Pathophysiological and Immunological Studies of Bovine Trypanosomiasis

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CONTENTS

GENERAL INTRODUCTION

Trypanosomiasis is one of the most important animal diseases in Ethiopia causing many thousands of deaths each year and the disease is not limited to Ethiopia alone. Approximately a quarter of the total land surface of the African continent is estimated to be infested by tsetse flies and virtually all of this infested area is south of the Sahara.

The geographical distribution of the various species of trypanosomes and their vector hosts, the tsetse flies, has been studied by a number of investigators. While all the species of the cyclically transmitted trypanosomes are distributed over Africa south of the Sahara, the predominant species vary between east, central, and west Africa.

Ford (1971) has made a review of the epizootiological and ecological picture of the African trypanosomes. A review of the east African picture has also been made by Willet (1970). The predominant trypanosome species affecting cattle in east Africa is Trypanosoma congolense (Shaw, 1958; Lawrence and Bryson, 1958; Randal, 1958).

The distribution of trypanosomes is in close correlation to the distribution of the vector Glossina. Figure 1 shows the distribution of tsetse flies in Ethiopia. For the cyclical transmission of T.congolense, the morsitans group of tsetse flies are the most important vectors with the palpalis group playing an insignificant role (Godfrey, 1961). The other important factor affecting the distribution of tsetse is the availability of suitable reservoir hosts, the wild ungulates.

Studies on the distribution of trypanosomiasis, the vector tsetse flies, and the wild animal hosts in Ethiopia are far from adequate. There are many reasons for this lack of information, the most important ones being the lack of trained Ethiopians and the unavailability of roads and other transport facilities into the vast interior of the country. This is especially true of the trypanosome infested southern and western provinces.

The published reports on the incidence of trypanosomiasis in Ethiopia before 1961 were solely concerned with Eritrea and Shoa provinces. The seriousness of trypanosomiasis in these areas as a major economic factor is not great, mainly because neither of the provinces has a large cattle population and most of Shoa is situated on the Ethiopian highland plateau free from tsetse flies.

Peck (1961) and Knowles (1967) investigated the problem of trypanosomiasis in the southern and western provinces where the disease is of major significance. It has been shown since, that the distribution of trypanosomiasis, the vector tsetse flies, and the reservoir wild animal hosts, is much wider than previously reported (Balis and Bergeon, 1968; Blower, 1968). According to reports published by the Ethiopian Ministry of Agriculture (1972) the disease is progressing farther and farther north and east from the south west. The epizootiology of cattle trypanosomiasis in south-west Ethiopia has been reviewed by Krug (1971).

In the 1972 report of the Ethiopian Ministry of Agriculture the known distribution of tsetse flies and trypanosomes is summarised as follows.

A. Tsetse flies

1. Morsitans group

a. Glossina morsitans submorsitans

Found in the west of Ethiopia along the rivers Muger, Guder, Blue Nile, Dedessa, Anger, Dabous, Baro, Gilo, Akobo, Omo, and the vicinity of Lake Marguerita. The specimens have always been found in savannah near water courses.

b. G.pallidipes

Found in the west and south west especially in the area of the rivers Omo, Baro, and Akobo.

c. G.austeni

Found in adjacent Somalia in the Bidi forest and adjacent to the Bidi forest.

2. Palpalis group

a. G.tachinoides

Found along the Dedessa river in Wollega province and nearby forests; in the region of Gambela along the rivers Baro, Gilu and Takaw.

b. G.fuscipes fuscipes

Found along the rivers Gibe, Gojeb, Omo, Keto and Birbir always below 1500 metres altitude.

3. Fusca group

a. G.longipennis

Found along the Welmel river in adjacent Somalia, in the Bur Fuleh and Bier Mudu regions of the Ogaden and along the Deghoto and Juba rivers. In the Ogaden these flies attack cattle at night.

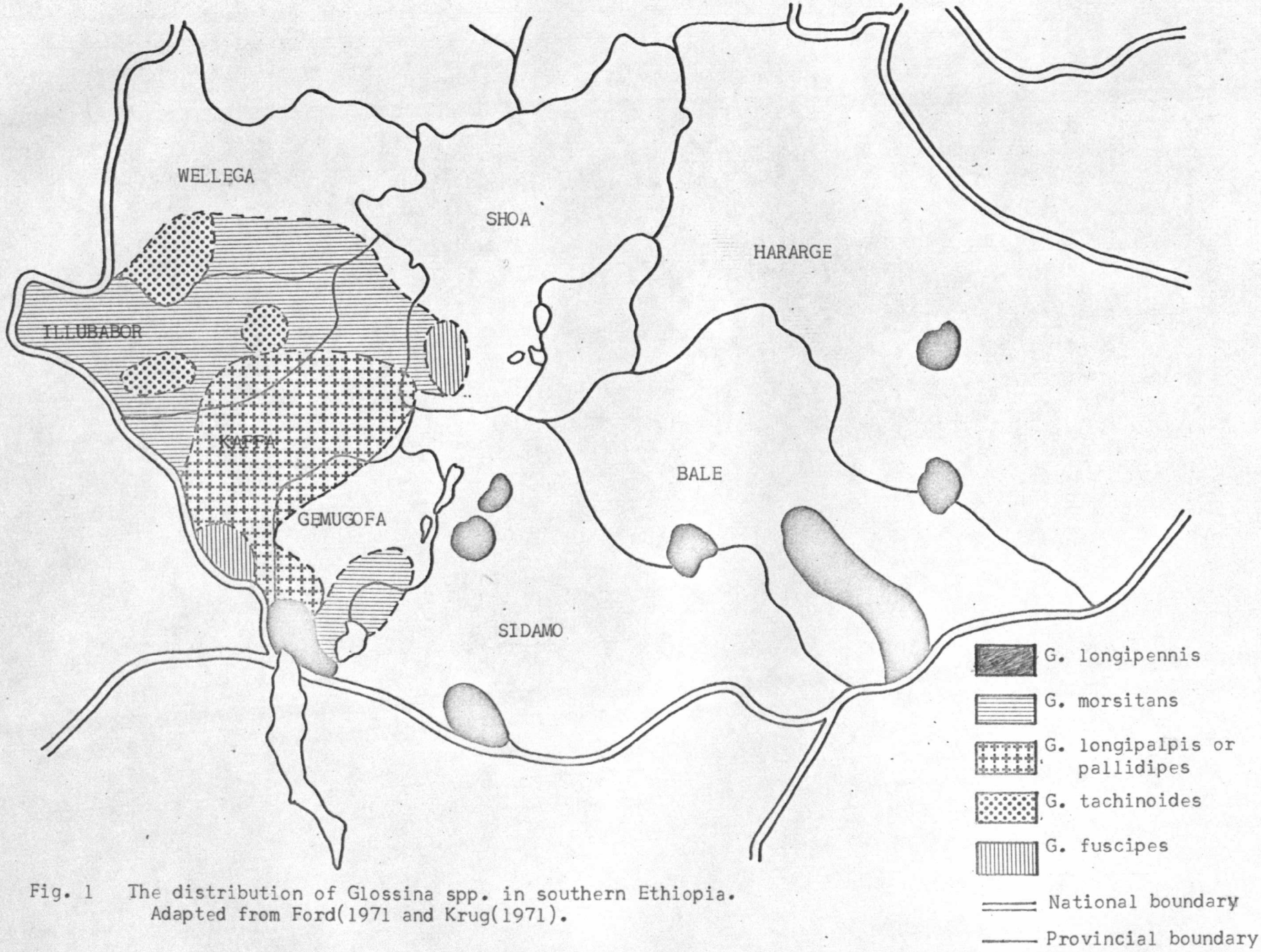


Fig. 1 The distribution of Glossina spp. in southern Ethiopia. Adapted from Ford(1971 and Krug(1971).

b. G.brevipalpis

Found along the Omo and Juba rivers in large numbers.

B. Trypanosomes

The trypanosomes identified so far include T. congolense dimorphon in large ruminants, equines and swine, T.vivax in ruminants and equines, T.brucei in large ruminants, T.equiperdum in equines and T.evansi in camels.

T.congolense is the most important of the trypanosome species affecting cattle in Ethiopia. The disease is cyclically transmitted to cattle by blood sucking tsetse flies, Glossina spp. On feeding on an infected animal the tsetse aquires the trypanosomes along with blood. These pass into the midgut where they assume the elongated trypomastigote form characterized by the position of the kinetoplast not far behind the nucleus. In this stage the flagellates multiply for some days and then move on into the cardia by the twentieth day or earlier. Here they become elongated and migrate through the esophagus to the proboscis. In the proboscis they are transformed to the epimastigote forms and attach themselves to the walls of the labrum and food canal. Finally they find their way to the hypopharynx in which the cycle is completed by transformation of the flagellates into the metatrypanosomes. The entire cycle of development in the tsetse fly may take 19 to 53 days. During feeding on a new host the tsetse injects the metatrypanosomes into the blood stream. In addition to cyclical transmission by tsetse flies the parasites can be mechanically transmitted by other blood sucking flies the

most important of which belong to the genera Stomoxys and Tabanus.

Three forms of T.congolense are recognized based mainly on their size and pathogenicity, the two factors having a direct relationship (Hoare, 1959). The short form with a mean size of 12.2 to 14.4 microns is characterized by low parasitaemia and low pathogenicity. The intermediate form has a mean length of 14 to 15 microns and is characterized by high parasitaemia and a low pathogenicity. The long or dimorphon form has a mean length of 15.3 to 17.6 microns and is characterized by high parasitaemia and high pathogenicity producing the acute form of the disease.

The experimental contents of the present thesis are presented in three general categories. In the first section the anaemia in acute, subacute and chronic trypanosome infections has been studied using conventional haematological, biochemical and isotopic methods. In the second section aspects of the immunology of T.congolense infections including the protective value of radiation attenuated organisms, and the immunosuppressive effect of trypanosome infections have been evaluated. In the third section a short description of the pathological findings are presented.

Anaemia is among the most important features of bovine trypanosomiasis. Fiennes (1954) and Edwards et al (1956) among others have described the haematological picture in some detail. French (1935) described some of the major biochemical changes taking place in the host during infection.

To date the nature of the anaemia is not fully understood. Basically anaemia may be caused due to decreased production or increased loss due to intravascular destruction, haemorrhage, or both. Fiennes (1954) showed that during peaks of parasitaemia the urine was positive for the indirect van den Bergh test and Murray et al (1973) found haemoglobinuria in experimentally infected rabbits. These findings tended to suggest intravascular haemolysis as the major cause for the increased loss of red blood cells. Erythrophagocytosis has also been introduced as a very important factor in the loss of red blood cells from the circulation (Mackenzie and Cruickshank, 1973).

Observations on the loss of the red marrow of the long bones (Fiennes, 1953) gave rise to the view that there is depression of erythropoesis in trypanosome infected animals. Thus both decreased production and increased loss of red cells have been suggested as causes of the anaemia.

To resolve the relative importance of each in the development of anaemia and also to confirm whether the depression of the marrow of the long bones could be extrapolated to general medullary dishaemopoesis, it is obvious that kinetic approaches have to be used. Isotopic tracers have in the past been found to be very useful for kinetic studies on anaemias.

In the present thesis isotopes of iron and chromium were employed in the study of the anaemia. ^{51}Cr labelled red cells were used to study the kinetics of the erythrocytes. Such a study helps not only in estimating the erythrocyte life span but also indicates the site and manner of loss of

the labelled cells.

Iron being an integral part of haemoglobin, ^{59}Fe can be used to estimate the amount of new haemoglobin formed and therefore haemopoesis. More detail on each subject is given in the appropriate section of each experiment.

Splenomegally is a common finding in trypanosomiasis and the role of the spleen in the anaemia is not well understood. Experiments were conducted on splenectomized animals to help elucidate some of the parts played by the spleen in ferrokinetics and erythrokinetics of infected animals.

