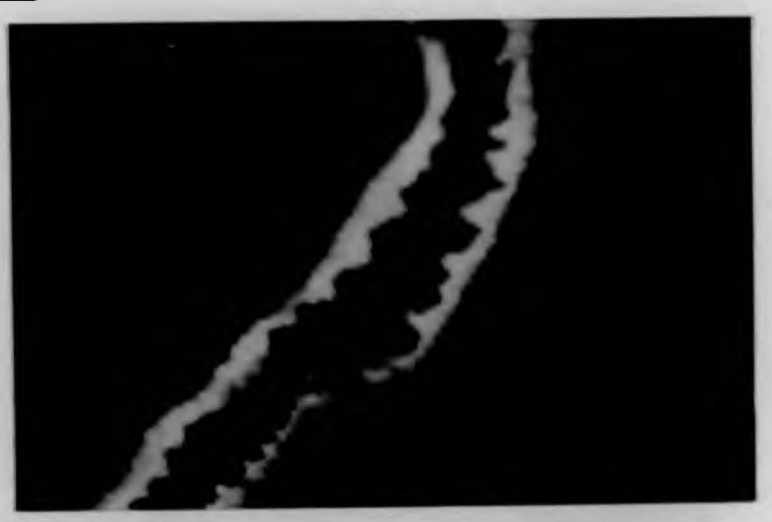
Worms were then washed in PBS for 30 minutes and counter-stained with Evans blue 1 to 10,000 for 5 minutes, then washed several times in PBS; mounted on slides and examined by a U.V. microscope.

Results:

Most of the female but not the male worms stained with fluorescein conjugated anti-mouse C3 sera showed clear fluorescence of their tegument (fig. 28) and some of their eggs (fig. 29). Fluorescence was also noticed when the worms were incubated with anti-human immunoglobulin and palin flourescein but the results were variable. Fluorescence was readily abolished by the addition of Evans blue in those stained with anti-human immunoglobulin (fig. 30). However, this did not regularly happen in those stained with the plain fluorescein solution which appears to stain the whole field (fig 31).

From this experiment, it was concluded that using anti-mouse C3 fluorescein conjugated sera may be a reliable method for detecting complement deposits in mouse tissues.

Figure 28



"a" Female S.mansoni adult worm with anti-mouse (C3) deposited on its tegument

"b" Male S.mansoni adult worm showing that anti-mouse (C3) deposits are absent

Figure 28

"a" Female S.mansoni adult worm with anti-mouse (C3) deposited on its tegument

"b" Male S.mansoni adult worm showing that anti-mouse (C3) deposits are absent

Figure 28

"a" Female S.mansoni adult worm with anti-mouse (C2) deposited on its tegument

"b" Male S.mansoni adult worm showing that anti-mouse (C3) deposits are absent

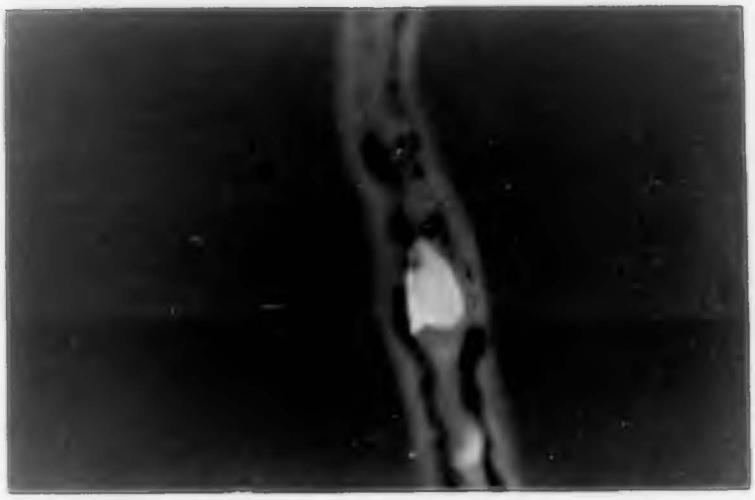
Figure 29

Anti-mouse (C3) deposited on the egg of S. mansoni inside the female worm

Figure 29

Anti-mouse (C3) deposited on the egg of S. mansoni inside the female worm

Figure 29

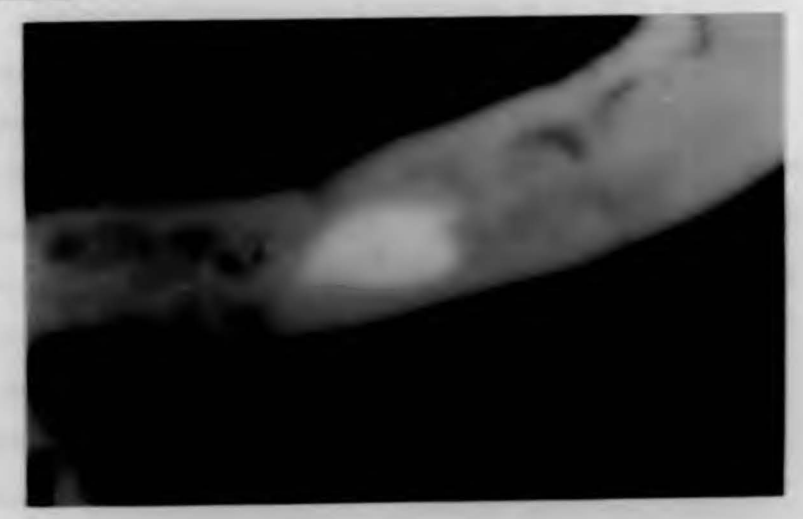


Anti-mouse (C3) deposited on the egg of S. mansoni inside the female worm

Figure 30

Anti-human (C3) on the tegument of a female worm. The fluorescence abolished by the additon of Evans blue

Figure 31

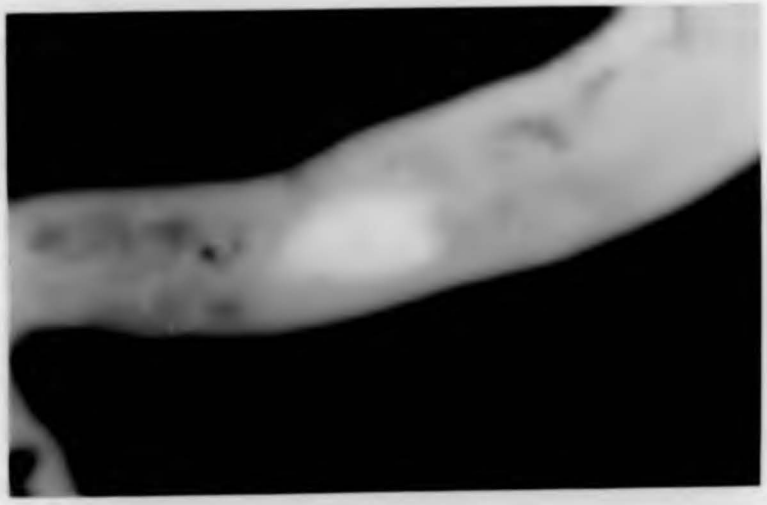


Female worm stained with plain fluorescein solution

Figure 30

Anti-human (C3) on the tegument of a female worm. The fluorescence abolished by the additon of Evans blue

Figure 31

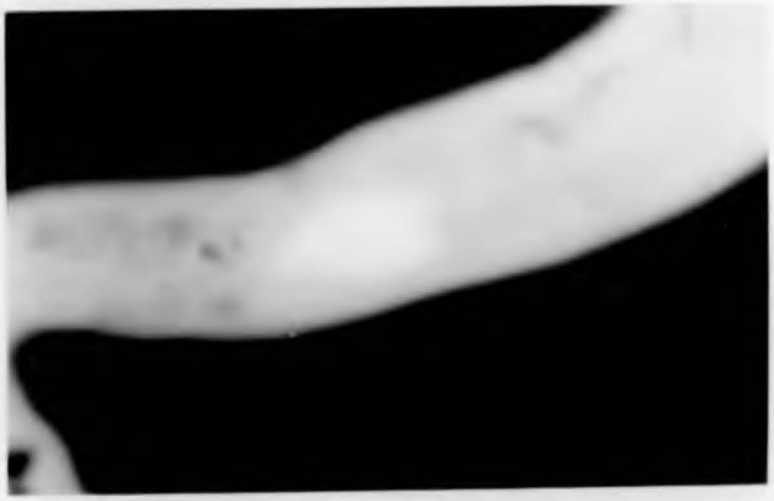


Female worm stained with plain fluorescein solution

Figure 30

Anti-human (C3) on the tegument of a female worm. The fluorescence abolished by the additon of Evans blue

Figure 31



Female worm stained with plain fluorescein solution

Test

A method similar to that of (Ehrich et al, 1972) was used to stain and examine the kidneys and the livers. After the mice were killed, kidneys and livers were removed instantly, snap-frozen in liquid nitrogen and kept at -30°C. When processed, sections of 4Um were cut in a cryostat and kept on siliconed glass slides at -25°C. To remove serum proteins non-specifically bound to the tissue the slides were washed with phosphate buffered saline PH = 7.2 (PBS) for 15 minutes. The slides were subsequently stained for 40 minutes with a 1 in 40 dilution of PBS of fluorescein labelled swine anti-mouse C3 sera (obtained from Nordic Pharmaceutical Ltd., Tilbury). The slides were then washed with PBS for 30 minutes to remove the excess of non-conjugated stain. Contrast staining by Evans blue at the usual concentration of 1 to 1,000 (Niel et al, 1971) for 3 minutes followed by washing in PBS 3 times 5 minutes each was found to be satisfactory as a background. The slides were then mounted in buffered glycerol mounting medium pH 7.2. The sections were examined (using a Leitz fluorescence microscope with U.V. blue illumination) and photographed. The test was carried out on kidneys and livers obtained at various regular intervals after the infections were induced. A similar technique was regularly performed on uninfected mice. Plain fluorescein was also used to stain some kidney sections of mice infected with T.rhodesiense.

RESULTS

1. Kidneys

a. Schistosomiasis

Complement was demonstrated in the kidneys of most infected animals after different periods of infection. Those infected with 50 or 100 cercariae exhibited complement in their kidneys after 12 or 13 weeks of infection (figs. 32 and 33). Complement was demonstrated as early as the 8th week of infection in those infected with 150 cercariae (fig. 34, control - fig. 35; table 20).

More glomeruli showed fluoresence (measured by counting the number of fluorescent glomeruli per Low Power field) in the kidneys of the mice infected with 150 cercariae than in the kidneys of the other two groups (table 20).

b. Trypanosomiasis

Two weeks after infection glomerular fluorescence was observed in most of the mice kidneys (table 21 and fig. 36). Different patterns of fluorescence (irregular patches) were also occasionally observed (fig. 37) in the kidneys stained with plain fluorescein. As with schistosomiasis, the drop in complement precedes the fluorescence of the glomeruli.

2. Livers (both infections)

None of either the infected or control mice livers examined showed specific tissue fluorescence. However, all the livers of mice infected

with S.mansoni showed a fluorescence pattern similar to a schistosomal egg. The fluorescence was mostly in the centres of granulomas (figs. 38 and 39, control - fig. 40). Fluorescence of schistosomal eggs inside the female worm when stained with fluorescein anti-mouse (C3) sera was reported earlier by Kabil (1976).

Association between complement activity in the serum and nephropathy

In schistosomiasis:

It was noticed that the specific presence and prevalence of glomerular fluorescence was associated and almost directly proportional to the increase in both the dose and duration of infection (table 20). It was noticed also that the fall of complement levels observed with the infection usually preceded the deposition of complement in the renal glomeruli (table 21).

Only one of the control animal's kidneys exhibited fluorescence when stained by the sera used.

In trypanosomiasis:

The very low complement levels observed with the infection was associated with greater prevalence of glomerular fluorescence than that observed with schistosomiasis. Similar to schistosomiasis, the glomerular fluorescences increased with the progress of infection (table 22).

The differences between the results in both schistosomal and trypanosomal infections and those of the control series are summarized in (table 23).

The main site in the reticuloendothelial system for sequestration of the damaged red cells, with C3 adhered to them, was suggested by Brown et al, (1979), to be the liver, but in the work here reported no complement deposits were detected in the liver. However, fluorescence of schistosomal eggs in the centres of the granulomas was obvious in all the groups of infected mice. This probably resulted from the high antigenicity of the eggs (Pelley et al, 1976), thus antibodies together with complement would be present in large amounts on the egg surface. It would thus appear that the complement molecules may pass the liver tissue but fail to filter through the renal glomeruli, hence its absence in the former and presence in the latter.

The absence of complement deposits from the liver granulomas rejects the suggestion of Warren et al, (1967) and Hang et al, (1974) that granuloma formation around schistosome eggs is a manifestation of delayed hypersensitivity reaction. Such granulomas might be inflammatory because if it is immunological in nature the presence of complement deposits would be expected in it.

Table 20

Number of mice infected with S.mansoni with immunofluorescent deposits in their renal glomeruli/the total number examined

Infecting number of cercariae	Weeks after infection			
	8 - 10	11 - 13	14 - 16	18 - 19
50	0/4	2/4	3/4	3/3
100	0/4	2/4	3/3	-
150	2/4	4/4	3/4	4/4
0	0/4	1/4	0/4	0/4

Table 21

Mean complement levels percentage/mean prevalence of immuno-fluorescent deposits demonstrated in the renal glomeruli in mice infected with S.mansoni and controls

Infecting number of cercariae	Weeks after infection			
	8 - 10	11 - 13	14 - 16	18 - 19
50	17.9/ve-	16.3/ve+	12.5/ve+	9.9/ve++
100	11.6/ve-	7.0/ve++	8.7/ve++	-------
150	8.0/ve++	5.6/ve+++	7.5/ve++++	8.9/ve+++
0	43.9/ve-	47.8/ve-	42.7/ve-	44.2/ve-

Table 22

Nephropathy and complement activity in mice infected with *T.rhodesiense* and their controls

Sources of variations	Animals	Weeks after infection				
		1	2	4	6	7
1. Number	Infected	0/3	3/4	4/4	4/4	1/1
	Controls	0/2	0/2	0/2	0/2	0/1
2. Prevalence	Infected	ve-	ve+++	ve++++	ve++++	ve++++
	Controls	ve-	ve-	ve-	ve-	ve-
3. Complement %	Infected	1.7	1.0	1.3	2.0	0.8
	Controls	36.0	54.1	36.8	38.3	66.3

1. Number of animals with immunofluorescent deposits in their renal glomeruli/number of animals examined
2. Prevalence of immunofluorescent deposits in the renal glomeruli
3. Mean complement levels percentage

Table 23

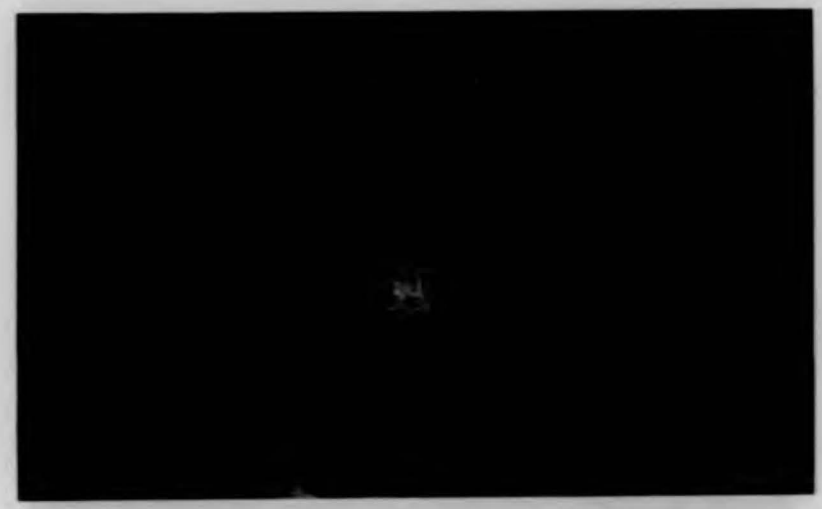
Summary of the differences in the results between schistosomiasis, trypanosomiasis and the controls

Infection	1. Number	2. Prevalence	3. Complement %
Schistosomiasis	50%	ve++	9.3
Trypanosomiasis	75%	ve++++	1.4
Controls	4%	ve —	45.6

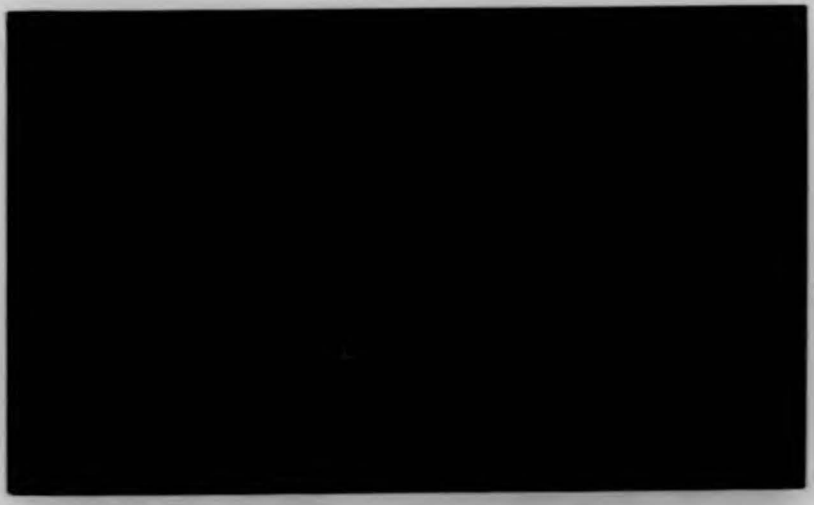
1. Percentage of the animals with positive glomerular deposits out of the total number examined.
2. Mean of prevalence of glomerular fluorescence.
3. Mean complement levels percentage.

Figure 32

Anti-mouse (C3) deposited in a renal glomerulus of a mouse



"a" Low power Magnification 1 : 488



"b" High power Magnification 1 : 800

Figure 32

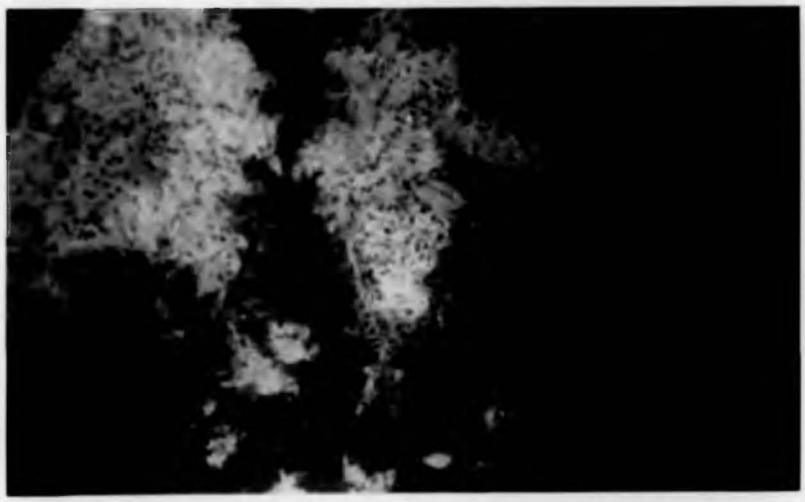
Anti-mouse (C3) deposited in a renal glomerulus of a mouse

"a" Low power Magnification 1 : 488

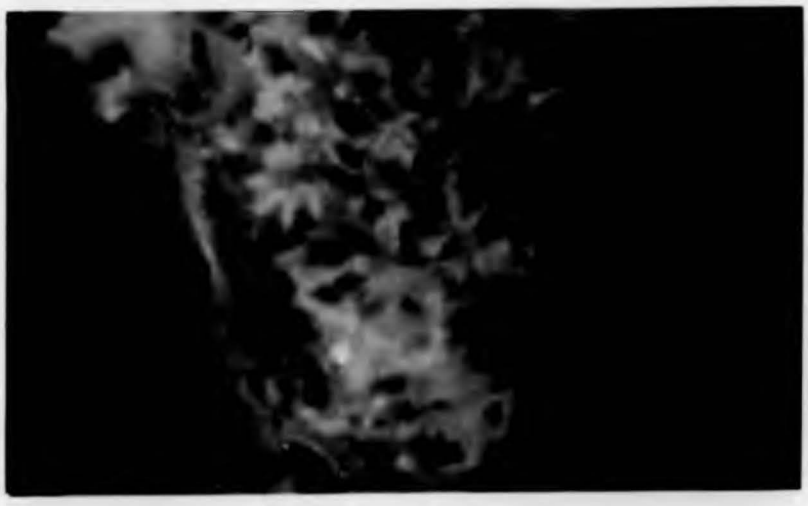
"b" High power Magnification 1 : 800

Figure 32

Anti-mouse (C3) deposited in a renal glomerulus of a mouse



"a" Low power Magnification 1 : 488



"b" High power Magnification 1 : 800

Figure 33

Anti-mouse (C3) deposited in 3 renal glomeruli of a mouse infected with 100 cercariae of S.mansoni for 10 weeks

"a" Low power

"b" High power

Figure 33

Anti-mouse (C3) deposited in 3 renal glomeruli of a mouse infected with 100 cercariae of S.mansoni for 10 weeks

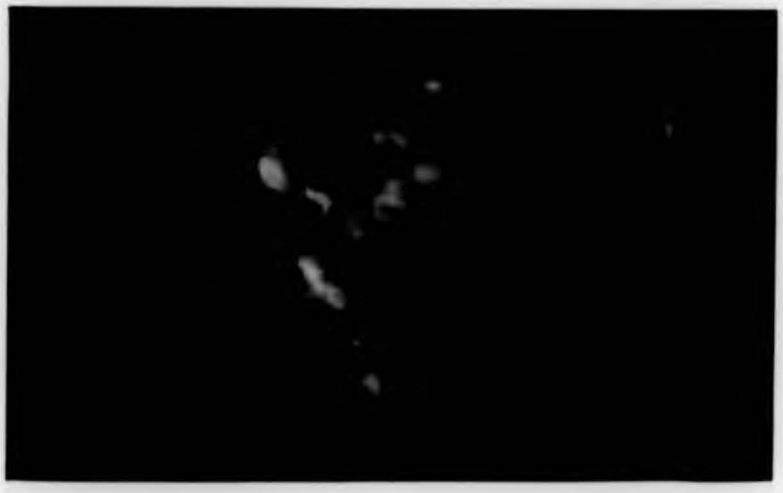
"a" Low power

"b" High power

Figure 33

Anti-mouse (C3) deposited in 3 renal glomeruli of a mouse infected with 100 cercariae of S. mansoni for 10 weeks

"a" Low power



"b" High power

Figure 34

Anti-mouse ($C3$) deposited in 4 renal glomeruli of a mouse infected with 150 cercariae of *S. mansoni* for 10 weeks

"a" Low power

"b" High power

Figure 34

Anti-mouse ($C3$) deposited in 4 renal glomeruli of a mouse infected with 150 cercariae of *S. mansoni* for 10 weeks

"a" Low power

"b" High power

Figure 34

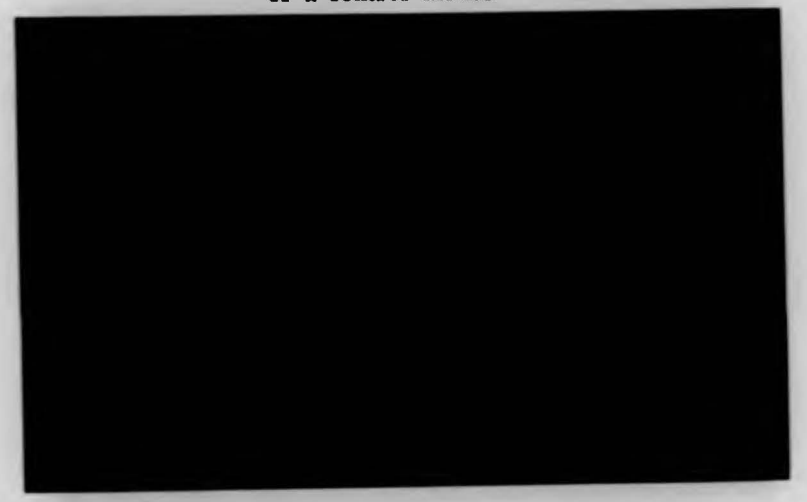
Anti-mouse (C3) deposited in 4 renal glomeruli of a mouse infected with 150 cercariae of S. mansoni for 10 weeks