From the immunological point of view, as has been pointed out earlier, the experiments presented are on the use of radiation-attenuated organisms and on immunosuppression as a result of trypanosome infections.

Immunological control has been a major area of research in trypanosomiasis. Attempts at producing active immunity have been made using various approaches. Intentional infection of animals and treating them after predetermined time intervals was shown to have some protective values (Taliaferro, 1929; Cunningham, 1967), but the degree of such protection was inadequate. The use of chemically killed trypanosomes for active immunization has been investigated by Soltys (1964, 1967) but the protection level of such a vaccine was also shown to be limited.

The only effective vaccines to date against helminths and protozoa are those produced by radiation attenuation of the infective agent. Jarret *et al* (1958) demonstrated the value of irradiated helminth parasites for

immunization of cattle against lungworms. Mulligan et al (1961) showed the protective capacity of irradiated larvae of Haemonchus contortus and Trichstrongylus colubriformis in sheep. Urquart (1961) showed the immunization capability of irradiated Taenia saginata eggs against bovine cysticercosis. More recently Bitakaramire (1973) reported the use of irradiated metacercarine for vaccination against Easciola gigantica infections in cattle.

For the protozoan parasites among the earlier radiation attenuated vaccines reported was that against Plasmodium gallinacium in chickens (Ceithmal and Evans, 1946). In the case of trypanosomiasis various degrees of success have been reported depending on the species of the host, the species of the trypanosome, the virulence of the trypanosome strain and the size of the challenge dose.

Stubbs et al (1958) found little or no protection of rats using irradiated T.equiperdum. On the other hand irradiated T.lewisi (Sanders and Wallace, 1966) and T.rhodesiense (Duxbury and Sadun, 1969) were shown to confer a fair degree of immunity on experimental mice.

Duxbury et al (1973) reported the use of radiation attenuated organisms in protecting cattle against a very mild strain of T.congolense and based on this finding it was considered desirable to investigate the possibility of vaccination against a virulent strain of T.congolense isolated from cattle in Gambela, Illubabor province. In addition, pathophysiological studies were performed in the vaccinated cattle to determine whether any measurable improvement over non inoculated cattle has been attained

following challenge.

In recent years marked suppression in the immune response of laboratory animals infected with trypanosomes have been observed. Goodwin (1970) showed that mice and rabbits infected with chronic trypanosomiasis showed a suppressed response to the production of haemaglutinins to sheep red blood cells. Urquart et al (1973) demonstrated the same phenomenon using Nippostrongylus braziliensis as their model. Longstaffe et al (1973) in their study on immunoglobulin response observed that in early T.brucei infections of mice IgG producing splenic cells were markedly reduced and that later on in the infection both IgG and IgM producing cells were reduced significantly as compared to the control groups.

The phenomenon of immunosuppression has so far not been demonstrated in cattle. Obviously it is very important, especially from the standpoint of the response of such cattle to vaccinations against other diseases and their ability to withstand other secondary invaders, to determine whether such an effect takes place in the bovine. The immunosuppression experiments described in this thesis show that such a phenomenon occurs in cattle infected with T.congolense.

As mentioned earlier the last part of this thesis consists of a short description of the major pathological findings in cattle experimentally infected with T.congolense. The pathology of trypanosomiasis especially in the large animals has not been adequately investigated. Jubb and Kennedy (1970) stated that "It is impossible yet to give a

connected account of the pathology of trypanosomiasis in the domestic animals". Fiennes et al (1946) described some of the pathological changes in bovine trypanosomiasis. Infection is generally followed by a rise in temperature which coincides with the appearance of parasites in the blood stream and a drop in temperature occurs with a fall in the level of parasitaemia. Later during the infection temperature and parasitaemia remain elevated and massive trypanolysis and red blood cell destruction takes place. Symptoms of anaemia and wasting away of the animals begin to show. Appetite is generally not affected until the last stages of the disease when the animals are in extremis.

The state of cachexia produced in infected cattle is of considerable economic significance. In addition to direct losses of cattle due to mortalities other factors of loss may be incurred due to decreased reproductive efficiency, loss of libido of infected bulls, abortion, failure of infected calves to reach sexual maturity, lowered milk yield, and poor feed conversion efficiency.

An understanding of the basic mechanisms of the disease is therefore essential and though much work has been done in the laboratory animals, as pointed out earlier, the results cannot be directly applied to the large animals in which the ultimate understanding lies.

The present thesis describes the extention and development of such studies in the bovine host.

II. GENERAL MATERIALS AND METHODS

Experimental animals

Cattle

The cattle used in the experiments described were 8-12 month old male zebus obtained from the countryside around Addis Ababa on the Ethiopian highland plateau. This area is over 2000 metres above sea level with no endemic trypanosomiasis or tsetse flies. All cattle were checked for general physical condition and mass treated for liver fluke and intestinal parasites on arrival at the experiment site at least four weeks prior to use.

Mice

These were random bred laboratory white mice obtained from the Central Laboratory and Research Institute of the Ethiopian Government in Addis Ababa.

Maintenance of experimental animals

The cattle were maintained solely on grass pasture during the rainy season (July to September). During the rest of the year they were on pasture supplemented with hay and local commercial balanced concentrate dairy ration.

The mice and rats were maintained on barley and wheat supplemented with carrots and cabbage. Water was supplied ad lib.

Trypanosomes

Sub-acute strains

The Gemugofa strain of T.congolense was kindly supplied by W. Langridge and A. Alemu of the Ethiopian Ministry of Agriculture. This strain was isolated from cattle by passage in mice from Gemugofa province, one of the endemic southern provinces.

The Gambela I strain of T.congolense was isolated from cattle in Gambela in Illubabor province. It was designated Gambela I to differentiate it from the Gambella II strain which is an acute strain isolated from the same district later.

Dedessa strain was isolated from cattle in the Dedessa valley in Wollega province by M. Scot (O.D.A.) and maintained in cattle by serial passage.

Acute strain

This was isolated from cattle in Gambella district and maintained in mice by serial passage.

Chronic strain

This was supplied by W. Langridge and A. Alemu of the Ethiopian Ministry of Agriculture. It was isolated from cattle in Gemugofa province.

Parasitological techniques

Enumeration of trypanosomes

The number of trypanosomes in a specific volume of blood was determined by first diluting the blood 10 to 25 fold depending on the haematocrit of the animal and counting in a haemacytometer.* The number present in the four large corner squares (4 sq mm) was counted and the number per mm^3 arrived at using the following formula.

$$\text{number of trypanosomes per } mm^3 \text{ of blood} = \frac{\text{trypanosomes counted} \times \text{dilution} \times 10 \text{ (0.1mm depth)}}{4 \text{ (no. of 1mm squares counted)}}$$

Routine determination of infection

This was carried out by placing one drop of a well mixed heparinized blood on a slide with a cover slip and examining under the microscope with 400x magnification. The level of infection was then determined according to the following schedule (Desowitz and Watson, 1953)

-	no trypanosomes observed
+	average 1-5 trypanosomes per field
++	average 6-10 trypanosomes per field
+++	average 11-20 trypanosomes per field
++++	average more than 20 trypanosomes per field

* Improved Neubaur, Hawksley and Son, U.K.

Trypanosome infectivity determination

The ID_{63} was determined according to the method described by Lumsden *et al* (1963). The ID_{63} is essentially the estimated number of trypanosome doses necessary to infect 63% of a population of mice. It is based on the theory that one trypanosome can infect one mouse. If the trypanosomes in a particular suspension follow the Poisson distribution curve 63% of the mice will receive an infective dose. The method used involves serial dilution of a suspension of trypanosomes and inoculation of each dilution into mice. The highest dilution at which infection occurs is then converted to the ID_{63} on the basis of mice infected out of the total number inoculated at that dilution.

Haematological techniques

Pack Cell Volume (PCV)

The PCV was determined by the microhaematocrit method. Capillaries containing heparinized blood were sealed at one end and centrifuged in a microcentrifuge* for five minutes. A direct reading of the PCV was made with the aid of the Hawksley haematocrit reader.

Haemoglobin

The cyanmethemoglobin method described by Drabkin and Austin (1935) as recommended by the International Committee for Standardization of

*Hawksley and Son, U.K.

Haematology (1967) was used for haemoglobin determination. The principle of this method involves laking of erythrocytes and converting the haemoglobin to cyanmethaemoglobin by the cyanide in the diluting solution. The optical density is read off in a spectrophotometer and the concentration of cyanmethaemoglobin is directly proportional to the optical density. The spectrophotometric reading is multiplied by a factor to get the concentration of haemoglobin per unit volume of blood.

Total red cell counts

This was performed as described by Benjamin (1961).

Mean corpuscular volume (MCV)

This was derived from the PCV and total erythrocyte count as follows:

$$MCV = \frac{PCV \times 10}{\text{rbc count in millions per mm}^3}$$

Mean corpuscular haemoglobin concentration (MCHC)

This was derived from the haemoglobin and PCV values as follows:

$$MCHC(\%) = \frac{\text{haemoglobin(Gm\%)} \times 100}{PCV(\%)}$$

Statistical methods

All statistical analyses were based on the method described by Bishop (1966). Standard deviations (sd) were

used for presentation of deviations from the means. Differences between means were subjected to the "t" test and were regarded as significant where $P < 0.05$.

Other materials and methods

The materials and methods described above apply to all the experiments presented in this thesis. Specialized materials and methods are presented under the appropriate experiments.

SECTION I

THE ANAEMIA IN *T.CONGOLENSE* INFECTIONS OF CATTLE

A. HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS

B. ISOTOPIC STUDIES

A. HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN *TRYPANOSOMA CONGOLENSE* INFECTIONS OF CATTLE.

Introduction

Haematological observations on cattle infected with *Trypanosoma congolense* have been made by Fiennes *et al* (1946), Fiennes (1950, 1954, 1970), Edwards (1956), Naylor (1971) and Wellde *et al* (1973). There are virtually no studies recorded in the literature pertaining to the pathophysiological effects produced in the various hosts by the various strains of *T.congolense* in Ethiopia. The approximate distribution of trypanosomiasis and the vector tsetse flies in Ethiopia has been presented in the general introduction.

Anaemia is a general feature of trypanosomiasis. In chronic *T.congolense* infections of cattle the anaemia produced is of normochromic normocytic nature which in the later stages reverts to normochromic microcytic anaemia (Fiennes, 1954, 1970). Reticulocytosis has been shown to occur in infected cattle (Fiennes, 1954) although this was not confirmed by Naylor (1971) who observed that the literature apart from some work by French(1935) on biochemistry and some superficial investigations by a number of workers, is confined to the writings of Fiennes and his co-workers. This comment underlines the limited volume of reference material especially on *T.congolense* infections of large animals, and this fact obviously makes repeated reference to the above sources unavoidable.

Fiennes (1954) divided the development of anaemia due to T.congolense into four different courses : (1) The hyperacute course in which severe haemolysis occurs and death ensues early in the infection. (2) The acute course in which the occurrence of haemolysis is less severe and macrocytic anaemia develops. (3) The chronic course in which hydraemia and microcytic anaemia develop and the haemolytic crisis is usually not fatal. During chronic infections three stages were observed. In the first stage increased blood volume, plasma volume and circulating red cell volume were recorded even though the total red cell concentration per unit volume of blood was reduced. This state he referred to as hydraemia and not anaemia. During the second stage there was shown to be a fall in circulating red cell volume but the plasma volume remained at normal levels. In the third stage of chronic infections acute haemolysis (usually not fatal) and increased plasma specific gravity were observed and the rise in plasma specific gravity was said to be due to dehydration. (4) The recovery course in which non-significant hydraemic changes in the host take place.