"a" Low power

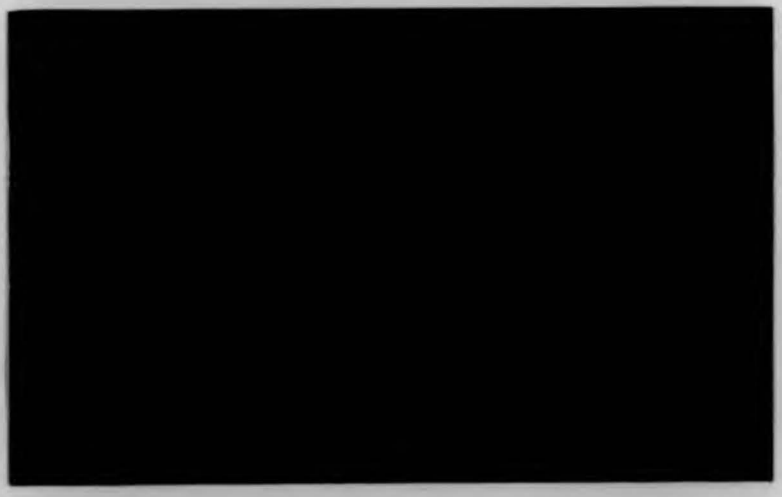
"b" High power

Figure 35

Absence of anti-mouse (C3) deposits in the renal glomeruli of a control mouse



"a" Low power

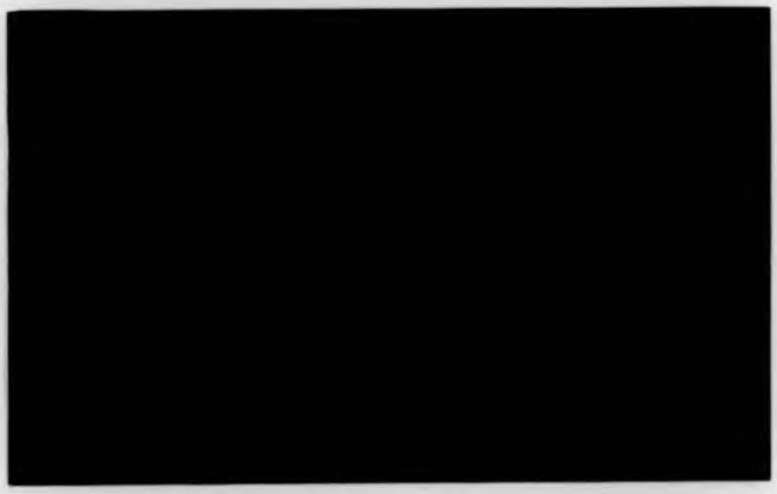


"b" High power

Figure 35

Absence of anti-mouse (C3) deposits in the renal glomeruli of a control mouse

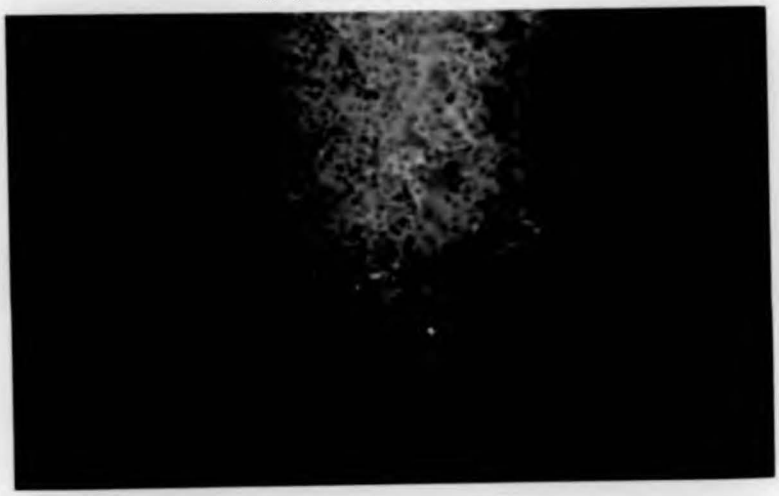
"a" Low power



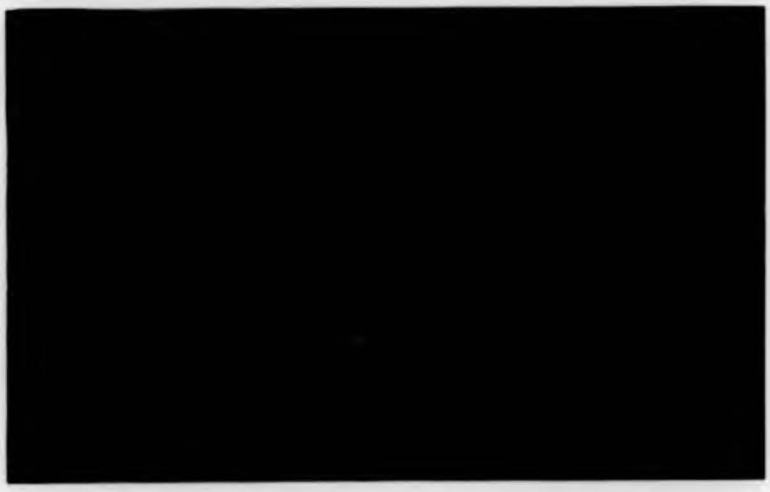
"b" High power

Figure 35

Absence of anti-mouse (C3) deposits in the renal glomeruli of a control mouse



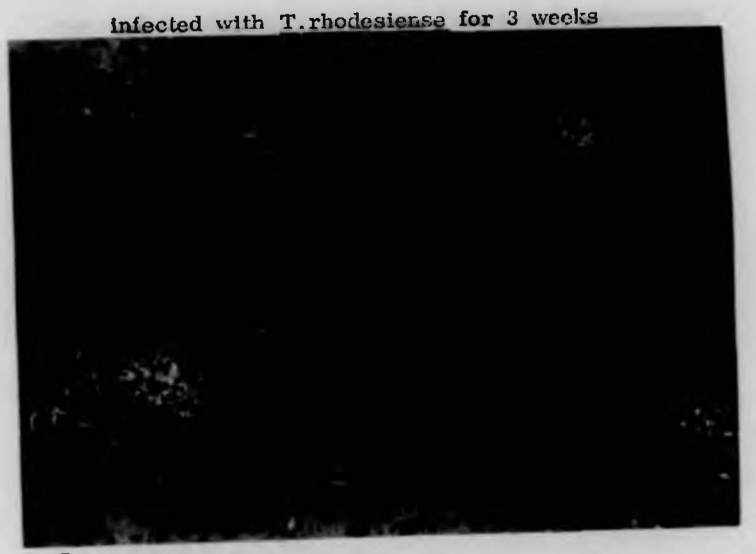
"a" Low power



"b" High power

Figure 36

Anti-mouse (C3) deposited in G renal glomeruli of a mouse infected with T.rhodesiense for 3 weeks

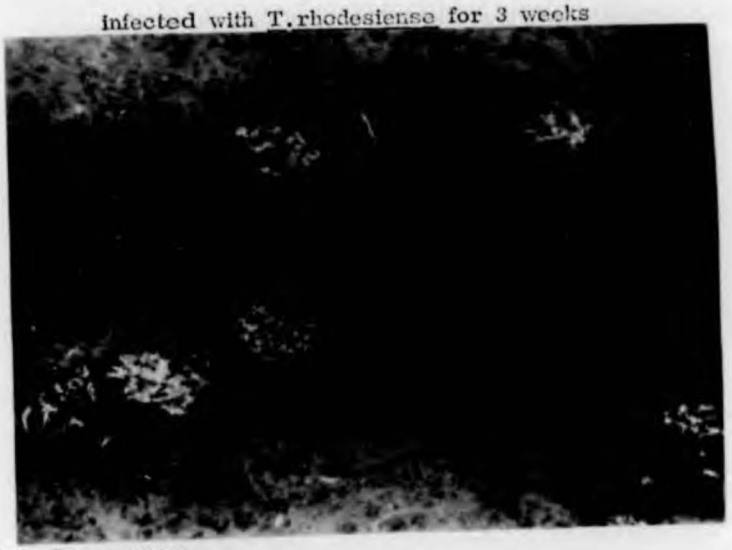


"a" Low power

"b" High power

Figure 36

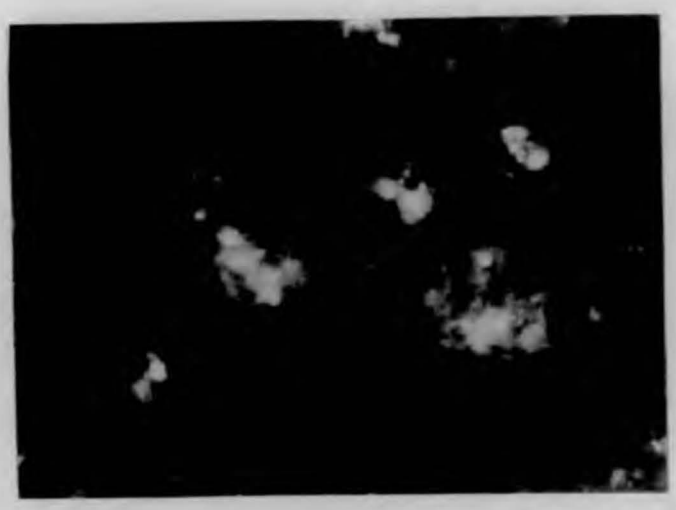
Anti-mouse (C3) deposited in G renal glomeruli of a mouse infected with T.rhodesiense for 3 weeks



"a" Low power

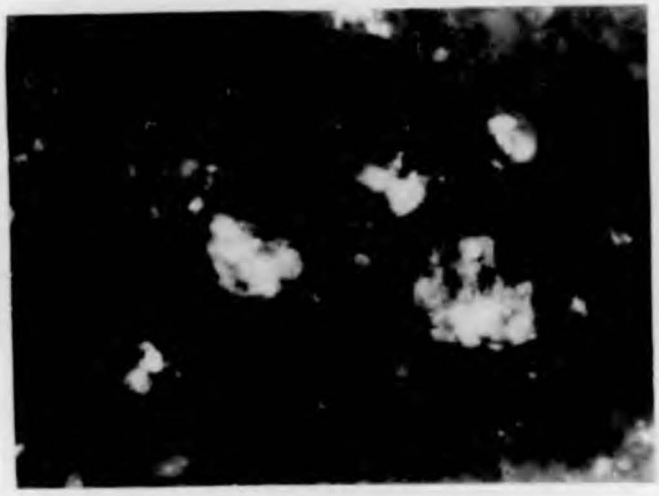
"b" High power

Figure 37



Kidney section of a mouse infected with T. rhodesiense for 3 weeks showing different pattern of " patches " of fluorescence when stained with plain fluorescein solution

Figure 37



Kidney section of a mouse infected with T. rhodesiense for 3 weeks showing different pattern of " patches " of fluorescence when stained with plain fluorescein solution

Figure 38

Anti-mouse (C3) deposited on the egg surface in the centre of a liver granuloma of a mouse infected with S.mansoni. The (C3) deposits were absent from all liver tissues.

"a" Low Power

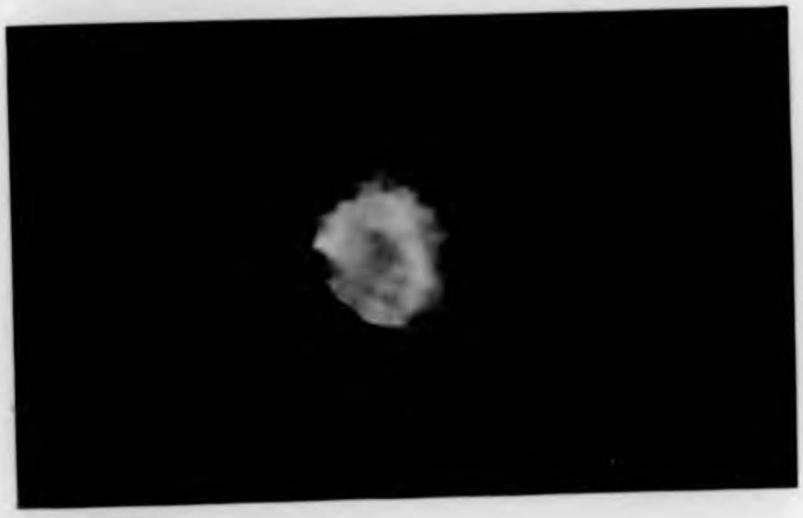
"b" High Power

Shows absence of fluorescence from the granulomatous tissue.

Figure 38

Anti-mouse (C3) deposited on the egg surface in the centre of a liver granuloma of a mouse infected with S.mansoni. The (C3) deposits were absent from all liver tissues.