In cattle infected with T.congolense total white cell counts fell significantly at 6 weeks post infection though this value rose to above preinfection levels at 14 weeks. Eosinophil, lymphocyte and neutrophil counts fell in the blood smear early in the infection, monocytes remained within normal levels, and plasma cell counts increased significantly (Naylor, 1971).

One of the problems in evaluating pathological effects in terms of changes in the blood indices is the physiological variation. "Great disparity occurs with regard to mean erythrocyte counts and this in turn causes the erythrocytic indices to be at variance. No doubt personal errors are in part responsible, but the differences in red cell numbers of cattle reported from the different regions of the world are of such magnitude as to suggest that breed, climate and level of nutrition may have a significant influence on the red cell numbers and size of the cow" (Schalm, 1965). This statement underlines the fact that because these indices are used in the measurement of both physiological and pathological changes one could not rely totally on the "normal" values published in the literature. Nevertheless certain generalizations could be made regarding haematological indices. For example in the cow total red cell and haematocrit indices generally decline with age whereas the mean corpuscular volume generally increases with age (Greatorex, 1954; 1957; Holman, 1955; 1956). Other factors that may affect the haematological indices include the nutritional status, the presence of other parasites and altitude.

Obviously the same generalizations could be made for the biochemical indices. Animals maintained on pasture are known to show significant variations in their biochemical indices depending on the season of the year and the quality of the pasture. For example blood total nitrogen and vitamin C levels are known to rise during the rainy season

when the pasture is green and these values decline during the dry season. On the other hand blood chloride levels are known to increase during the dry season and fall off during the rainy season (Braun, 1946). Pasture deficiencies in certain minerals such as iron, copper or cobalt either singly or in combination would be reflected in declining haematological values as these elements are involved directly or indirectly in the formation of erythrocytes. Cattle receiving concentrate supplement generally show higher haemoglobin concentration than those maintained on pasture alone (Thomas et al, 1954).

For some time it has been recognized that the effects produced by trypanosome infections varied not only with the strain of the trypanosome but also with the breed or strain of cattle. A good example is the N'dama and Muturu cattle of west Africa which are considered to be more resistant to trypanosome infections than other breeds in that part of the continent (Chandler, 1952; 1958). Bangham and Blumberg (1958) observed that resistance to trypanosome infections may be related to blood groups. Cattle with homozygous A blood group, eg N'dama and Muturu, were shown to be more resistant than AB and homozygous B groups.

The biochemical estimations performed in the present experiment were mainly on blood proteins, serum iron and serum iron binding capacity as these factors are most directly involved in the process of haematopoesis. Albumen and globulin are the major constituents of plasma proteins. Other plasma proteins include the various

conjugated proteins, nucleoproteins and fibrinogen. Albumen is the most abundant of the plasma proteins and has the smallest molecules. Because of these two properties albumen contributes a major portion of plasma colloid osmotic pressure and hence a major influence in the maintenance of plasma volume. The plasma protein globulin has several components among which are the gamma globulins and beta globulins. The gamma globulins are generally involved in the body defence mechanisms. The beta globulins contribute such an essential protein as transferrin which is an iron binding beta globulin essential in the iron transport mechanism of the body.

Plasma protein alterations are known to occur during infections with trypanosomes (French, 1935). Wellde et al (1973) observed that a sharp fall occurred during the first five weeks and then gradually increased to low normal levels by the 12th week and remained at this level for the duration of the experiment. Serum albumen was shown to fall and remain at the low level throughout the infection period while globulin fell early and then rose to above preinfection levels in the later stages.

Tartour and Idris (1973) reported on iron metabolism in cattle infected with T.congolense. Among their findings are hypoferraemia during the early course and in some cases in the later stages of chronic infections. At the terminal stages of the infection hyperferraemia was observed and they associated this finding with haemolytic crisis and excessive transferrin saturation. In their view complete disappearance of the unsaturated iron binding

capacity resulting in full saturation of transferrin seemed to constitute a rather constant feature of the premortal collapse both in acute and chronic cases.

The present section is concerned in particular with the changes in the haematological picture of circulating red cells, plasma proteins including albumens, globulin and total protein, and plasma iron of Ethiopian cattle infected with T.congolense.

Materials and Methods

Trypanosomes

Three strains of T.congolense were used (acute, subacute and chronic). Origin and maintenance of these strains has been described in general materials and methods.

Experimental animals

These were approximately one year old cattle purchased and maintained as described in general materials and methods.

Haematological methods

These were performed as described in general materials and methods.

Biochemical methods

Plasma proteins

The biuret method (Weischelbaum, 1946) was used in the determination of total protein. Serum albumen was determined according to the method of Rodkey (1965). Globulin values were derived from the difference between total protein and albumen concentrations.

Serum iron and iron binding capacity

Blood samples were collected in non-heparinized tubes and allowed to clot at room temperature. The serum was then separated and frozen. Following completion of the experimental period all samples were analysed.

Iron determination kits were obtained from Roche (Roche Products, Basle). This method was described by Sanford (1963) and is essentially a modification of the method of Ramsay (1957; 1958). The values obtained according to this method have been shown to be in close correlation and not significantly different from that recommended by the International Committee for Standardization of Haematology (1971) by Meerkin (1973). The principle of the method is the splitting of the iron binding protein, transferrin and the bound ferric ions by sodium dithionate. The addition of bathophenanthroline disulfonate produces a coloured complex which is read at 546 millimicrons in a spectrophotometer. The intensity of the colour produced is directly proportional to the concentration of iron.

The principle of the method used in estimating total iron binding capacity (Lauber, 1965) involves treating the serum with excessive iron to saturate the transferrin and unbound iron is precipitated with basic magnesium carbonate. Transferrin bound iron is determined spectrophotometrically at 546 millimicrons.

Latent iron binding capacity was arrived at by subtracting the serum iron from the total iron binding capacity.

Results

Normal values of Ethiopian zebu, Ugandan Ankole and Friesian cattle.

Because of the possibility of variation in haematological indices due to breed, nutritional and

environmental differences in "normal" cattle, investigations were carried out on uninfected Ethiopian cattle of the age group and sex used in the experiments described in the thesis.

There were no significant differences in the mean PCV, haemoglobin and total red cell counts between "normal" Ethiopian zebu cattle, Ankole cattle of Uganda and American Friesian cattle maintained on pasture, though the mean values of Ethiopian cattle were apparently lower than both of the other groups (table 1).

Haematological findings in infected animals

A total of 22 animals were used in this investigation on acute (8 infected and 3 control), subacute (5 infected and 5 control) and chronic (3 infected and 3 control) trypanosomiasis. The scatter in the individual values is relatively large and the numbers of animals small; therefore the results should be taken guardedly.

In acute infections the sequential changes in pack cell volume, haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration were not significantly different from the control cattle on the basis of the "t" test (table 2). However on observation of the changes in the infected cattle one could see that there was a progressive fall in the pack cell volume and haemoglobin during the first and second weeks of infection. The animals died during the third week of infection.

In subacute infections there was a progressive and significant fall in pack cell volume, red blood cell concentration and haemoglobin values. The mean MCV and MCHC did not appear to be drastically affected throughout

the experimental period (table 3).

In chronic infections there was a significant fall in PCV starting at three weeks post infection and thereafter the fall was very gradual. Haemoglobin values fell significantly starting at 2 weeks post infection and thereafter maintained more or less at a low level. There were no significant alterations in the MCHC. A significant rise in MCV was observed at 6 and 8 weeks post infection (table 4).

Serum iron studies

Serum iron in chronic infections tended to be elevated to above preinfection levels for the first few weeks and this period of elevation above normal varied considerably between individual calves (fig 2, 3, 4). In the later stages the serum iron fell drastically and this appeared to be associated with impending death. A fall in total iron binding capacity throughout the infection period was observed in calves 18 and 19 though in the case of calf 20 such decline did not take place until after the sixth week of infection.

In subacute infections serum iron concentration was shown to be not significantly altered as the disease progressed until the final stages when the level rose significantly (table 5). Total iron binding capacity fell significantly on the 26th day but rose to approximately preinfection levels later on (table 5).

Serum protein studies

Serum total protein in acute infections declined progressively throughout the infection period(table 6). Total protein in subacute infections fell progressively and the fall increased in significance as the disease progressed (table 5). Values beyond 33 days were omitted as most of the infected calves died soon after. Albumen values fell significantly below the controls starting 12 days post infection and maintained at this low level throughout the infection period and this resulted in a corresponding increase in the globulin values (table 5).

Serum protein values in chronic infections followed the same pattern as subacute infections for the first 5 to 7 weeks and after that the concentration of protein rose gradually to approximately preinfection levels by week 13 and maintained above this level for the duration of the experiment (fig 5).

Table 2

Mean values for Pack cell volume (PCV), Haemoglobin (HB), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in calves infected with an acute strain of T.congolense and in control calves.

Group		weeks post infection											
		0 - 1				1 - 2				2 - 3			
		PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %
CONTROL	Mean	28	9.2	45.0	33.0	25	8.5	53.1	32.0	28	9.0	42.8	32.8
	sd	5.7	1.6	10.3	0.6	5.5	1.9	1.2	1.3	4.9	1.4	3.1	1.9
INFECTED	Mean	32	9.8	43.3	31.0	29	8.2	53.3	31.5	21	6.7	47.1	33.6
	sd	11	3.5	0.3	0.3	5.0	1.1	3.1	3.6	2.7	0.6	3.5	1.3
	"t"	NS	NS	NS		NS	NS	NS	NS	NS	P< 0.05	NS	NS

Table 3

Haematological Values in Calves following infection with subacute T.congolense and in Normal Controls (5 per group)

		DAYS POST INFECTION								
		-3	4	11	18*	25	32	39*	46*	53*
PCV	Inf.	30	30	25	20	18	16	14	14	8
(%)	Con.	28	29	29	25	24	23	24	25	26
Rbc.	Inf.	7.99	7.48	6.83	5.76	4.45	4.40	3.85	3.25	2.03
(10^6 mm^3)	Con.	7.26	6.95	6.86	6.79	6.31	6.09	5.65	6.39	5.86
Hb	Inf.	10.9	9.3	7.9	5.8	5.3	4.6	4.6	4.4	2.9
(gms%)	Con.	9.5	9.3	8.8	7.1	7.0	7.0	7.5	8.1	9.3
MCV	Inf.	38.8	40.1	36.6	34.7	40.0	36.3	36.4	43.0	49.3
(μ^3)	Con.	41.3	41.7	42.3	36.8	38.0	37.7	42.5	39.1	44.3
MCHC	Inf.	35.2	31.0	31.6	29.0	29.4	28.8	32.9	31.4	29.0
(%)	Con.	31.7	32.1	30.3	28.4	29.1	30.4	31.3	32.4	35.7

* denotes death of infected calf

Table 1

Haematological values of "normal" Ethiopian cattle (mean of 49 male approximately 8 - 12 months old animals) used in the present experiment as compared to Ugandan Ankole cattle (Jones, 1943) and Friesians (Brown, 1946) maintained on pasture (sd = standard deviation, se = standard error).

Breed	PCV %	HB gm %	Red cells 10^6	MCV u^3	MCHC %
Ethiopian zebu					
mean	30.0	9.9	7.2	44.4	32.1
range	24-40	5.7-13.6	4.3-9.7	30.6-57.6	23.8-38.2
sd	3.9	2.0	1.4	7.1	4.2
se	0.55	0.28	0.20	1.20	0.71
Friesian					
mean	35.2	12.2	7.5	46.9	34.6
sd	3.8	1.2	0.9	-	-
Ankole					
mean	-	11.7	8.8	-	-
sd	-	2.3	1.7	-	-

Table 4

Haemotological studies in cattle infected with chronic T.congolense (HB= haemoglobin in gm%, PCV= pack cell volume in %, MCHC= mean corpuscular haemoglobin concentration in %, MCV= mean corpuscular volume in cubic microns)

	Weeks post infection						
	0	2	3	4	6	7	8
infected mean	9.0	6.6	7.5	6.8	5.3	5.5	4.6
sd	0.4	1.7	1.2	0.7	0.8	0.6	0.4
Hb(gm %)							
control mean	10.2	10.3	11.6	11.6	9.2	7.9	9.3
sd	0.9	0.6	1.8	1.8	1.1	3.1	1.9
"t" test	NS	P < 0.005	P < 0.02	P < 0.02	P 0.001	NS	P < 0.01
infected mean	28.8	26.9	20.7	19.1	18.7	16.0	16.7
sd	0.9	3.6	0.3	0.9	2.3	0.9	1.2
PCV(%)							
control mean	38.8	33.5	35.6	31.1	32.6	32.0	33.7
sd	8.9	6.9	5.4	4.6	2.7	5.4	4.5
"t" test	NS	NS	P < 0.01	P < 0.001	P < 0.001	P < 0.001	P < 0.001
infected mean	35.9	34.6	35.7	34.3	30.1	35.3	27.6
sd	4.9	5.3	5.9	7.1	1.3	3.7	1.2
MCHC(%)							
control mean	32.7	32.3	33.8	37.9	27.7	24.5	28.3
sd	2.8	4.3	2.9	3.9	0.5	7.2	4.5
"t" test	NS	NS	NS	NS	NS	NS	NS
infected mean	46.8	51.3	59.3	51.8	52.7	48.9	60.0
sd	2.8	9.8	7.2	1.6	2.1	6.4	4.5
MCV							
control mean	42.0	41.5	52.5	45.6	44.4	45.8	49.4
sd	7.1	3.9	12.1	7.3	1.5	4.4	5.5
"t" test	NS	NS	NS	NS	P < 0.01	NS	P < 0.05

Table 5

Blood Biochemical Values in Calves following infection with subacute T.congolense and in Normal Controls (5 per group)

BIOCHEMICAL STUDY		DAYS POST INFECTION								
		0	5	12	19*	26	33	40*	47*	54*
Serum Iron	Inf.	90	-	112	-	89	-	90	-	200
(fe µg%)	Con.	122	-	151	-	119	-	99	-	90
Total Iron Binding	Inf.	42-	-	446	-	263	-	375	-	372
Capacity(fe µg%)	Con.	480	-	444	-	391	-	386	-	383
Serum Albumen	Inf.	2.5	2.7	2.3	2.3	2.1	1.9	2.1	2.1	1.8
(gm%)	Con.	2.7	2.9	2.7	2.8	2.5	2.3	2.4	2.3	2.5
Total Serum Protein	Inf.	6.0	6.4	6.0	5.7	5.3	5.3	6.0	6.0	5.5
(gm %)	Con.	7.0	7.1	7.1	7.2	7.0	6.8	7.7	7.6	7.1

* denotes death of infected calf

Table 6

Sequential changes in serum total protein (gm %) of calves infected with acute T.congolense

		Weeks post infection			
	Calf	0	1	2	3
INFECTED	12	6.88	6.43	5.84	5.51
	13	7.21	6.82	6.04	6.23
	32	7.39	6.04	5.35	died
	35	7.01	6.04	5.75	died
	42	7.91	6.58	6.47	5.71
	mean	7.28	6.38	5.89	5.81
	sd	0.38	0.33	0.40	0.36
CONTROL	17	7.33	6.62	7.21	7.40
	21	7.60	7.79	8.08	7.88
	22	7.88	8.36	8.36	7.31
	31	7.16	7.24	7.37	7.50
	mean	7.49	7.50	7.75	7.52
	sd	0.30	0.73	0.53	0.24
"t" test		NS	$P < 0.02$	$P < 0.001$	$P < 0.001$

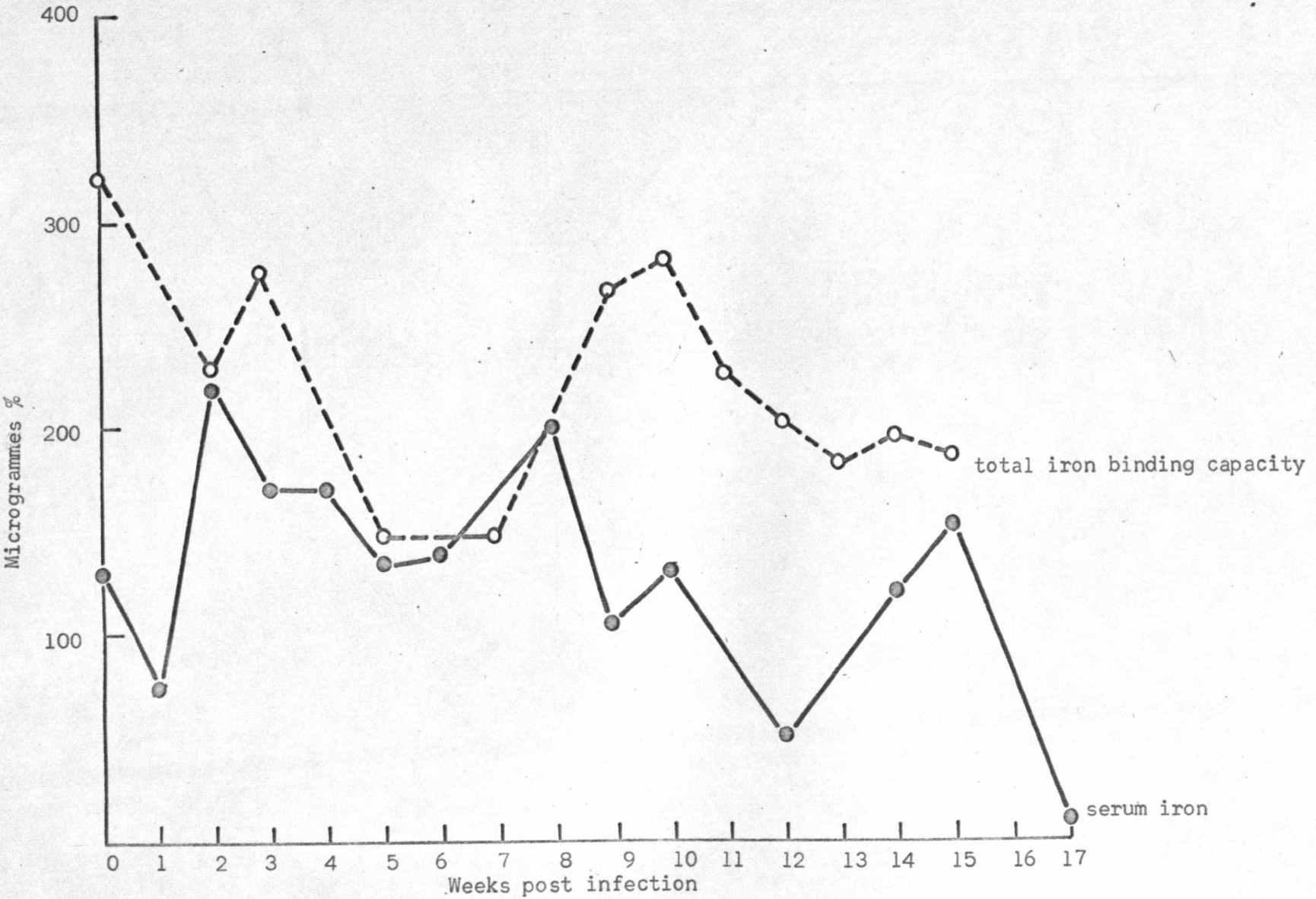


Fig. 2 Sequential changes in serum iron and iron binding capacity in calf no.18 infected with I. congolense.

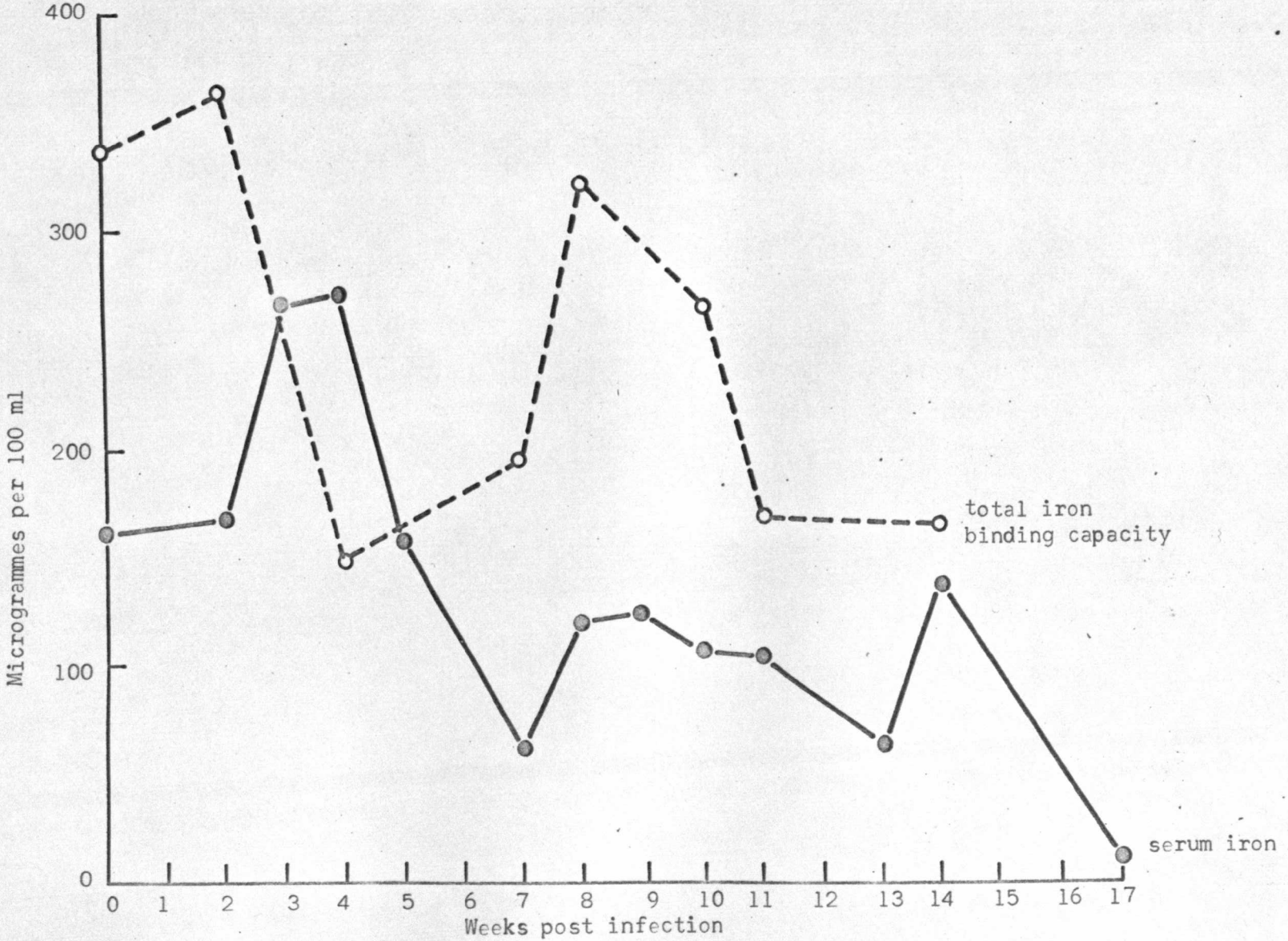


Fig. 3 Sequential changes in serum iron and total iron binding capacity in calf no 19 infected with T. congolense.