"a" Low Power

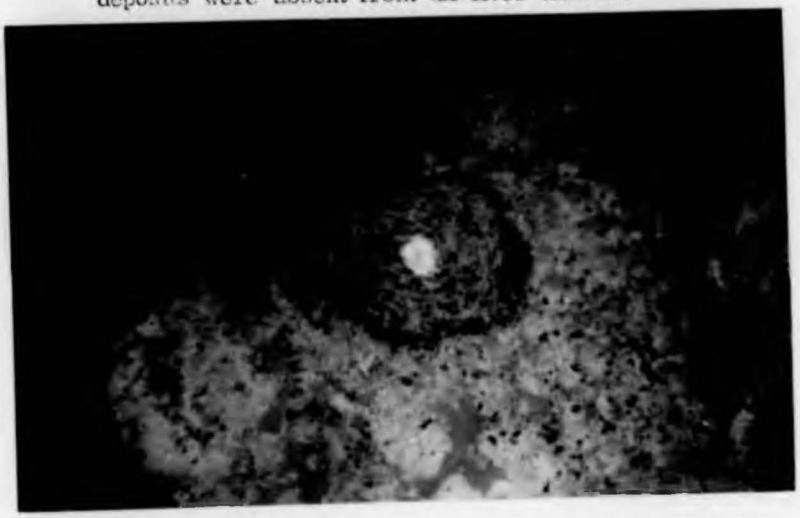


"b" High Power

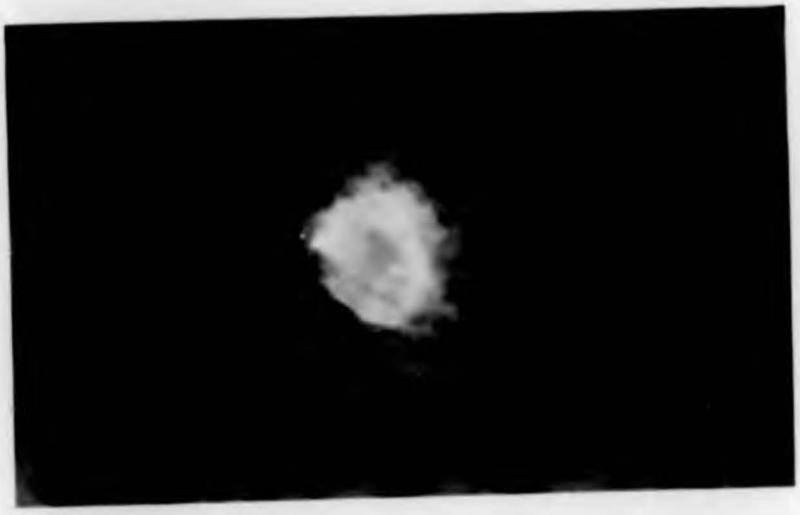
Shows absence of fluorescence from the granulomatous tissue.

Figure 38

Anti-mouse (C3) deposited on the egg surface in the centre of a liver granuloma of a mouse infected with S.mansoni. The (C3) deposits were absent from all liver tissues.



"a" Low Power

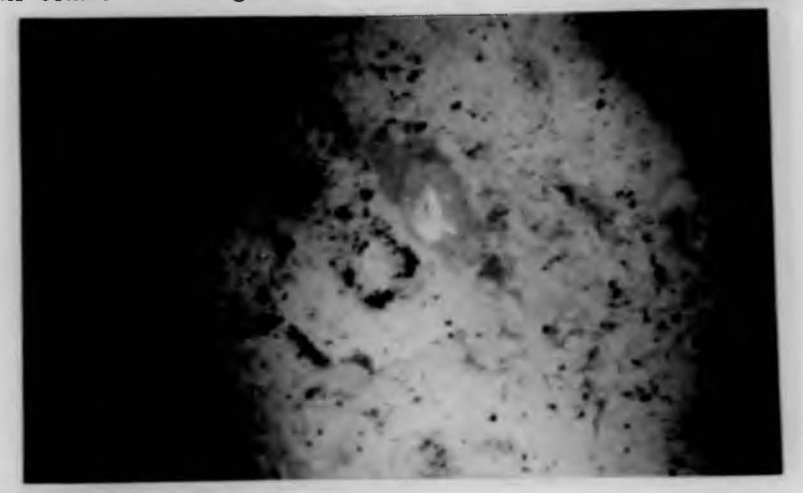


"b" High Power

Shows absence of fluorescence from the granulomatous tissue.

Figure 39

Anti-mouse (C3) deposited on all the egg surface with the spine in the centre of liver granulomas of a mouse infected with S. mansoni

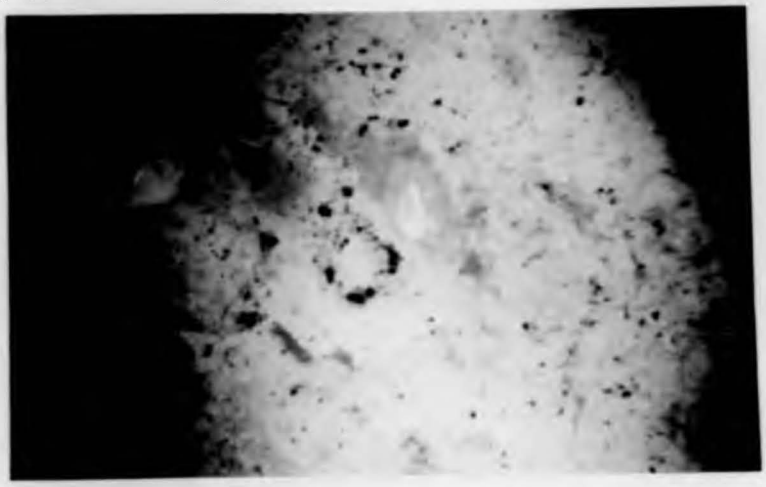


"a" Low power

"b" High power

Figure 39

Anti-mouse (C3) deposited on all the egg surface with the spine in the centre of liver granulomas of a mouse infected with S.mansoni

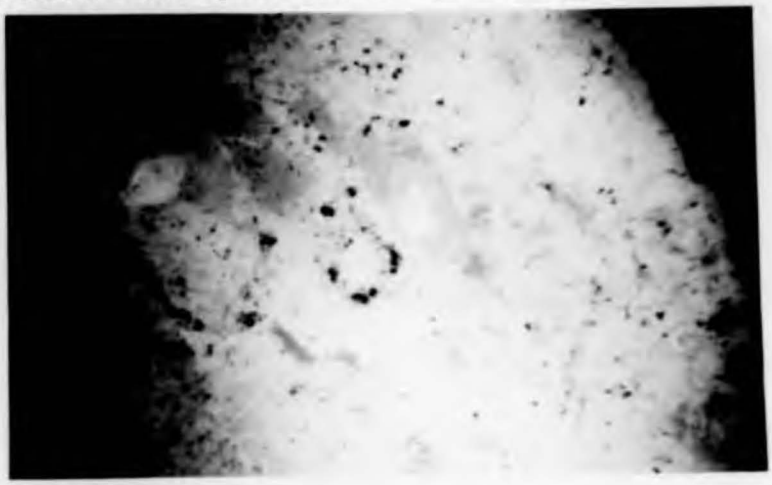


"a" Low power

"b" High power

Figure 39

Anti-mouse (C3) deposited on all the egg surface with the spine in the centre of liver granulomas of a mouse infected with S.mansoni



"a" Low power

"b" High power

Figure 40

Liver section of a control mouse stained with anti-mouse (C3) fluorescein solution

"a" Low power

"b" High power

Figure 40

Liver section of a control mouse stained with anti-mouse (C3) fluorescein solution

"a" Low power

"b" High power

Figure 10

Liver section of a control mouse stained with anti-mouse (C3) fluorescein solution

"a" Low power

"b" High power

CHAPTER 2

DISCUSSION AND ANALYSIS OF THE RESULTS

The findings reported in this part of the study confirm the presence of complement deposits on the renal glomerulus in mice infected with S.mansoni. In heavily infected animals (150 cercariae) such deposits were demonstrated 8 weeks after infection, while these deposits developed more slowly in the animals infected with 100 or 50 cercariae. With lighter infection (25 cercariae), complement deposits appeared on the renal glomeruli after 40 weeks of infection as found by Mahmoud et al, (1975). This was as early as the 7th week of infection in mice infected with 80 - 100 cercariae as suggested by Natali et al, (1976). In support of these proposals, the experimental model described in the present study showed that complement deposits on the renal glomeruli increased directly in proportion to the duration and the dose of infection.

The renal glomerular deposits also increased as the serum complement levels fell. There are several mechanisms whereby the complement depletion could be associated with the renal lesions. In patients with acute post-streptococcal nephritis, the acute nephritic serum causes (C3) breakdown and this reaction could be responsible for the fall of serum complement level (Williams et al, 1972). However, many workers consider that, as found in large groups of patients with renal diseases, prolonged reduction in serum complement

can cause the so-called hypocomplementaemic persistent nephritis (The Lancet, 1972). Brown et al (1970) suggested that in rabbits the (C3) dependent lysis of the red cells in the circulation probably causes release of the complement from the damaged red cell membrane. Our observation is that the fall in complement levels occured before it had been possible to demonstrate complement deposits in the renal glomeruli. This, however, might merely indicate that complement had by that time not accumulated on the glomerular membrane in sufficient quantities to be demonstrated- probably because large quantities of it are first adsorbed on the more extensive erythrocytic membrane. It is suggested, therefore, that the complement dependent haemolysis of red cells demonstrated by Woodruff (1973) occurs first and this consuming complement and depletes it from the serum. With the progress of infection, the haemolysis increases and complement containing deposits start to appear in the renal glomeruli. Complement deposition then causes renal lesions in 60% of the animals 3 - 4 weeks later (Natali et al, 1976). Thus, the renal lesions associated with the infection are unlikely to consume sufficient complement to be responsible for the fall in serum complement. Other causes of primary nephrosis in schistosomiasis such as the renal amyloidosis reported by Omer et al, (1976), have been rejected by workers studying large groups of patients (Sadigursky et al, 1976). Such lesions were only found in

10 out of 60 renal biopsies obtained from nephrotic subjects with schistosomiasis (Barsoum et al, 1979). The development of amyloidosis on top of schistosomal nephropathy may be explained in accordance with the concept originally proposed and developed by Telium (1964), and subsequently modified by other workers (Mandema et al, 1968), that immunologically competent cells, when overwhelmed by persistent antigenic stimulation resort to more primitive or alternate pathways of protein synthesis leading to the formation of amyloid. In patients with hepatosplenic disease caused by S.mansoni infection evidence of glomular lesions of immunological origin was reported by Rocha et al (1976).

In patients with S.mansoni, immunoglobulins (Da Silva et al, 1970); complement containing immune complexes (Hoshino-Shimizu et al, 1976) and probably complement only via glomerular (C3) receptors (Girard et al, 1977) became demonstrable on the renal glomeruli. It would appear, therefore, that the glomerular lesion in schistosomiasis (Sabour et al, 1972; Rocha et al, 1976) result from immunological injury to the kidneys with primary renal dysfunction.

The complement deposits in renal glomeruli in mice infected with T.rhodesiense are reported in this study for the first time. Almost similar results to those found in schistosomiasis were observed here, but the fall in serum complement levels and complement deposits in the renal glomeruli were greater in trypanosomiasis.

PART 7

THE ROLE OF COMPLEMENT IN THE PRODUCTION OF ANAEMIA IN HUMAN SCHISTOSOMIASIS

ROLE OF COMPLEMENT IN THE CAUSATION OF ANAEMIA IN HUMAN SCHISTOSOMIASIS

From previous studies, and work on animals in the preceding chapters, there is a wealth of evidence indicating that in mice infected with S.mansoni anaemia with a haemolytic component develops. In order to assess whether this mechanism is applicable to patients with schistosomiasis it was decided:

1. To examine the question whether the serum complement level is normal or not in such patients.
2. To determine whether there is any relationship between serum complement and the haemoglobin level in those patients.

Methods and persons studied

In the Department of Tropical and Endemic Diseases, Ain-Shams University Hospitals, Cairo, Egypt, 18 adult Egyptian patients were selected for this study. Of these patients 14 had active S.mansoni and 4 had active S.haematobium infections and all were known to be anaemic and had splenic enlargement, but no other illness.

Serum from those patients, together with 18 Egyptian controls who had no schistosomiasis, were examined for their complement levels.