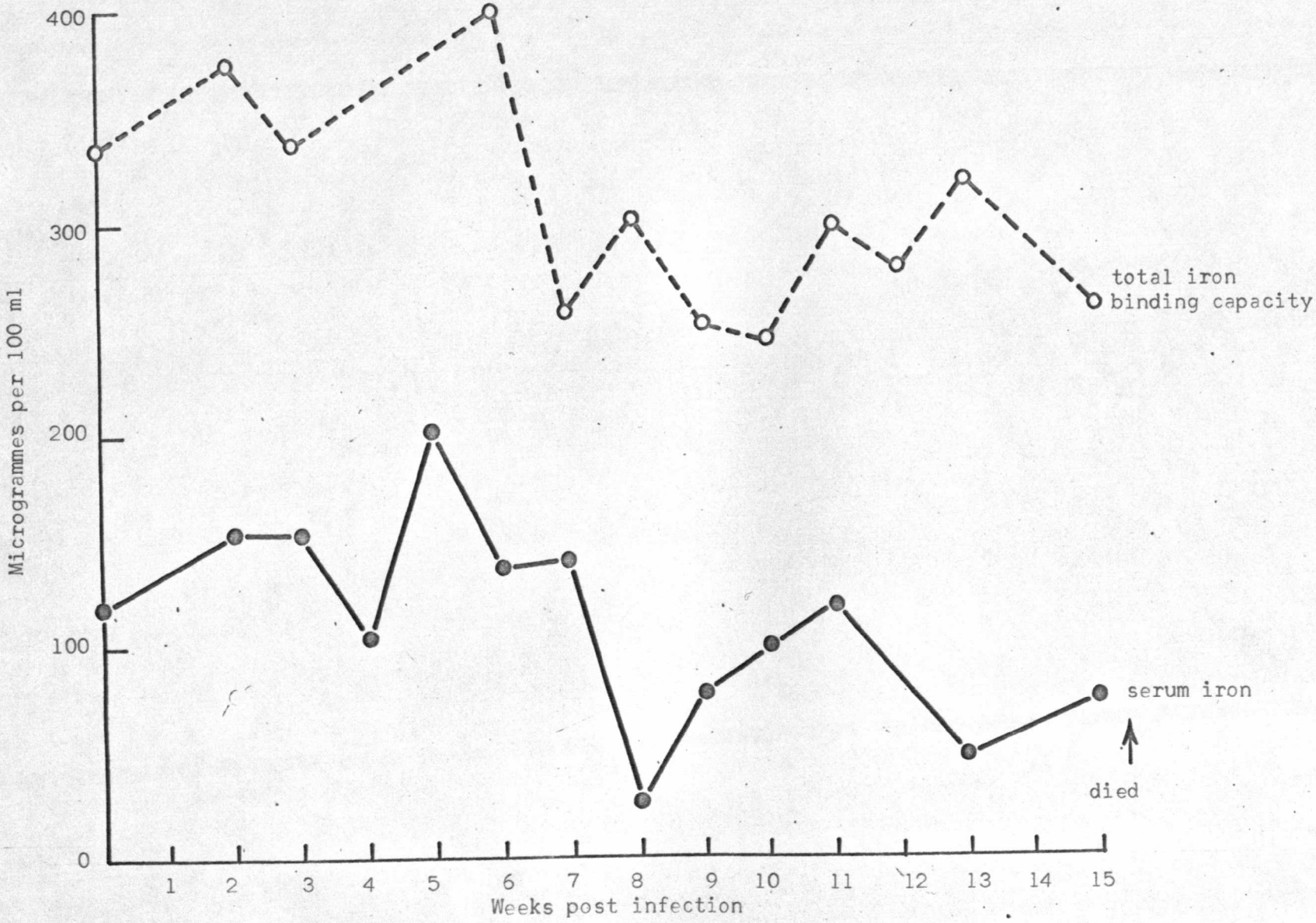


Fig. 4 Sequential changes in serum iron and total iron binding capacity in calf no.20 infected with T. congolense.

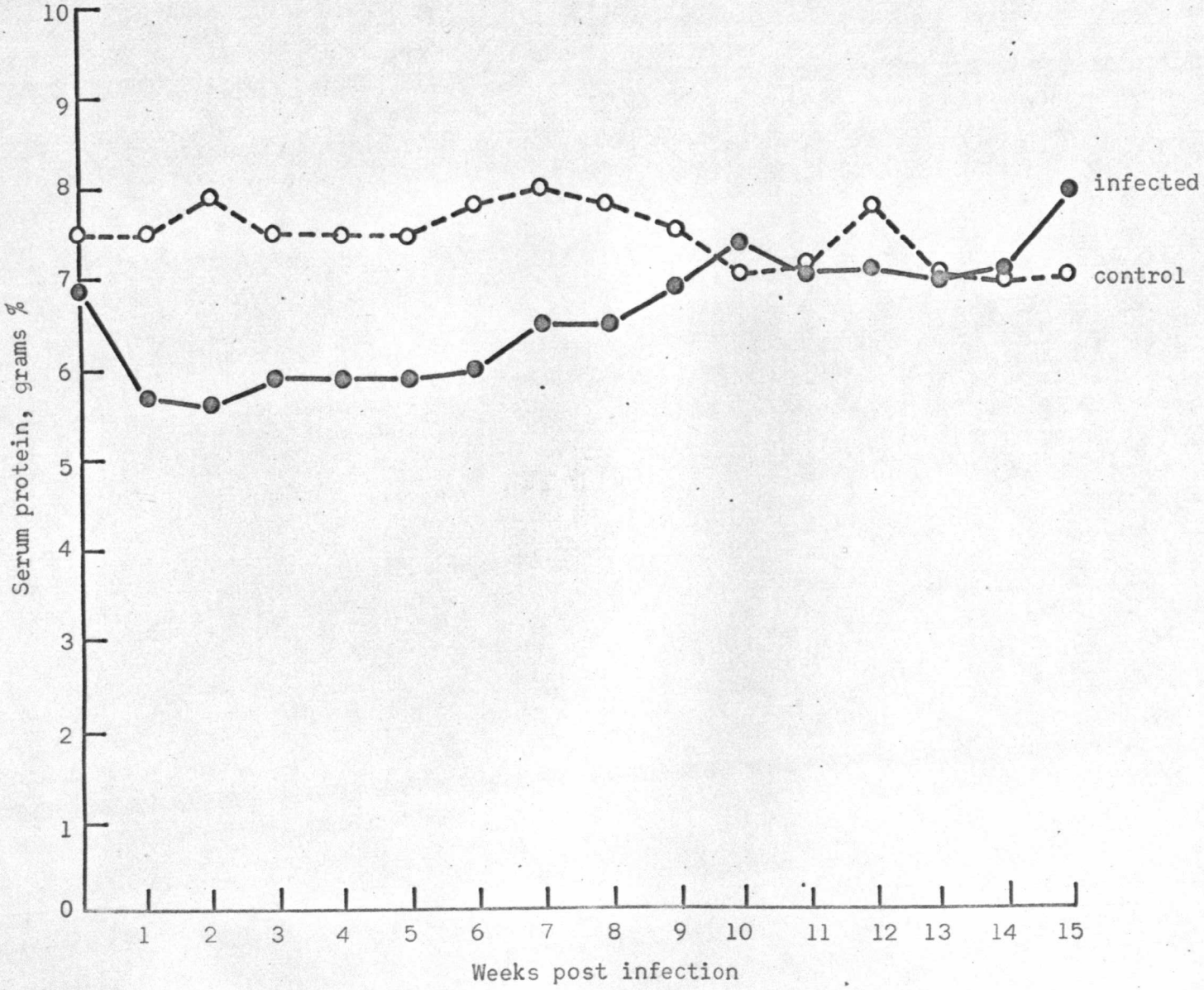


Fig. 5 Sequential changes in serum protein concentration of cattle infected with I. congolense.

Discussion

It is interesting that the "normal" haematological values for the number of calves examined were not significantly different from those of Ankole or Friesian cattle despite differences in breed, nutrition, altitude and other factors. Some of the factors that may affect the total red cell and haemogolbin concentration in the blood are age, severe exercise, dehydration, nutrition and altitude. Generally total red cell counts decline with age and the same pattern applies to the PCV and MCV (Greatorex, 1954). Severe exercise produces increased erythrocyte concentration in the blood circulation mainly through activation of the splenic reservoir pool to meet the higher demand by the body. Dehydration obviously results in haemoconcentration i.e. excessive numbers of erythrocytes per unit volume ofmblood.

Nutritional factors including the quality and the quantity of roughage feed, the quality and quantity of protein supplements and related factors such as adequate minerals and vitamins reflect directly or indirectly on the blood picture. Thomas et al (1954) observed that cattle given liberal amounts of milk, grain and alfalfa showed a higher haemoglobin level than those with limited milk and grain but unlimited alfalfa. It should be kept in mind that the comparative values given in table 1 are for animals maintained on pasture.

Altitude above sea level is normally considered to have a direct effect on the erythrocyte concentration (Best and Taylor, 1966). At higher altitudes there is decreased oxygen tension resulting in hypoxia the severity of which depends on the altitude. The state of hypoxia has a stimulatory effect of erythropoesis. It has been shown that the total circulating red blood cells are affected by altitude and that the blood volume remains normal (Miale, 1972).

It is not known what the distribution of fascioliasis and gastrointestinal parasites of cattle in Ethiopia are like, but personal observations have shown that the incidences are high. It has already been indicated that the calves purchased for trypanosome experiment purposes were mass treated for fascioliasis and gastrointestinal parasites a few weeks prior to use.

Fears of a physiological variation in the blood indices due to the altitude at Addis Ababa (over 2000 metres) and some of the other factors mentioned above have somewhat been allayed by the results shown in table 1.

Unsworth (1949) observed cyclical physiological changes in the haematological picture of normal cattle and showed periods of decreased counts and increased counts alternating without external influences. In addition to cyclical changes diurnal variations in the haematological picture are known to occur. Blood samples taken early in the morning before the animals began activities were shown to be higher than values taken later in the day and this was shown to be due to a physiological increase of circulating

blood volume during activity (Fiennes, 1970). In order to minimise such variations we took daily blood samples between 9 and 10am.

In trypanosome infected animals of the present experiment the anaemias observed fall into the normocytic normochromic class. Blood parasites generally are known to cause a normocytic normochromic anaemia which may later become macrocytic normochromic if bone marrow response is marked (Benjamin, 1961). Changes in the erythrocyte indices (MCV and MCHC) showed variations between individuals and did not conform to any specific pattern i.e. some of the infected calves showed the pattern described by Fiennes et al (1946) and Naylor (1971) and others did not. The above workers showed that the total red cell count fell for the first few weeks and then gradually rose to above normal values. The former investigator writes of "hyperchromic tendencies" of red cells in the early parts of the infection period. Both authors reported increased MCV during the initial stages of the infection. In our experimental animals although such a rise could be shown it was not statistically significant except in the final stages of acute infection.

Normocytic anaemias may be caused by haemolysis, haemorrhage, bone marrow hypoplasia or a combination of these. Macrocytosis is usually associated with increasing activity of the bone marrow or deficiencies of some haematopoetic factors such as vitamin B12 or cobalt. Vitamin B12 deficiency in ruminants does not occur as such. What may occur is cobalt deficiency which directly reflects

on the state of the vitamin B12 as cobalt forms part of the vitamin B12 molecule. Microcytosis in animals is usually associated with deficiencies of iron, copper or other factors concerned with haemopoesis.

The MCHC may be within the normal range (normochromic) or below the normal range (hypochromic). According to Benjamin (1961) and Miale (1972) there are no conditions in which the MCHC is increased above the normal range since the erythrocyte could not be supersaturated with haemoglobin. In the so-called hyperchromic anaemia there is an increase in the weight of haemoglobin in the average erythrocyte but the concentration of haemoglobin per unit volume is not increased.

The changes in total serum protein concentration showed a decline during the first few weeks and this finding was true for acute, subacute and chronic infections. In the chronic infections the serum protein concentration rose to approximately preinfection levels in the later stages. This finding is in good agreement with the findings of French (1938) who observed that there was a fall in total protein in cattle infected with T.congolense and T.brucei and that in the chronic infections there was an initial fall followed by a rise to above preinfection levels in the later stages of the disease. It has not been determined whether the elevation of serum protein concentration was relative or absolute. It has been shown that in the later stages of the disease there is dehydration and haemoconcentration (Fiennes (1954). Perhaps this

increase in total protein was indirectly the result of dehydration assuming that the same changes took place in our experimental animals. In the above mentioned experiment French showed that in acute infections the changes in total protein were greater than in chronic infections and that in T.brucei infections the changes were greater than in T.congolense infections.

Serum albumen concentrations in the present experiments were shown to fall steadily in the subacute infections more than in the chronic infections. There was a corresponding increase in the globulin fraction. These findings are also in agreement with the previous work of French in cattle infected with T.congolense.

French (1937; 1938a, b, c) also investigated other biochemical changes taking place in trypanosome infections. He showed increased excretion of body bases, chlorides and phosphates in the urine and faeces of cattle and sheep infected with T.congolense and T.brucei. He observed that lactic acid levels in the blood increased thus depleting the alkali reserve of the infected host. Hypoglycemia was reported in terminal cases and disturbances in carbohydrate metabolism were reported to be a common finding in moribund animals regardless of cause (Kronfield and Medway, 1969). Comperts (1969) showed that decreased levels of plasma inorganic phosphates resulted in depression of glycolysis due to limitations of glyceraldehyde 3 phosphate dehydrogenase reaction. The net effect is reduced metabolic activity of the red cell and subsequent disintegration.

Our findings of serum iron changes during chronic infection were different from those of Tartour and Idris (1973). Whereas we observed that in the terminal stages of infection plasma iron concentration and iron binding capacity were significantly reduced these workers reported an increase over preinfection levels at this stage. Iron from erythrocyte destruction is normally not lost but returned to the body pool. Haemosiderosis in trypanosome infections have been reported by various investigators. Perhaps a possible explanation for the fall in the serum iron as the disease progressed lies in the fact that there is chronic loss of blood (See Section IB). Excretion of blood breakdown products over a long period may result in a loss of the body iron stores (Miale, 1972).

None of these values should be interpreted separately as they are part of an interacting system. Haemoglobin is essential for the transport of oxygen and carbon dioxide and it also acts as a buffer. It is synthesized in the body from glycine and succinate and iron incorporated. Haemoglobin formation takes priority over plasma protein formation (Campbell *et al*, 1965). It is obvious that the continuous loss of haemoglobin demands a continuous supply of proteins for further synthesis. In chronically developing anaemia the haemoglobin level may drop to below 50% of minimum normal value without significant signs of anoxia developing (Schalm, 1965) unless the animal is exerted. Exertion due to long travel is a constant feature of nomadic life as practised by various groups in trypanosome areas of Ethiopia.

In conclusion the present work showed the development of normochromic normocytic anaemia in all groups of infected animals. Significant biochemical changes in chronic infections included a fall in serum iron and iron binding capacity, an initial fall in serum total protein which later on in the infection rose to preinfection levels, and a reversal in the albumen globulin ratio.

B. ISOTOPIC STUDIES ON THE ANAEMIA OF *TRYPANOSOMA CONGOLENSE* INFECTIONS IN CATTLE

1. ERYTHROCYTE SURVIVAL STUDIES IN CATTLE INFECTED WITH *T.CONGOLENSE* USING ^{51}CR LABELLED RED CELLS

2. FERROKINETIC STUDIES IN CATTLE INFECTED WITH *T.CONGOLENSE*

1. ERYTHROCYTE SURVIVAL STUDIES IN CATTLE INFECTED WITH T.CONGOLENSE USING ^{51}Cr LABELLED RED CELLS

Introduction

As discussed earlier the aetiology of the anaemia in trypanosomiasis is not well understood. In the chronic and subacute forms of the disease gross pathological examinations show bone marrow depression of the long bones characterized by gelatinous marrow as a result of the disappearance of the red bone marrow. The red marrow however does not disappear from all bones. It is retained in the bones of the sternum and the ribs (Fiennes, 1954) thus enabling the animal to continue production of blood cells. Gray (1970) reviewed the anaemia of trypanosomiasis.

Fiennes (1954) made two important observations on the aetiology of the anaemia in trypanosomiasis of cattle. A positive indirect van den Bergh test suggested that the infected animals became anaemic as a result of intravascular haemolysis, and he also noted that the erythrocytes of infected animals were more fragile than those from uninfected control animals. Murrey *et al* (1973) observed haemoglobinuria in rabbits infected with *Trypanosoma brucei* suggesting that the anaemia was of a haemolytic nature. Goodwin and Boreham (1966) observed an increased loss of integrity of the blood vessels in laboratory animals infected with *T.brucei*, and it is possible that the increased permeability may be of such magnitude as to allow loss of whole blood through damaged capillaries.

^{51}Cr is a very useful isotope in the study of erythrocyte survival as well as giving unique evidence on the site and manner of red cell loss. Erythrocytes may be tagged using this isotope. Sterling and Gray (1950) used this isotope to study blood volume, and since then different workers have used it in various experimental investigations involving red blood cells. Among its main advantages are that it is fairly firmly bound to erythrocytes and it is a gamma emitting isotope thus simplifying sample preparation and counting. The route of excretion of ^{51}Cr released from erythrocytes also provides useful information since ^{51}Cr released into the gut is not reabsorbed and appears quantitavely in the faeces (Roche *et al*, 1957) while ^{51}Cr released by elution or haemolysis appears in the urine and does not become bound to other erythrocytes. Erythrocytes tagged with ^{51}Cr have in the past been used successfully to study blood loss in various parasitic infections (Roche, 1957; Jennings, 1962; Pearson, 1963; Dargie, 1969; Holmes, 1969). In these studies homologous red blood cells were incubated with ^{51}Cr as sodium chromate *in vitro*. The tagged cells were then reinjected into the experimental animals from which they were taken. Several workers have demonstrated that labelling with less than 10 microgrammes per ml of red cells does not significantly alter the integrity of the erythrocytes (Ebaugh *et al*, 1953).

The mechanism of binding ^{51}Cr by the erythrocytes is not well understood. It is postulated that chromium in its hexavalent anionic form penetrates the erythrocyte

membrane and is reduced to the trivalent cationic form. The reduced cationic form is then firmly bound to the haemoglobin of the erythrocyte.

In the present experiments the kinetics of red blood cell loss was studied using homologous erythrocytes tagged with ^{51}Cr. These studies not only involved the rate of disappearance of ^{51}Cr from the circulatory system but its subsequent appearance in the faeces and urine were also studied. Two experiments were performed. In the first experiment 8 calves were used to study the changes in the advanced stages of subacute trypanosomiasis (12 to 16 weeks post infection). In the second experiment 10 calves were used to study the sequential changes from the time of infection until death.

EXPERIMENT I

Studies with ^{51}Cr Labelled Red Cells in Calves 12 to 16 Weeks Post Infection with T.congolense

Materials and methods

Male yearling Ethiopian zebu calves were purchased and maintained as described in general materials and methods.

Holding crates constructed from locally available timber with supporting straps were used to control movement of the animals. Plastic funnels fitted with rubber hoses were fixed over the prepuce of the calves so that the excreted urine could be collected in plastic buckets at the sides of the cages. The cages were elevated from the floor to allow faecal collection in aluminium dishes directly behind the calves. During defaecation the calves dropped their faeces directly into the dishes and were thus collected uncontaminated by urine.

The $Na^{51}CrO_4$ used in this experiment was obtained from the Radiochemical Centre (Amersham). Fifty ml of blood was taken from each calf into heparinized syringes and the haematocrit of each calf determined. The blood of each calf was then centrifuged in separate containers at 1500 r.p.m. and the plasma removed and retained. The erythrocytes were then washed twice in normal saline prior to labelling.

Labelling was performed by adding approximately 100 microcuries of ^{51}Cr per ml of red cells. The mixture

was mixed thoroughly and incubated in a water bath at 37°C for 30 minutes. Following incubation the cells were washed in normal saline until excess unbound ^{51}Cr was removed. The washed cells were then reconstituted in their own plasma and loaded into syringes. Approximately 2ml of each calf's labelled reconstituted blood was taken in appropriately labelled syringes for preparation of the standard.

All syringes were then weighed carefully on an analytical balance. The syringes for the preparation of the standards were emptied into 100ml volumetric flasks and the volume made up to 100ml with 0.02N NaOH. The labelled autologous cells were then injected into the calves from which they were taken. Injection was via an intravenous canula (Portex Ltd., U.K.) placed in the jugular vein. The time of injection was recorded for sampling purposes. All syringes were weighed after their contents were emptied to determine the net weight injected.

Fifteen minutes following the injection of labelled erythrocytes the first blood sample was taken in a heparinized 5ml evacuated tube from the opposite jugular. The second sample was taken at 4 hours and subsequent samples were taken at 24 hour intervals from the time of injection for approximately 2 weeks.

The pack cell volume of the blood was determined soon after each sampling. For radioassay one ml of well mixed whole blood was pipetted into counting vials and the volumes in the vials made up to 5ml with 0.02N NaOH.

The faeces of each calf was collected on a 24 hour basis. The total weight of the faeces was recorded and then manually stirred thoroughly with a wooden rod in a bucket. An aliquot of approximately 200 grams was then taken, placed in a weighed aluminium sheet and weighed. This weight represented the gross wet weight. The faeces was then dried in an oven and weighed. The weight of the dry faeces less the weight of the aluminium sheet represented the dry net weight. The total dry weight was determined as follows :

$$\text{Total dry weight} = \frac{\text{net dry weight}}{\text{net wet weight}} \times \text{total wet weight}$$

The dry faeces was then ground in a blender and a 2 gram sample placed in a counting tube. A total of 6 grams (3 tubes) was taken from each sample for assay of radioactivity.

Urine samples were collected on a 24 hour basis. The total urine volume was determined in a graduated cylinder and 5 ml samples pipetted into counting tubes for radioactivity assay.

All blood samples were assayed for radioactivity in an automatic well-type scintillation spectrometer (Packard Instruments). Calculations were made according to the following formulae :

1. Total injected activity (counts per min) =

$$\frac{\text{weight of suspension injected}}{\text{weight of suspension for standard}} \times \text{activity of standard in cpm/ml} \times \text{dilution of standard}$$

2. Activity per ml of red cells (c.p.m. per ml.) =

$$\text{Blood activity (c.p.m./ml)} \times \frac{100}{\text{PCV}}$$

3. Blood volume (ml) =

$$\frac{\text{Total activity injected (c.p.m.)}}{\text{Activity of 15 min blood sample (c.p.m./ml)}}$$

4. Circulating red cell volume (ml) =

$$\frac{\text{Total activity injected (c.p.m.)}}{\text{Activity \% red cells (c.p.m./ml)}}$$

5. Total faecal activity (c.p.m.) =

$$\frac{\text{Activity of the 2gm sample (c.p.m.)}}{2} \times \text{total dry weight(grams)}$$

6. Faecal blood clearance (ml per day) =

$$\frac{\text{Total daily faecal activity (c.p.m.)}}{\text{Activity of blood at the beginning of the 24 hour collection period (c.p.m./ml)}}$$

7. Faecal red cell clearance (ml per day) =

$$\frac{\text{Total daily faecal activity (c.p.m.)}}{\text{Activity of red cells at the beginning of the 24 hour collection period (c.p.m./ml)}}$$

8. Total faecal activity as % of total injected activity =

$$\frac{\text{Total faecal activity (c.p.m.)}}{\text{Total injected activity (c.p.m.)}} \times 100$$

9. Total urine activity (c.p.m.) =

$$\frac{\text{Activity of 5ml sample (c.p.m.)}}{5} \times \text{urine volume(ml)}$$

10. Total urine activity as a % of total injected activity =

$$\frac{\text{Total urine activity (c.p.m.)}}{\text{Total injected activity (c.p.m.)}} \times 100$$

The apparent half life of ^{51}Cr labelled red cells was obtained by plotting on semilogarithmic graph paper each value expressed as a percentage of the 15 minute activity. The time in which the activity of ^{51}Cr fell by 50% was calculated from the logarithmic part of the curve by regression analysis.