The haemoglobin values of all patients and controls were

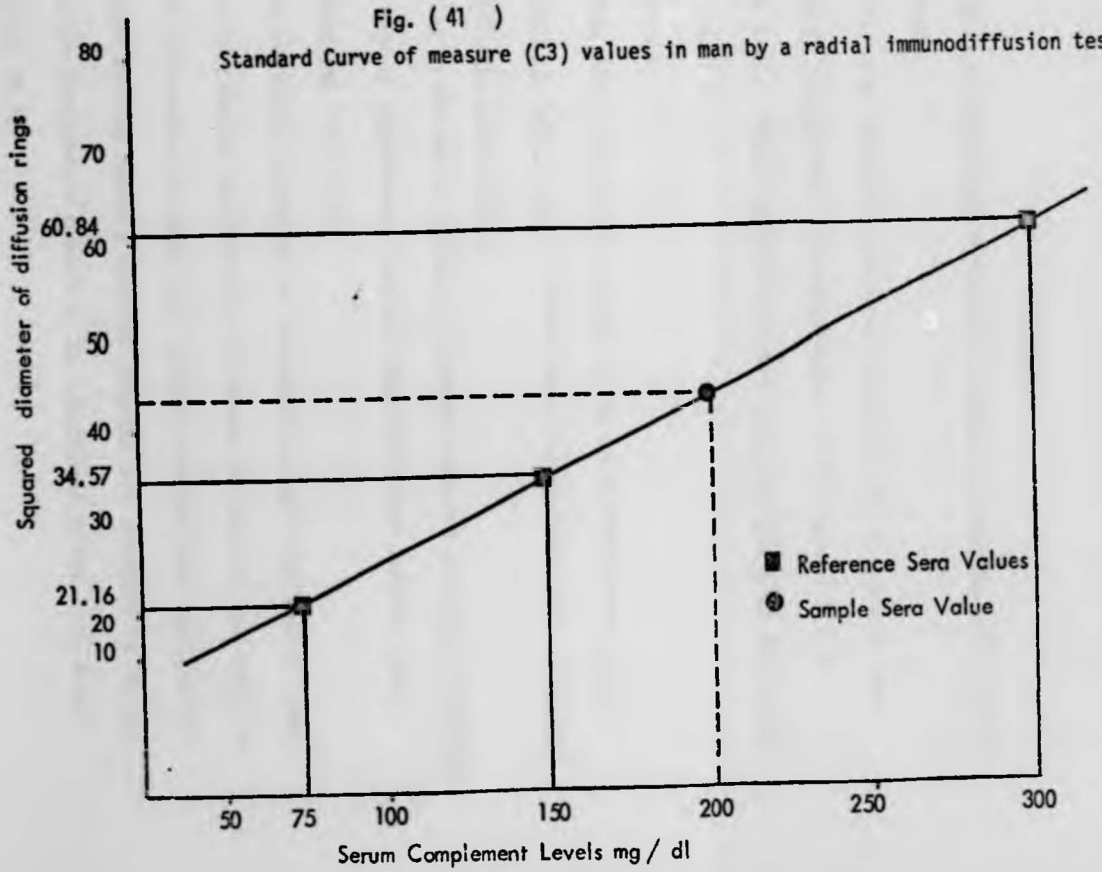
determined by the cyanmethaemoglobin method (Dacie et al., 1975).

Serum complement was measured quantitatively by the radial immunodiffusion technique of Mancini et al. (1965). Using commercially prepared agar gel plates containing specific anti-human (C3) sera (list number 83161, ICL scientific, 18249 Euclid, Fountain Valley Calif. 92708). The test was performed as follows:

1. The frozen serum was left at room temperature for 15 - 20 minutes then mixed thoroughly after thawing and before withdrawing the test sample.
2. Using disposable calibrated capillaries with a wire plunger. 2 uL samples of the test sera and standard reference sera containing known amounts of complement (75,150 and 300 mg/dl) were withdrawn and delivered into the wells of the plates.
3. The plates were then incubated in a moist chamber at room temperature for 48 hours and at the end point the diameters of the precipitation rings were measured to tenths of a millimeter by a precision viewer using indirect lighting.
4. Ring diameters of the three reference sera (fig. 42) were measured, squared and plotted as a standard curve (fig. 41). They were compared with squared ring diameters produced by sera from patients and controls (fig. 42). These diameters were directly proportionate to their complement contents which,

Fig. (41)

Standard Curve of measure (C3) values in man by a radial immunodiffusion test.



by refering to WHO standards, was expressed in international units/ml.

Results

1. Mean complement and haemoglobin levels in patients and controls

Patients:

The mean serum complement levels for the 18 patients was 90.27 mg/dl with a standard error of the mean (S.E.) of 3.48. Their haemoglobin was 8.85 mg/100 ml (S.E. 2.08) (table 24).

Controls:

The mean complement levels of the 18 controls was 134.22 mg/dl (S.E. 7.34). Their mean haemoglobin was 14.83 gm/ 100 ml (S.E. 0.12).

Owing to the small number of patients infected with S.haematobium no test of significance between them and those infected with S.mansoni was valid.

The individual values of the haemoglobin and complement levels for the patients and controls are shown in table 24 and table 25. The differences between both complement and both haemoglobin values for the patients and the controls are revealed by the two sample comparison t-tests to be significant at the 0.1% level (table 26).

2. Relationship between the haemoglobin values and serum complement levels in the patients and the controls

In the patients with schistosomiasis, there was a marked relationship between complement and haemoglobin levels (r. 0.89) significant at the 0.1% levels (table 26). Low complement levels were associated with low haemoglobin values (fig. 43).
In the controls, there was no such relationship (r. 0.38 and P n.s.). Thus 3 separate controls with haemoglobin values of 14.4 gm/100 ml or more had respectively complement levels of 102, 136 and 195 mg/dl (table 25 and fig. 43) .

Discussion and Conclusion

Complement components 5 to 9 (C5-C9) are now recognized to constitute the membrane attack mechanism (Götse et al, 1970; Kolb et al, 1972; Arroyave et al, 1973). The eighth and the ninth (C8-C9) in particular are considered to be the responsible components for bringing about the damage to the erythrocyte surface (Woodruff, 1973). In the same study (Woodruff, 1973), it was emphasized that (C3) is the most readily recognized complement component on the red cell surface, some hundreds of particles of (C3) become attached to the red cell surface for each C4-C2 complex present on it. Moreover, there is good evidence from in-vivo studies that

(C3) is the essential complement component for promoting uptake of complement coated red cells by the reticuloendothelial system (Brown et al, 1970). Furthermore, it has been reported that immunoglobulins (IgG, IgM and many cold antibodies) are capable of red cell destruction by fixing (C3) on the red cell surface or by direct (C3) mediated intravascular lysis (Hoffbrand et al, 1975). Finally, it was reported that (C3) is the bulk component of the complement system and all the reactions of the system can be conveniently related to its cleavage (activation), inactivation and subsequent part in the causation of lytic lesion and of the biological phenomena (Lachmann et al, 1978). Hence, the importance of (C3) as a reliable measure of serum complement activity.

In the present study it was found that patients with low complement (C3) levels are those with low haemoglobin values and vice versa. Thus, there is evidence that in schistosomiasis complement is consumed and consequently its concentration in the serum tends to be lower than in normal controls. This consumption of complement may have been on the red cell surface with consequent haemolysis leading to the observed anaemia as suggested by Woodruff (1973) and Sund (1978).

A fall in (C3) levels has been reported in patients with salmonellosis and schistosomiasis (Farid et al, 1972). There have,

however, been no previous reports on the relationship between serum complement and the haemoglobin in patients with schistosomiasis. Cheever (1969) found that livers of mice infected with one pair of S. mansoni worms for one year contained 75 times more eggs/gm than livers of most heavily infected "asymptomatic" humans. Gillett et al (1978) also found in mice infected with S. mansoni that the plasma cholesterol and phospholipids were significantly reduced, while in patients with S. mansoni only plasma cholesterol was reduced. However, syndromes similar to human hepatosplenomegaly, portal hypertension and anaemia have been described in mice infected with S. mansoni (Warren et al, 1958). In the present study, the relationship between complement and haemoglobin in both infections is similar. It is, therefore, suggested that the haemolytic mechanism for anaemia is closely analogous in both human and animal schistosomiasis.

Table 24

The individual values among the patients with Schistosomiasis

Number	Age	Sex	Infected with	Complement mg/dl	Haemoglobin gm/100 ml
1.	16	M	S. mansoni	52	5.3
2.	17	M	"	90	9.6
3.	23	M	"	88	8.2
4.	23	M	"	104	10.8
5.	24	F	"	75	6.9
6.	26	M	"	102	9.0
7.	26	F	"	82	5.7
8.	30	F	"	102	11.4
9.	32	M	"	85	7.2
10.	32	M	"	104	10.5
11.	34	F	"	82	8.6
12.	35	M	"	94	10.2
13.	38	F	"	106	11.6
14.	40	M	"	70	6.2
15.	19	M	S. haematobium	100	10.5
16.	36	M	"	94	9.2
17.	39	F	"	110	11.0
18.	53	M	"	85	7.4
Mean	30.2			90.27	8.85
S.E.	2.2			3.48	2.08

Table 25

The individual values among the controls

Number	Age	Sex	Complement mg/dl	Haemoglobin gm/100 ml
1.	24	M	136	14.4
2.	24	M	126	14.9
3.	24	M	202	15.8
4.	25	M	150	15.4
5.	26	M	102	13.2
6.	28	M	150	15.4
7.	29	M	195	14.4
8.	29	M	155	15.6
9.	29	M	160	14.5
10.	29	M	145	14.8
11.	36	M	155	15.2
12.	37	M	110	15.9
13.	46	M	115	15.2
14.	52	M	98	14.9
15.	58	M	102	14.5
16.	28	F	105	13.8
17.	34	F	106	14.6
18.	42	F	104	14.5
Mean	33		134.22	14.83
S.E.	2.3		7.34	0.12

Table 26

Statistical differences between the results of patients with schistosomiasis and their controls

Sources of variations	Correlation Coefficient		t-test
	Patients	Controls	
Hb gm/dl	P < 0.001	P n.s.	P < 0.001
Complement mg/dl			P < 0.001

Figure 42

A radial immunodiffusion test showing ring diameters of patients with schistosomal anaemia and controls

"a" 1. Patient serum
2. Patient serum
3. Control serum
4. Reference serum 300 mg/dl
5. Reference serum 150 mg/dl
6. Reference serum 75 mg/dl

"b" 1. Patient serum
2. Patient serum
3. Patient serum
4. Patient serum
5. Control serum
6. Control serum

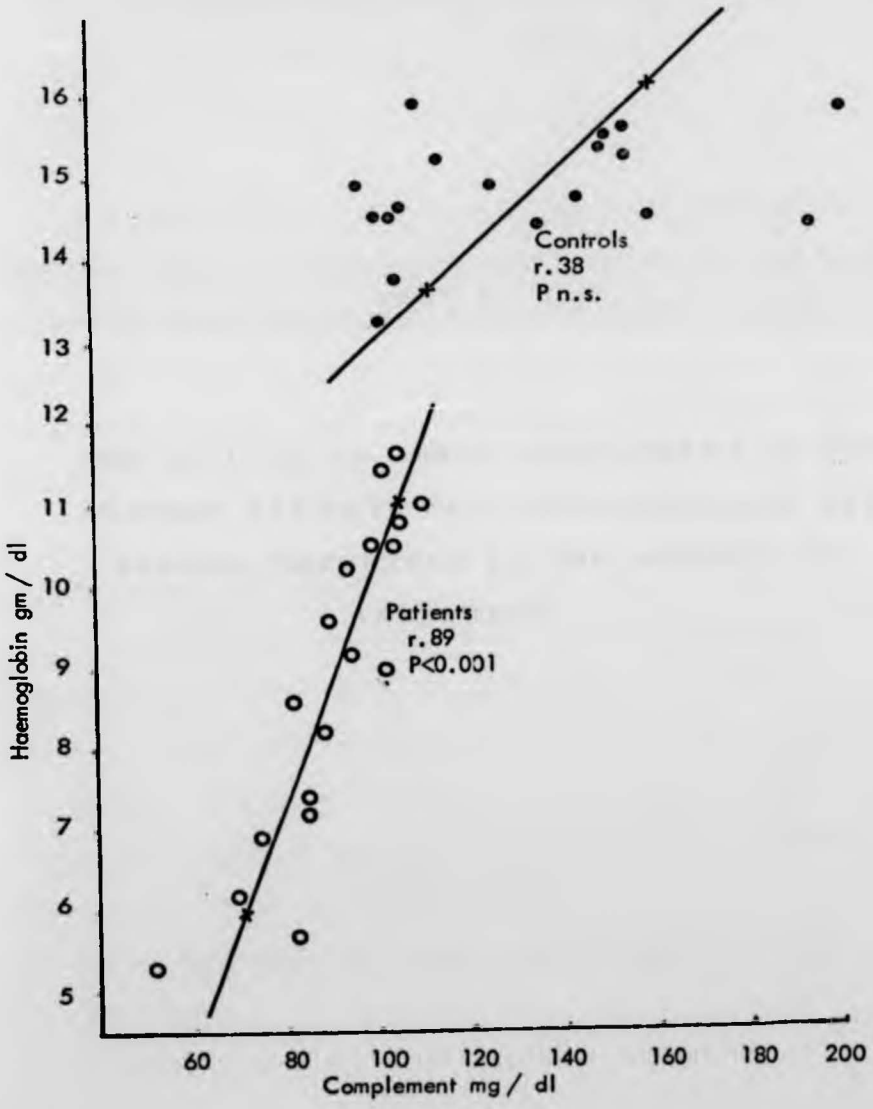
Figure 42

A radial immunodiffusion test showing ring diameters of patients with schistosomal anaemia and controls

"a" 1. Patient serum
2. Patient serum
3. Control serum
4. Reference serum 300 mg/dl
5. Reference serum 150 mg/dl
6. Reference serum 75 mg/dl

"b" 1. Patient serum
2. Patient serum
3. Patient serum
4. Patient serum
5. Control serum
6. Control serum

Fig (43)
Correlation between haemoglobin and Complement
in patients with Schistosomiasis and Controls.



PART 8

THE ACTIVITY OF SERUM COMPLEMENT IN NON-ANAEMIC PATIENTS WITH SCHISTOSOMIASIS WITH SPECIAL REFERENCE TO THE EFFECTS OF TREATMENT

CHAPTER 1

SERUM COMPLEMENT LEVELS IN NON-ANAEMIC HUMAN S.MANSONI AND S.HAEMATOBIUM INFECTIONS, WITH SPECIAL REFERENCE TO THE EFFECTS OF CHEMOTHERAPY

In anaemic patients suffering from S.mansoni and S.haematobium infections, serum complement levels were reduced and there was strong positive relationship between such reduction and the fall in the haemoglobin values. For control purposes it was decided to examine the complement levels in non-anaemic patients with schistosomiasis and the effect of treatment on such levels.

Persons studied

The sera samples were provided by co-operation between the Department of Clinical Tropical Medicine, Hospital for Tropical Diseases, London University and the Department of Medicine, Faculty of Medicine, University of Khartoum, Sudan. Out of 159 patients, sera samples brought frozen from Sudan, 41 samples were selected. Those chosen fulfilled the following criteria:

1 - Absence of anaemia - the group included only 1 or 2 persons with anaemia.

2 - Absence of malaria and intestinal parasitic infection.

3 - The patients had reported for follow-up 6 weeks after successful hycanthone treatment with a single intramuscular dose of 3 mg per kilogramme body weight administered in hospital.

All patients had active schistosomiasis, those with S.mansoni infection had the intestinal, hepatic and hepatosplenic clinical forms of schistosomiasis.

Using the radial immuno-diffusion method previously described in Part 7 of this thesis (Mancini et al, 1965), complement levels (C3) were measured in the patients before and after treatment, and also in 33 local Sudanese controls (figure 44).

RESULTS

The patient's haemoglobin values, complement levels before and after treatment, together with complement levels in the Khartoum controls are shown in table (27) for males, and table (28) for females.

In the female patients the complement levels before treatment were significantly more than those levels of the female controls ($P < 0.05$). After treatment, the complement fell but not to a significant level. However, the significant difference between the complement levels of the patients in the pre-treatment stage and those levels of the controls became not significant after treatment (table 29).

In the male patients, the complement levels before treatment were also more than those levels of the male controls (means of serum complement were 141.7 mg/dl , S.E. 4.8 and 131 mg/dl, S.E. 3.7 respectively) but the difference between those levels was not significant. After treatment the complement levels fell significantly ($P < 0.01$), and the difference with the controls remained not significant (table 29).

Most of the male (72%) and the female (67%) patients showed a fall in their complement levels after treatment. Moreover, there was no significant difference between the male and female patients as regards their complement levels either before or after treatment (table 29). However, in order to study the effects of the age; sex or infection on the patient's complement levels, they were divided into groups according to their age, and the differences in the complement levels of the pre- and post-treatment stages were measured for each age group (table 30). In the patients with schistosomiasis mansoni this difference in the complement levels between one age group and the other, or within the same age group (i.e. between males and females) was not significant. However, the total difference in the complement levels of all the male patients, or all the patients, was significant ($P < 0.02$ and $P < 0.05$ respectively); In the female patients, such difference was not significant. All the

patients with schistosomiasis haematobium were in the first age group (0-24 years old); the difference between their complement levels and such levels in the patients with schistosomiasis mansoni in the same age group was not significant. However, the differences in the complement levels for all the patients with schistosomiasis haematobium was significant ($P < 0.05$). In fact, this statistical analysis has, therefore, indicated that neither the age, sex nor the infection (S.mansoni or S.haematobium) affects the complement levels in the patients with schistosomiasis reported in this study.

The difference between the haemoglobin values of the male and female patients was also not significant.

In view of these considerations, all the data was used as one group. Comparing the patients with the controls, significantly high levels of complement (C3) were seen in the patients before, but not after treatment ($P < 0.05$ and P n.s. respectively, table 29). Meanwhile, there was no significant relationship between the haemoglobin values and serum complement levels (r. 0.22, P n.s., figure 45).

Table 27

Results of the male patients and the male controls

Number	Patients					Controls
	Age	Infected with	Hb gm/dl	Complement mg/dl		Complement mg/dl
				1	2	
1	17	S. mansoni	12.8	177	148	121
2	18	" "	13.3	116	137	131
3	18	" "	6.0	189	142	137
4	20	" "	15.1	127	111	137
5	20	" "	12.3	131	157	121
6	21	" "	16.7	127	116	111
7	22	" "	16.0	127	116	142
8	23	" "	14.7	142	137	142
9	24	" "	12.9	131	137	137
10	26	" "	14.9	154	142	165
11	28	" "	13.6	116	116	137
12	29	" "	13.1	106	101	148
13	29	" "	16.7	183	121	121
14	29	" "	17.3	106	121	87
15	30	" "	14.9	127	101	142
16	30	" "	15.2	121	110	116
17	30	" "	16.1	157	142	148
18	32	" "	14.6	177	148	142
19	35	" "	14.4	111	116	107
20	36	" "	13.6	131	116	142
21	48	" "	14.0	183	157	116
22	50	" "	15.2	177	154	131
23	14	S. haematobium	12.7	148	127	-
24	19	" "	16.2	127	137	-
25	19	" "	14.9	195	171	-
26	20	" "	16.0	137	127	-
27	21	" "	14.4	101	97	-
28	21	" "	15.2	157	157	-
29	23	" "	15.6	127	107	-

Complement : 1 Before treatment

2 After treatment

Table 28

Results of the female patients and the female controls

Number	Patients					Controls
	Age	Infected with	Hb gm/dl	Complement mg/dl		Complement mg/dl
				1	2	
1	15	S. mansoni	8.3	157	148	137
2	16	" "	12.5	131	130	148
3	20	" "	13.6	154	148	121
4	21	" "	13.8	142	157	154
5	22	" "	14.4	154	165	142
6	22	" "	13.4	131	116	116
7	29	" "	11.2	154	142	157
8	30	" "	12.3	127	131	107
9	38	" "	12.2	148	116	131
10	40	" "	12.9	148	157	121
11	60	" "	16.1	157	142	127
12	13	S. haematobium	13.1	148	127	-

Complement : 1 Before treatment

2 After treatment

Table 29

Differences between all the results of the patients with S.mansoni and S.haematobium infections and their controls

No. of cases	Sources of variations	Age		Infection		Haemoglobin gm/dl		Complement mg/dl							
								Before treatment			After treatment			% showing fall	*P
		Mean	S.E.	S.man.	S.hae	Mean	S.E.	Mean	S.E.	P	Mean	S.E.	P		
29	Male patients	25.9	0.63	22	7	14.4	0.39	141.7	4.8	n.s.	130.0	3.44	n.s.	72.4	<0.01
22	Male controls	-	-	0	0	-	-	131.0	3.64		131.0	3.44			
12	Female patients	27.3	0.23	11	1	12.8	0.54	145.9	3.1		139.1	4.63	n.s.	66.7	n.s.
11	Female controls	-	-	0	0	-	-	132.8	4.9	<0.05	132.8	14.9			
74	P Males & Females	n.s.		n.s.		n.s.		Pat. P n.s. Cont. P n.s.			P n.s.			-	

*P Paired observation t-test

P Two samples t-test

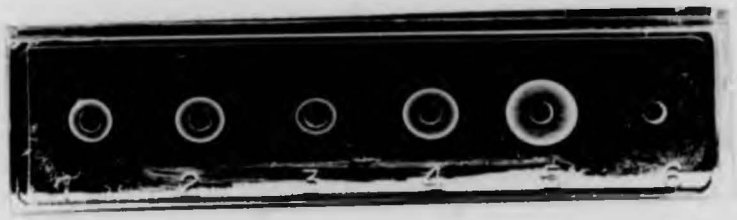
Table 30

Differences between the complement levels in patients with schistosomiasis before and after treatment with regard to their age, sex and infections

Sources of Variations	Age Groups				All Groups		
	0 - 24	25 - 34	35 - 44	45 +	0 - 45 +	P	
S. mansoni							
Males	7.3 (9)	16.1 (9)	5 (2)	24.5 (2)	12 (22)	<0.02	
Females	0.67 (6)	4.0 (2)	- 20 (2)	15.0 (1)	-1 (11)	n.s.	
All	4.7 (15)	13.9 (11)	- 7 (4)	21.0 (3)	7.8 (33)	< 0.05	
P	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.
S. haematobium							
Males	9.9 (7)	-	-	-	9.9 (7)	n.s.	
Females	21.0 (1)	-	-	-	21.0 (1)	n.s.	
All	11.3 (8)	-	-	-	11.3 (8)	<0.05	
P	n.s.	-	-	-	n.s.		

Figure 44

A radial immunodiffusion test showing the ring diameters of patients with schistosomiasis before and after treatment and controls

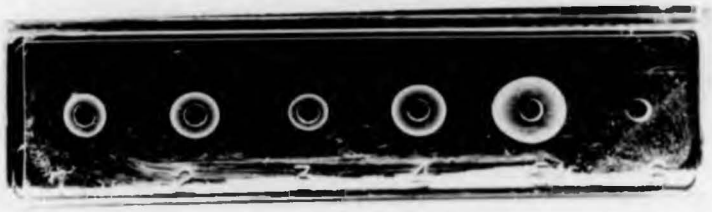


"a"
1. Control serum
2. Patient serum before treatment
3. Reference sera (75 mg/dl)
4. Reference sera (150 mg/dl)
5. Reference sera (300 mg/dl)
6. Normal PBS

"b"
i - 1 ; 3 and 5 patients sera before treatment
ii - 2 ; 4 and 6 sera obtained from the same patients after treatment

Figure 41

A radial immunodiffusion test showing the ring diameters of patients with schistosomiasis before and after treatment and controls



"a"

1. Control serum
2. Patient serum before treatment
3. Reference sera (75 mg/dl)
4. Reference sera (150 mg/dl)
5. Reference sera (300 mg/dl)
6. Normal PBS

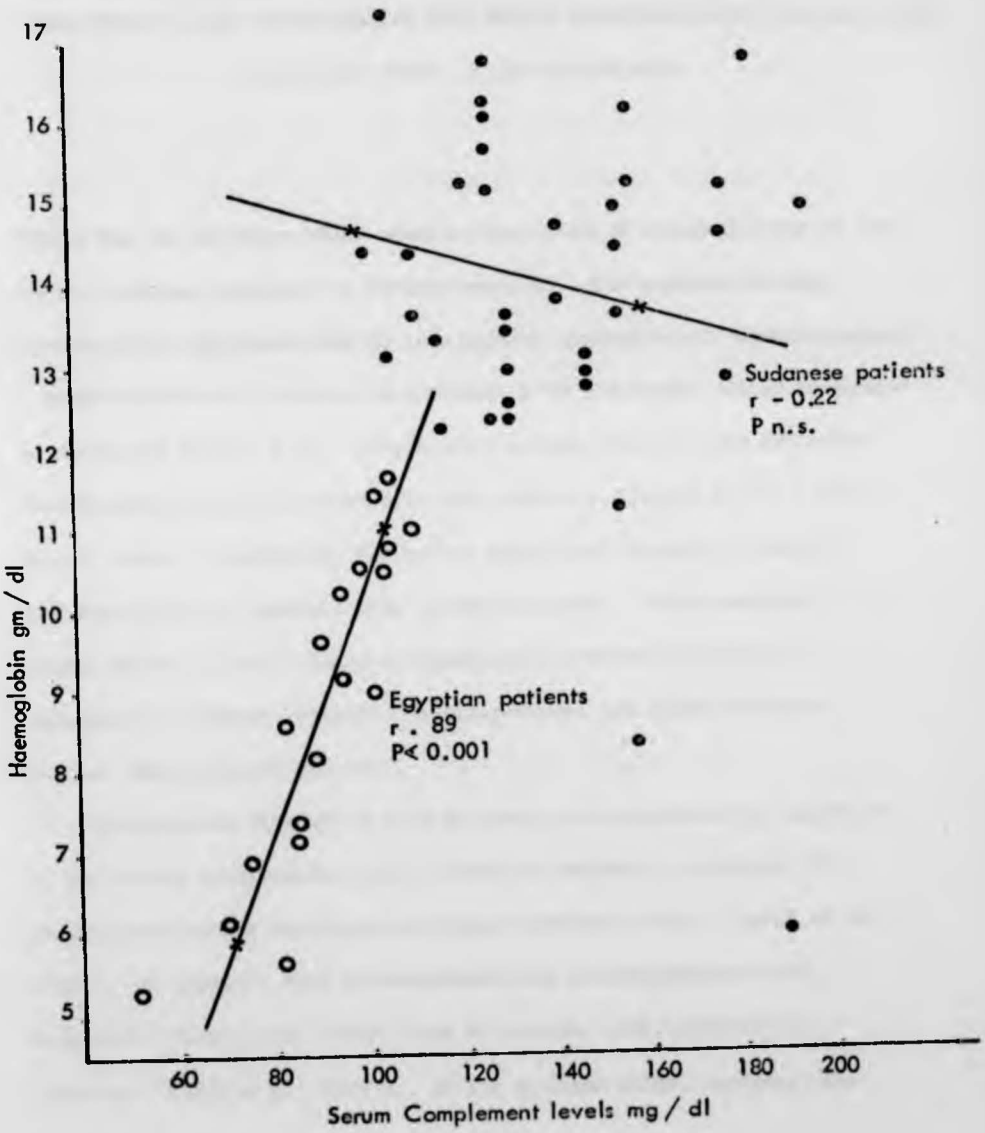
"b"

i - 1 ; 3 and 5 patients sera before treatment

ii - 2 ; 4 and 6 sera obtained from the same patients after treatment

Fig (45)