Results

The time (mean) taken for ^{51}Cr red cell activity to fall by 50% was 3.7 $\pm$ 0.7 days in the infected calves as compared to 6.1 $\pm$ 0.8 days in the control calves $P < 0.05$ (table 7). From this it could be shown that approximately 138ml of red cells were being lost from the circulation daily.

A mean daily faecal blood clearance ranging from 180ml to 480ml was recorded in the infected calves. The mean daily urinary red cell clearance in the infected calves excluding the elution period was approximately 46 $\pm$ 19ml daily (table 8).

An estimated mean daily loss of 25 ml of red cells was shown in the faeces per animal excluding the elution period (table 8).

The amount of ^{51}Cr recovered in the faeces of infected animals ranged from 0.3% to 1.5% of the total injected activity per day (table 8).

The fraction of total injected activity recovered in the urine ranged from 0.4% to 21% per day with the highest loss occurring in the first day following administration of isotope. By the fourth day after injection the mean activity recovered was 2.4 $\pm$ 0.1% of the total injected activity (table 8).

The higher figure for faecal clearance of ^{51}Cr was for calf no. 18 which died on the fifth day following administration of ^{51}Cr (17 weeks post infection).

Figures for faecal and urinary ^{51}Cr recovery in control animals are not included because of failure to achieve complete collection on a number of occasions due to the wildness of the animals.

Table 7

Blood volume, plasma volume and circulating red cell volume in cattle twelve weeks following infection with T.congolense, using ^{51}Cr labelled red cells.

	Animal Identification	Blood volume ml	Blood volume ml/Kg	Red Cell volume ml	Red Cell volume ml/Kg	^{51}Cr $T\frac{1}{2}$ (days) Blood	^{51}Cr $T\frac{1}{2}$ (days) Red cells
INFECTED	18	5038	56	1008	11	2.6	3.0
	19	4743	103	806	18	4.1	4.4
	20	6991	93	1258	18	2.9	3.7
	mean	5591	84	1024	16	3.2	3.7
	s.d.	1222	25	226	4	0.8	0.7
CONTROL	129	5506	88	1432	23	10.0	6.9
	132	7027	90	1749	22	5.0	5.3
	133	4361	61	1177	17	9.1	6.1
	mean	5230	80	1452	21	8.0	6.1
	s.d.	1338	16	287	7	2.7	0.8
"t" test		NS	NS	NS	NS	$P<0.01$	$P<0.05$

Table 8

Studies on faecal and urinary clearance of erythrocytes (packed red cells) following injection of ^{51}Cr labelled red cells in cattle infected with T.congolense (values are ± standard deviation from the mean)

Days	^{51}Cr Red Cell Clearance						recovered ^{51}Cr as % of total injected activity		
	urine		faeces		cumulative		faeces	urine	cumulative
	ml	ml/Kg	ml	ml/Kg	ml	ml/Kg			
1	225±93	3.2	61±51	0.9	287±144	4.1	1.5±1.3	20.7±8.2	21.7±9.4
2	110±16	1.6	65±25	0.9	175±41	2.5	1.0±0.3	8.9±0.9	9.9±1.2
3	54±27	0.8	54±8	0.8	108±35	1.5	0.7±0.3	3.7±0.4	4.4±0.7
4	87±47	1.2	43±1	0.6	130±48	1.9	0.9±0.4	2.4±0.1	3.3±0.5
5	35±4	0.5	32±2	0.5	67±6	0.9	0.5±0.1	1.7±0.3	2.1±0.4
6	38±23	0.5	32±22	0.5	70±45	1.0	0.4±0.3	1.9±1.5	2.3±1.8
7	44±3	0.6	17±4	0.2	61±7	0.8	0.4±0.1	0.7±0.3	1.1±0.4
8	60±19	0.8	22±3	0.3	82±22	1.3	0.4±0.3	0.7±0.3	1.1±0.4
9	55±2	0.8	13±1	0.2	68±3	1.0	0.3±0.1	0.4±0.1	0.7±0.2
mean days 5 -9	46±19	0.6	26±8	0.3	70±21	0.9	0.4±0.2	1.1±0.5	1.5±0.8

EXPERIMENT II

The use of ^{51}Cr labelled red cells in the study of the sequential changes in cattle after infection with T.congolense

Materials and methods

A group of 10 male zebu calves weighing 80 to 100 Kg were used. Five of these were infected and five kept as controls. On the day prior to infection each animal received 20ml of its own red cells labelled with approximately 1 mCi of ^{51}Cr. This procedure was repeated at 3 weekly intervals during the course of the infection. All analyses performed in this experiment were performed as described in experiment I.

Results

A significant and progressive fall in pack cell volume was observed during the course of the disease. Associated with a fall in haematocrit there was a marked and progressive drop in the ^{51}Cr apparent half life of all infected calves (table 9). The fall in apparent half life values in the infected calves became evident early in the infection i.e. 10 days post infection.

Changes were also detected in the red cell and plasma volumes during the course of the disease (the plasma volumes were obtained from the ^{59}Fe injection as described in the later section on ferrokinetic studies). In the infected calves there was a 43% increase in the plasma

volume compared to only an 8% increase in the control animals. This increase in the plasma volume is not entirely due to the fall in the red cell volume because the total blood volume also increased by 21% in the infected calves as compared to only 6% in the controls (table 9).

Table 9

^{51}Cr labelled Red Cell Survival Studies in Calves infected with *T.congolense* and in Normal Controls (5 per group).

Weeks Post Infection		Cr-rbc $T_{\frac{1}{2}}$ (hr)	Change in PCV (%)	Blood Vol. (ml)	Rbc Volume (ml)
0 - 3	Inf.	181	-8	4710	1245
	Con.	287	-4	5496	1308
3 - 6	Inf.	127	-4	5682	868
	Con.	227	+2	5271	1113
6 - 8	Inf.	108	-4	5730	751
	Con.	226	+2	5848	1277

Discussion of ^{51}Cr experiments I and II

The disappearance rate of ^{51}Cr from the circulation of the infected calves in both experiments was significantly greater than that in the controls, the most likely explanation being that a large volume of blood was being lost per unit of time in the infected calves over and above normal red cell losses. Erythrocyte survival studies are essential in the evaluation of the aetiology of the anaemia. Before isotopes were introduced into pathophysiological studies Ashby's (1919) differential agglutination method used to be the only valid method of measuring erythrocyte survival. This method in addition to its inherent technical difficulties, cannot be used to study the life span of autologous red cells. Since the introduction of ^{51}Cr as a red cell label it has been widely used for the estimation of erythrocyte survival although it too has the drawback of elution, i.e. the constant leak of isotope from labelled erythrocytes. This is much greater in some species than others and is particularly marked in cattle. In addition to information on the survival of erythrocytes, the site and manner of loss can also be determined by radioactivity determinations on the faeces and urine.

In experiment I measurement of activity recovered shows a loss of radioactivity in the faeces and urine. It should be remembered that this experiment was carried out 12 to 16 weeks after infection. For the first four to five days following intravenous administration of ^{51}Cr labelled red cells most of the recovered ^{51}Cr activity appeared in

the urine. This corresponds to the large elution of isotope which occurs in the first few days in cattle following the injection of ^{51}Cr labelled red cells. After the fifth day the activities recovered were not significantly different between the two routes.

A very interesting finding in experiment I is the discrepancy between the activity of ^{51}Cr lost daily from the circulation and that recovered in the faeces and urine. The results show that an equivalent of 138ml of red cells were being lost from the circulation and radioactivity corresponding to only approximately 70ml of this appeared in the faeces and urine. That is, the activity recovered was only about 51% of the activity estimated to disappear from the circulation.

Similar findings on retention of ^{51}Cr were recorded by Jennings *et al* (1974) using whole body counting and blood sampling techniques in *T.brucei* infected mice. They showed that the apparent whole body half life of ^{51}Cr was significantly more extended than that of the blood activity. The apparent half life of circulating ^{51}Cr in control and *T.brucei* mice were 8.3 ± 3.2 days and 1.5 ± 0.9 days respectively and 31 days and 24 days respectively on a whole body count basis. This finding corresponds to a mean disappearance ratio of 1 : 5.5 (control : infected) in the circulation and 1 : 1.3 in the whole body. More discussion on this topic is presented in the section on the pathological findings as it is postulated to bear relevance to excessive erythrophagocytosis shown by histopathological studies of our experimentally infected cattle.

Fiennes (1954) demonstrated positive indirect van den Bergh reaction in cattle infected with T.congolense during the terminal stages of the disease. Murray et al (1973) observed haemoglobinuria in rats infected with T.brucei. The present experiment partly lends further support to the above findings of intravascular haemolysis. On the other hand the appearance in the faeces of ^{51}Cr activity corresponding to approximately 37% of the excreted activity from day five on in experiment I suggests a loss of erythrocytes via the gastrointestinal tract also though the mechanism of this loss is not understood.

The clearance rate for calf no. 18 was interesting in that the calf was in its terminal stages and the results are markedly different from the other infected calves. The faecal loss of erythrocytes in this calf was increasing daily from an equivalent of 25ml in the first day to approximately 141ml on the fourth day following the administration of isotope. Urine clearance during the same period did not show this pattern. This calf died on the fifth day of the isotopic experiment. While it may be unjustifiable to present evidence on the basis of one calf it is interesting to note that in the terminal stages of T.congolense infection of calves blood loss via the gastrointestinal tract may be an important factor. The cause of the increased loss of ^{51}Cr labelled red cells from the circulation of the infected calves in both experiments is most probably due to excessive erythrophagocytosis by the reticuloendothelial system and haemolysis, though the ^{51}Cr results give no precise information on the mechanisms

of destruction. Many workers have suggested that intravascular haemolysis is the primary factor in the aetiology of the anaemia though this has not been fully substantiated.

Various factors including trypanosome toxin, mechanical damage by trypanosomes, and increased red cell fragility have been suggested as possible causes of intravascular haemolysis. Fiennes (1970) suggested that the important factor both in the haemolytic stage of the disease and in the chronic, is the presence of dead and not living trypanosomes in the blood circulation. In the chronic stages of the disease living trypanosomes are apparently protected against attack by the body's defence system. These are continuously replenishing the animal's system with daughter trypanosomes which are destroyed, thereby imposing a continuous drain on the body's defences and contributing to capillary dilatation and increased permeability. Jubb and Kennedy (1970) observed that there is not enough evidence available to support the trypanosome toxin theory. A slight increase in osmotic fragility of red cells of infected animals has been observed by Naylor (1971a).

In vivo haemolytic mechanisms have been classified on the basis of alterations in the integrity of the red cell membrane (Weed and Reed, 1966) as follows: (1) fragmentation of erythrocytes due to extrinsic damage (eg. uremia), intrinsic damage (eg. iron deficiency, antibody damage) or phagocytic damage; (2) changes in the permeability of the red cells which may consist of increased cation permeability leading to colloid osmotic lysis or macromolecular

permeability leading to direct haemoglobin loss; (3) phagocytosis of damaged cells. There is evidence to show that red cell survival declines considerably in disease conditions where the capacity for the metabolism of glucose by red cells is reduced (Tanaka et al, 1962; Schneider et al, 1965; Harris and Pressman, 1967) and thus predisposing the cells to phagocytosis.