Correlation between haemoglobin and Complement in anaemic and non-anaemic patients with Schistosomiasis



CHAPTER 2

DISCUSSION AND COMPARISON BETWEEN ANAEMIC AND NON-ANAEMIC PATIENTS WITH SCHISTOSOMIASIS

There has so far been little work on the effect of chemotherapy on the serum immune response in schistosomiasis. Our present finding, however, has indicated that in non-anaemic patients with schistosomiasis a significant rise in serum complement (C3) occurs. After treatment a significant fall in (C3) levels also occurs, thus it does not differ significantly from these levels in the controls. Dennis et al, (1972) found either a significant fall or no significant changes in serum anti-body titre in patients with schistosomiasis. More recently, Camus et al, (1977) found a significant decrease in humoral response in patients treated with splenectomy and worm filtration but not with splenectomy only.

Complement fixation in vivo is commonly expressed by decrease of the serum complement (C3) levels as noticed in patients with glomerulonephritis and systemic lupus erythematosus (Gottoff et al, 1969); in patients with salmonellosis and schistosomiasis with anaemia (Farid et al, 1972) and in patients with schistosomal anaemia (Kabil et al, 1977). In the present study, however, the

ordinary immune response of complement consumption with anaemia appears, at times, to be overcompensated for both the fall in complement and the anaemia.

Bassily et al (1972) suggested that an increase in immunoglobulin G (IgG) and immunoglobulin M (IgM) occurs in patients with schistosomiasis. In the same samples of sera used in the present work, a significant increase in (IgG) and (IgM) in the pre-treatment stage was found. A rise in (IgG) and a decline in (IgM) levels have been found 6 weeks after successful treatment with hyacanthone, but the different between pre- and post-treatment levels was not significant (Salih et al, 1978).

This decline in (IgM) and the fall of (C3) following the treatment may be inter-related. The high ability of (IgM) molecules to bind complement is known and has been reported several times, (Woodruff, 1973 and Hoffbrand et al, 1975). The molecule of (IgM) has also been suggested to be more efficient than other immunoglobulin molecules in initiating the complement activity (Frank et al, 1977). The fall in serum complement (C3) and (IgM) in the post-treatment stage could be, therefore, a response to an immunological mechanism attacking the dying and dead worms. Such mechanism is not unlikely, as the maximal adherence of white cells to schistosomules is only achieved in the presence of antibody

and complement (i.e. with immune sera from patients with chronic S.mansoni infection). Even with complement alone (i.e. normal sera), this adherence was statistically indistinguishable from that with the immune sera (Ottesen et al, 1977).

The data reported in the present study confirm and extend our earlier observation that anaemia in schistosomiasis is associated with a decline in serum complement concentration. Thus, in the non-anaemic Sudanese patients examined here, the complement levels were normal. The absence of anaemia might be a result of the infections being mild, or perhaps the patients had acquired their schistosomiasis long enough and had gradually developed resistance and immunity against it. The gradual development of immunity to schistosomiasis has been suggested by Smithers et al (1969). This immunity may also be related to the phenomenon on self-cure as suggested by Smithers (1972), thus the patients may have developed a compensatory anaemia. This suggestion is probably in agreement with the finding reported by Walker et al (1954) that in fit workers with schistosomiasis mansoni, there was no significant degree of anaemia.

Whatever the reasons are for the absence of anaemia, however, it may be useful to compare these results with those obtained from anaemic Egyptian patients with schistosomiasis. The positive

relationship between the haemoglobin values and serum complement levels was highly significant in the Egyptian patients ($P < 0.001$ and controls P n.s.). In the Sudanese patients, there was no such relationship (P n.s.) (Fig. 45). Strong evidence of a fall of haemoglobin associated with a fall of complement is, therefore, confirmed.

It is important, however, to note that many of the Sudanese patients had splenic enlargement without anaemia. On the other hand, all the Egyptian patients had splenomegaly and anaemia. It seems very likely, therefore, that splenomegaly per se is only a contributory factor in the causation of anaemia in schistosomiasis.

Our findings have not previously been reported, but they compare favourably with those obtained by other workers who found complement activity could cause red cell lysis in many illnesses and particularly in schistosomiasis (Woodruff, 1973 and Suad, 1978).

PART 9

TROPICAL SPRUE - IS IT A COMPLEMENT-MEDIATED SYNDROME?

COMPLEMENT ACTIVITY IN MALABSORPTION SYNDROME (TROPICAL SPRUE)

In schistosomiasis and trypanosomiasis, it has been consistently shown that complement is an important factor in mediating damages to cell membranes upon which it is deposited. This observation stimulated further interest into whether similar damages occured in sprue. Much of the interest arises from the challenge of the unknown actiology of sprue, but perhaps equally significant is the fact that in both schistosomiasis and sprue anaemia is almost invariable.

For these reasons, it was thought possible that the determination of the complement levels in sprue could be a clue to the fundamental manifestations associated with the small bowel disorder.

PERSONS STUDIED

Among patients admitted to the Hospital for Tropical Diseases, University of London, in 1977-1978 for investigation of abdominal pain, flatulence, diarrhoea and loss of weight, six with tropical sprue were selected for this study. The diagnosis of tropical sprue was confirmed by careful study of the patients to establish the presence of intestinal malabsorption. Four of these patients were

British and had acquired sprue abroad during overland trips to Asia. The other two patients were Indian, one of them known to have had sprue for one year. Three of the British patients had Giardia intestinalis infection proved by stool examination or duodenal intubation, the other three patients were free from the infection at the time of admission to the Hospital.

The haemoglobin and the PCV values of these patients were measured according to the methods of Dacie et al (1975) described in part 2 of this thesis. The complement (C3) levels were measured by the radial immunodiffusion test of Mancini et al (1965) described in part 7, (fig. 46). Six equivalent controls were also examined for their haemoglobin, PCV and complement levels.

RESULTS

The patients examined were not anaemic. The differences between the haemoglobin, PCV and MCHC values of the patients and the controls were not significant as revealed by the t-test (table 31). The patients were found to have lower serum complement levels (mean 108 mg/dl, S.E. 9.3) than the controls (mean 152 mg/dl, S.E. 6.4) (fig. 46). Using the t-test, the difference between the complement levels in both the patients and

the controls was significant at the 2% level (table 31). There was no relationship between this low complement level and either of the haemoglobin or the PCV values (P n.s.), probably as the anaemia was absent.

DISCUSSION AND COMPARISON WITH SCHISTOSOMIASIS

Although all the patients examined in this study were symptomatic, they were not anaemic. One of them was known to have had sprue for about one year and she was receiving treatment for anaemia, the others had not acquired sprue for long enough to become anaemic. Nevertheless, serum complement was found to be decreased in all these patients to a significant level. An analogous saturation has been observed in patients with leprosy (Saha et al, 1976) and the question of complement consumption remained unanswered. However, the fall of serum complement in sprue may be important in view of the many doubts existing about its aetiology. The initiating agents are still unknown, so in some instances at least it may be an effective agent, possibly bacteria as suggested by Leishman (1945) and Mathan et al (1968). The association of malabsorption syndrome with G.intestinalis infection in some previously healthy adults (Hoskins et al, 1967; Tewari et al, 1974 and Ridley, 1974) and in patients with immunoglobulin deficiency

(Brown et al, 1972) is now well established. Treatment of G.intestinalis with a drug like tinidazole was said to eradicate the infection and symptoms like loss of weight, milk intolerance and mucoid stools disappeared, (Salih et al, 1977). Nevertheless, in the present study, those patients who had no G.intestinalis infection appeared to have lower serum complement levels (mean 95.7 mg/dl, S.E. 25.7) than those who had it (mean 120.7 mg/dl, S.E. 14.8), but the difference was not significant. However, those patients with lower serum complement and who had no G.intestinalis infection for at least a year, were still suffering from sprue.

In view of these many reports and findings about sprue, it is almost certain that it is not a single disease entity, but a syndrome of multiple aetiology and probably multiple pathogenesis. The fall in serum complement in sprue might share, therefore, in bringing about the absorptive disorder. Comparing the fall in serum complement in both sprue and schistosomiasis (fig. 47) might yield useful information about the pathogenesis of sprue.

In schistosomiasis, the fall in serum complement was either associated with an immunological mechanism bringing about anaemia and nephropathy or the result of another immunological mechanism attacking the schistosome worms after treatment. Thus, the consumption of complement in schistosomiasis is a

result of an immunological process in which complement and probably (IgM) are associated in mediating damage to cells and membranes. The consumption of complement in sprue could also be a result of an immunological mechanism bringing about damage to cells and membranes responsible for absorption and leading to malabsorption. This suggestion agrees favourably with the findings of Ridley et al (1976) that in patients with a short history of giardiasis and moderately severe jujenal lesions, (IgM) molecules were deposited on jujenum surface more than other antibodies. Jarnum et al, (1968), observed normal or slightly raised levels of serum (IgM) in 8 patients with untreated tropical sprue. Samuel et al, (1970), also observed low levels of (IgM) in some patients with tropical sprue. Misra et al (1976) further observed that in 25 patients with tropical sprue, the rest had normal levels of serum (IgG) with either raised or reduced (IgM) levels.

These reports about the activity of serum (IgM) in tropical sprue together with consumption of complement observed by the present study are probably related. Thus, similar to the association between serum complement and (IgM) in mediating cell damage in schistosomiasis (Woodruff, 1973 and the present study), in sprue serum complement could also be associated with (IgM) in an immunological mechanism damaging the absorptive cells on the intestinal surface.

Table 31

The results of patients with malabsorption syndrome (tropical sprue) and their controls

Patients				Mb gm/dl		P C V %		M C H C %		Complement mg/dl	
Age	Nat.	Sex	G.int.	Pat.	Con.	Pat.	Con.	Pat.	Con.	Pat.	Con.
22	Bri.	M	ve -	15.5	14.9	43.7	41.0	35.5	36.3	84	164
28	Bri.	M	ve +	16.3	15.6	46.8	45.0	34.8	34.7	112	150
31	Bri.	F	ve +	14.1	17.3	39.9	51.0	35.3	34.0	136	158
23	Bri	F	ve +	12.5	16.7	37.0	46.8	33.8	35.7	114	172
55	Ind.	F	ve -	12.1	14.2	36.0	40.0	33.6	35.5	125	126
34	Ind.	M	ve -	15.6	15.2	44.5	43.0	35.0	35.3	78	144
Mean				14.4	16.3	41.3	44.7	34.7	35.3	108.2	152.3
S.E.				0.71	0.47	1.8	1.7	0.31	0.33	9.3	6.4
P				n.s.		n.s.		n.s.		<0.02	

Figure 46

A radial immunodiffusion test showing ring diameters of patients with tropical sprue and controls

1. Reference serum 300 mg/dl
2. Reference serum 150 mg/dl
3. Reference serum 75 mg/dl
4. Control serum
5. Patient serum
6. Patient serum

Figure 46

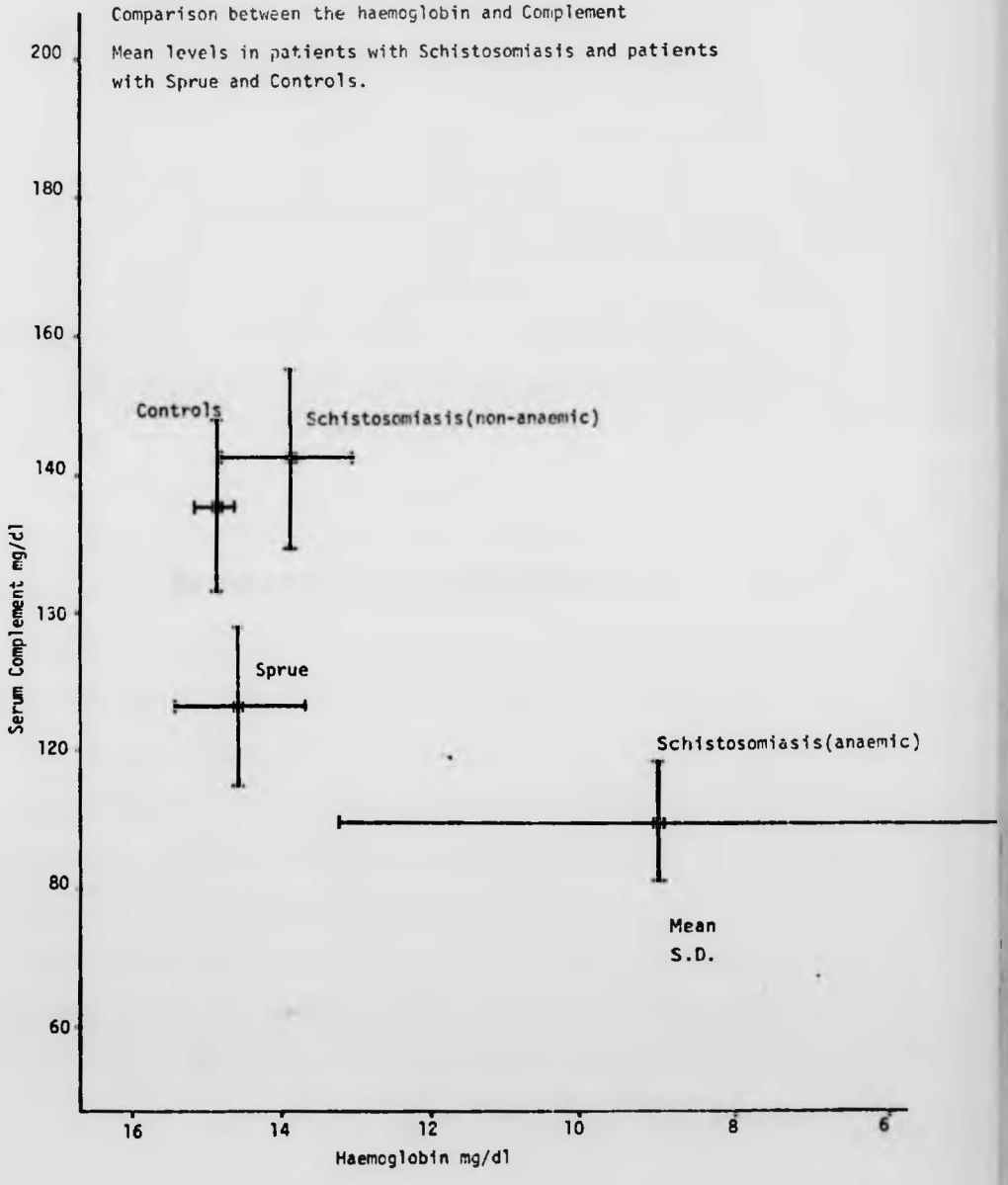
A radial immunodiffusion test showing ring diameters of patients with tropical sprue and controls

1. Reference serum 300 mg/dl
2. Reference serum 150 mg/dl
3. Reference serum 75 mg/dl
4. Control serum
5. Patient serum
6. Patient serum

Fig.(47)

Comparison between the haemoglobin and Complement

Mean levels in patients with Schistosomiasis and patients with Sprue and Controls.



PART 10

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Anaemia is almost invariable in schistosomiasis, and is more prominent in those with nutritional deficiencies. Various mechanisms have been cited in an effort to identify the causation of anaemia. Thus, chronic blood loss, splenomegaly, schistosomal toxins and haemolysis have been implicated. Reviewing these hypotheses, it was concluded that the fundamental concept for anaemia in schistosomiasis includes an immunological mechanism which holds serum complement responsible for erythrocyte haemolysis (Woodruff, 1973).

The main object of the present study was, therefore, to try to answer the question whether complement mediated haemolytic mechanism plays an important part in the genesis of anaemia in schistosomiasis. Thus, the work was designed to study anaemia, splenomegaly, nephropathy and liver lesions in relation to serum complement activities in mice infected with S.mansoni and to compare these results with those of other mice infected with T.rhodesiense. The relationship between the haemoglobin values and complement (C3) levels in anaemic and non-anaemic patients with schistosomiasis was also studied with reference to the effect of chemotherapy

on complement levels. Complement levels in tropical sprue were also studied, as it was suspected that malabsorption in sprue might result from a mechanism also involved in causing anaemia in schistosomiasis and trypanosomiasis.

In three groups of mice infected with 50, 100 and 150 cercariae of S.mansoni, anaemia was found to be mainly normochromic, although in some mice there was evidence of a slight degree of hypochromasia. This is not in agreement with the findings of Mahmoud (1971). He suggested that the anaemia is hypochromic. Using two-way analysis of variance, the severity of anaemia was found to be equally dependent on both the dose and the duration of infection. This compared favourably with the finding reported by Sauod (1965) and Suad (1978). The blood films examined showed evidence of polychromasia, a slight degree of macrocytosis and of some fragmented cells. This picture, particularly with the presence of the fragmented cells, suggests that the anaemia is haemolytic in nature (de.Gruchy, 1973 and Hoffbrand et al, 1975).

The role played by the spleen in the development of anaemia was investigated. First splenomegaly was expressed as the weight the spleen represented as a percentage of its body weight. Splenomegaly was found to increased in parallel with the anaemia

and with the increase in both the dose and duration of infection. A significant relationship was thus found between anaemia and splenomegaly. This did not, however, indicate that splenomegaly is the main cause for anaemia. Anaemia was reported in splenectomised mice when infected with S.mansoni (Mahmoud et al, 1972).

A method was developed to measure the complement levels in the mice sera by employing some modifications to the radioactive immunohaemolytic technique of Rosenberg et al (1962). Compared with controls, complement was significantly diminished in the infected mice. Even in mice infected with 150 cercariae it was less than in those infected with 50 cercariae. Like the relationship found between anaemia and splenomegaly, anaemia was also significantly related to the decrease in complement levels. Comparing these two positive relationships has indicated that the degree of anaemia is more associated with the variation of the complement levels than with the increase in splenic weight.

These results were compared with the similar results obtained from mice infected with T.rhodesiense. In trypanosomiasis, anaemia was normochromic and less severe than the anaemia observed with schistosomiasis. Complement levels were drastically reduced. Similar reduction was reported in rats infected

with T.lewisi (Jarvinen et al, 1976). This fall in serum complement was neither related to the increase in the splenic weight nor to the increase in the parasitaemia in the blood. An almost perfect relationship has, however, been found between the increase in the splenic weight and the increase in the number of trypanosomes/ml of blood. This suggests that the remarkably larger spleens observed in the infection could result from an immune response to the large numbers of trypanosomes present in the blood. However, despite the much larger spleens observed in trypanosomiasis as compared with those of schistosomiasis, anaemia was less severe in the former than in the latter. This observation suggests that splenomegaly per se is not the main cause for anaemia in both infections. Splenomegaly is a result of the destruction of erythrocytes rather than a cause of the destruction.

Kidneys and livers of mice from both infections were examined by a fluorescence test for the presence of complement (C3). Complement deposits on the renal glomeruli were present in both infections, though in trypanosomiasis there was more than in schistosomiasis. This may be related to the dramatic fall in serum complement observed in trypanosomiasis. In the two infections, it has been observed that the fall in serum complement

(C3) is associated with its deposition in the renal glomeruli. However, the fall in (C3) levels precedes such deposition by at least 1 or 2 weeks. This time may be necessary for the complement deposits to accumulate in sufficient amounts or to block some of the renal glomeruli or to activate the complement receptors probably present as that described in the human kidney by Girard et al (1977). The presence of (C3) deposits on the glomeruli of kidneys in mice infected with S.mansoni was also repoted by Mahmoud et al (1972) and Natali et al (1976).

In the liver, complement deposits were only present on the eggs lodged there and were also found on the eggs inside the female worms. It was thought that the high antigenicity of the eggs provokes an immunological reaction on their surface in which complement is actively involved. The effect of such reaction could initiate the formation of granuloma around the eggs. This observation, together with the absence of (C3) deposits from the liver granulomas, has indicated that the granulomatous response in schistosomiasis is rather inflammatory and not immunological in nature.

Despite the difficulties in performing human studies, it was particularly important to do it in view of the different aspects of pathology observed between human and experimental schistosomiasis

(Cheever, 1969 and Gillett et al, 1978). Thus, two groups of patients with active S.mansoni and S.haematobium infections, together with equivalent local controls were examined with respect to their haemoglobin and complement (C3) levels. The complement (C3) levels were measured by the radial immuno-diffusion method of Mancini et al (1965).

The first group were of Egyptian anaemic patients in whom the (C3) levels were found to be significantly lower than those of the controls. The second group comprised Sudanese non-anaemic patients. A strong positive relationship between the haemoglobin values and (C3) levels was found in the anaemic patients, but neither in the controls nor in the non-anaemic patients. Contrarily, those non-anaemic patients had significantly higher values of (C3) than their local controls. Six weeks after successful treatment with hycanthone, their complement levels declined significantly from its pre-treatment levels and at that time did not differ significantly from such values in the controls. Good evidence of a fall of haemoglobin with consumption of complement was obtained.

It was noticed that many of the Sudanese patients had splenomegaly without anaemia, and all the Egyptian patients had splenomegaly with anaemia. It seemed likely, therefore,

that splenomegaly may only be a contributory factor towards the causation of anaemia in human as in experimental schistosomiasis. In the same Sudanese sera, (IgG) and (IgM) have also been measured by other workers in the same department (Salih et al, 1978). Significantly higher levels of both (IgG) and (IgM) were found before treatment. In the post-treatment stage, (IgM) only fell, while (IgG) even rose though the rise did not differ significantly from its pre-treatment levels. The greater ability of (IgM) molecules than of the other immunoglobulin molecules to bind complement has been reported several times (Woodruff, 1973; Hoffbrand et al, 1975 and Frank et al, 1977). It was thought then that the fall of complement and (IgM) in the post-treatment stage is not coincidental and it could be a response to an immunological mechanism whereby the dead and dying worms are attacked. Such a mechanism is not unlikely as (C3) deposits have already been detected on the female worm tegument (Kabil, 1976). Moreover, the maximal adherence of white cells to the schistosomes has been reported only to be achieved in the presence of complement (Ottesen et al, 1977).

Haemoglobin and complement (C3) values were measured in patients with tropical sprue. The patients were not anaemic and there was no relationship between their haemoglobin and complement

levels, though their complement levels were significantly lower than in the controls. This fall in (C3) levels was apparently greater than in those patients who had no active infections with G.intestinalis. It has been suggested that the malabsorption syndrome develops in patients with immunoglobulins deficiency (Brown et al, 1972). It has also been suggested by the present study that the fall in complement (C3) levels in patients with schistosomiasis results from immunological mechanisms associated either with anaemia or the effect of treatment on the schistosomes. Thus, in sprue there could be also some other immunological mechanism consuming complement. The present study raises the possibility of an immunological mechanism bringing about the malabsorption. This mechanism probably brings about changes on the intestinal mucosa. In those changes complement is involved and consumed. It is therefore suggested that further investigations of the intestinal tissues of patients with tropical sprue may, therefore, yield useful information on the aetiology of small bowel syndrome in the tropics.

From all this work taken together, it is concluded that in human as in experimental schistosomiasis, a haemolytic component develops during the course of the infection. During the infection complement is actively involved and consumed and the role played

by splenomegaly is only contributory. The wider picture of this haemolytic process may include the presence of complement deposits on the renal glomeruli. The same mechanisms with some differences also occur in trypanosomiasis. In tropical sprue the immunological process consuming complement probably takes place mostly on the intestinal mucosa rather than on the erythrocyte surface.

PART 11

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