Zuckerman (1964) reviewed autoimmune response in trypanosome infections and presented two related concepts on the subject. The first is the reduction in charge of trypanosomes when coated with immune serum. Thus negatively charged trypanosomes may bear a positive charge following coating by immune serum (Brown and Broom, 1935; Broom, 1936). This reduction in charge is supposed to be responsible for the adhesion phenomenon observed between trypanosomes and the formed elements of the blood. Adhesion of trypanosomes to red cells is thought to be more common than to other formed elements of the blood. The above works showed that not only the charge of trypanosomes but also the charge of erythrocytes was reduced thus allowing both the trypanosomes and erythrocytes to be phagocytized by negatively charged phagocytes. Weitz (1960) demonstrated soluble trypanosome antigen in rats infected with T.brucei. This antigen could be adsorbed onto erythrocytes contributing further to erythrophagocytosis.

The second concept is that of autoimmune reactions developing as a result of the production of immunoconglutinins. This is an anticomplementary antibody which brings about the aggregation of particulate antigen-antibody complexes

(Coombs, 1953). The titer of immunoconglutinin was shown to be directly proportional to the severity of infection and it was shown to wane when the disease is overcome (Soltys, 1959).

Mackenzie and Cruickshank (1973) demonstrated erythrophagocytosis in sheep infected with T.congolense and suggested this factor to be the prime cause of the anaemia. They pointed out that possible causes of erythrophagocytosis may include the coating of red cells with parasitic antigen, the production of defective cells and autoimmune reactions.

In observing the sequential changes at different stages of the disease it was shown that the apparent half life of ^{51}Cr labelled erythrocytes became increasingly shorter as the disease progressed (experiment II). The life span of erythrocytes was thus shown to be markedly reduced and that the relative reduction in life span was greater with the advancing disease. The absolute circulating red cell volume fell by approximately 31% at six weeks and 22% at eight weeks post infection. This increasing gap between blood volume and red cell volume represented an expanding plasma volume. An increase in the plasma volume of 31% at six weeks and 43% at eight weeks over and above the preinfection value was observed (see section I.B.2). If the blood volume were constant these values of plasma volume are 11% and 21% greater respectively than if the plasma volume increase was only due to a corresponding decrease in circulating red cell volume. Clarkson (1968) and Naylor (1971) reported increased plasma

volumes in animals infected with T.vivax and T.congolense, as high as 30% in the latter. Roberts (1973) observed a 25% increase in plasma volume at the height of the infection and noted decreased serum lipid concentration. A number of workers have reported a fall in plasma protein concentration associated with trypanosome infections. Roberts pointed out that part of the fall in the plasma solids recorded is attributable to the dilution effects of increased plasma volume and part due to the effect of the trypanosome on the metabolism of the host. It is important that due allowance must be made for this dilution effect when considering the changes which occur in plasma constituents in trypanosomiasis. Wraight (1974) showed that in rats plasma volume increased in the face of decreased intravascular protein concentration and that there was a significant negative correlation between changes in capillary permeability and plasma volume. He suggested further investigations into hormonal control and also the existence of receptors sensitive to colloid osmotic pressure which are active in the control of plasma volume.

SUMMARY

Eighteen male zebu yearling calves were used to study the anaemia of T.congolense infection using ^{51}Cr labelled red blood cells. In the first experiment red cell survival studies were performed in the advanced stages of the disease. In the second experiment the sequential changes in red cell survival from the time of infection to death were studied.

In the first experiment it was shown that the apparent in vivo half life of ^{51}Cr labelled red cells in the infected calves was significantly shorter ($T\frac{1}{2}$ = 3.7 ± 0.7 days) than in the control calves ($T\frac{1}{2}$ = 6.1 ± 0.8 days). In one infected calf in its terminal stages the amount of ^{51}Cr recovered in the faeces increased significantly and progressively until the time of death suggesting the possibility that there may be significant blood loss via the gastrointestinal tract in the terminal stages of T.congolense infections. It was also shown that in all the infected calves the mean daily loss of ^{51}Cr from the circulation was significantly greater than the cumulative activity recovered.

In the study of the sequential changes it was shown that the fall in apparent half life became significant as early as 10 days post infection and continued to fall progressively. The total blood volume in the infected calves increased by 21% as compared to 6% in the controls and the plasma volume in the infected calves increased by 43% as compared to a mean of 8% in the controls.

From the findings it was concluded that severe loss of erythrocytes from the circulation complicated by a state of hydraemia was mainly responsible for the anaemia developing in T.congolense infections of cattle

2. Ferrokinetic Studies In Cattle Infected with T.congolense

a. Subacute infections

Experiment I

Studies at 12 to 16 weeks post infection using ^{59}Fe.

Experiment II

Sequential changes from the time of infection to death using ^{59}Fe.

Experiment III

Iron absorption studies at 12 weeks post infection using simultaneous ^{55}Fe and ^{59}Fe.

b. Acute infection

Studies on spenectomized and intact calves using ^{59}Fe.

a. Ferrokinetic Studies on Subacute Bovine Trypanosomiasis

Introduction

The two experiments described earlier in this chapter clearly demonstrated that a large loss of red cells occurs from the circulation of infected calves. It was postulated that the loss of red cells is of sufficient magnitude as to be the primary cause of the anaemia. However, the use of ^{51}Cr labelled cells gave no direct evidence of the rate of erythropoesis in these animals. The present studies using ^{59}Fe were undertaken to investigate whether dyshaemopoesis is an important complicating factor in the aetiology of the anaemia.

^{59}Fe (ferric citrate) has been used in studies involving the kinetics of blood iron by Hahn et al (1943), Huff et al (1950 and Bothwell et al (1957). More recently Dargie (1969) and Holmes (1969) used ^{59}Fe in their studies on the aetiology of the anaemia in fascioliasis of sheep and laboratory animals. Jennings et al (1973) used this isotope in their study of the aetiology of the anaemia in laboratory animals infected with T.brucei. ^{59}Fe has a physical half life of 45 days and decays with gamma and beta emission.

Plasma iron, although a very small fraction of the body iron, plays a major role in erythropoesis. In man approximately 3 milligrams out of a total body iron of 4 grams is bound primarily to the beta globulin, transferrin.

This represents transport iron and plays a vital role in the synthesis of haemoglobin. The introduction of trace amounts of ^{59}Fe into the blood stream leads to its binding to transferrin (Huff et al, 1956). The rate at which ^{59}Fe is transported from the plasma can then be used in the assessment of the quantity of iron transported provided that the serum iron level per unit volume of blood is known, and along with the rate at which ^{59}Fe reappears in the erythrocytes, provides a useful index of the measurement of erythrocytic activity of the bone marrow.

Sources of plasma iron include intestinal absorption, physiological breakdown of erythrocytes, and body stores. Serum iron turnover is the most important indicator of erythrocytic activity. Bothwell et al (1957) studied serum turnover in human patients under various pathological conditions and found that serum iron turnover is affected primarily by the state of the erythroid marrow.

Measurement of iron absorption from the gastrointestinal tract is an essential part of ferrokinetics. There are essentially three methods of measuring iron absorption. The faecal recovery method first described by Dubach et al (1948) employed oral administration of ^{59}Fe and subsequent faecal collection until ^{59}Fe excretion is at a minimum. This method is very laborious and complete collection of faeces could be difficult. The whole body counting method described by Price et al (1961) and van Hoeke and Conrad (1961) also performed by administering ^{59}Fe orally, has many drawbacks although it is simpler to perform. The double isotope method described by Saylor

and Finch (1953) was used in the present experiment. In this method specific amounts of intravenous ^{59}Fe and oral ^{55}Fe are administered simultaneously and after a suitable interval of time the ratio of radioactivity of the two isotopes in the blood measured. The two isotopes can be measured separately because of their different energies of radiation. Comparing the percent utilization (maximum incorporation into erythrocytes) of the intravenously injected isotope to that of the orally administered isotope it is possible to calculate the absorption of the oral dose.

The aetiology factors on the anaemia of trypanosomiasis have already been discussed in the introductory section of the ^{51}Cr experiment. Among the factors that are believed to be the causes of the anaemia are depression of bone marrow resulting in decreased production, intravascular haemolysis and erythrophagocytosis (Boycot and Price, 1913).

Ferrokinetic studies in bovine trypanosomiasis are lacking in the literature. Our studies seek to explain some of the factors associated with the anaemia in cattle affected with subacute T.congolense. In these studies ^{59}Fe has been used as a label to study the kinetics of plasma iron. Three experiments are described. In the first experiment 8 calves were used to study the pathophysiological changes in advanced trypanosomiasis 12 to 16 weeks post infection. In the second experiment 10 calves were used to study the sequential changes taking place from the time of infection to death. In the third experiment absorption studies were performed using simultaneous administration of ^{59}Fe intravenously and ^{55}Fe orally.

Experiment I : Ferrokinetic Studies In Cattle infected with T.congolense 12 to 16 Weeks previously.

Materials and Methods :

The animals used in this study were the same as those used in experiment I of the previous section (^{51}Cr red cell survival studies).

^{59}Fe was obtained from the Radiochemical Centre (Amersham, U.K.). This was diluted in a solution containing 10mg sodium citrate and 6mg sodium chloride per ml. The diluted isotopic solution was loaded into syringes and weighed carefully on an analytical balance. The content of each syringe was then injected intravenously into the corresponding calf using an intravenous cannula (Portex Ltd.) placed in the jugular vein and rinsed down with normal saline. The empty syringes were then weighed carefully to determine the net activity injected. For the standard, approximately 2ml of the isotopic solution was loaded into a syringe, weighed and emptied into a 100ml volumetric flask. The flask was filled to the 100 mark with the above mentioned diluent. The empty syringe was weighed to determine the net weight in the standard. Fifteen minutes following the injection of isotope the first blood sample was taken in a heparinized tube from the opposite jugular vein. Subsequent samples were taken at 30, 60, 90, 120, 150 and 180 minutes from the time of injection. All the blood samples collected during the three hours post injection were centrifuged at 1500 rpm for ten minutes and one ml of plasma was pipetted into counting tubes for

subsequent radioactivity determination. Subsequent one ml mixed blood samples were taken at 24 hour intervals during the course of the experiment. Each blood sample was made up to 5ml volume with 0.02N NaOH. One ml of the standard was treated in the same manner as the other samples for radioactivity assay. The measurement of activity of the standard was used for correction of decay, variations in counting of the scintillation spectrometer, and calculation of injected activity.

Measurement of radioactivity was performed in a well-type automatic scintillation spectrometer (Packard Instruments) and calculation of results was performed according to the following formulae:

$$\text{Injected activity (c.p.m.)} = \text{Wt. injected} \times \frac{\text{Activity of St (cpm/ml)}}{\text{Wt of St.}} \times \text{dilution of standard}$$

$$\text{Plasma volume (ml)} = \frac{\text{Injected activity (c.p.m.)}}{\text{Sample activity extrapolated to "0" time (cpm/ml)}}$$

$$\text{Daily serum iron turnover (}\mu\text{g/24 hours} = \frac{\text{Serum iron (}\mu\text{g/100ml)}}{T\frac{1}{2}\text{ (min)}} \times 0.693 \times 1440 \times \frac{100 - \text{PCV}}{100}$$

where $T\frac{1}{2}$ = plasma ^{59}Fe half life

0.693 = natural log of 2

1440 = number of minutes in 24 hours

The "apparent half life" of plasma ($T\frac{1}{2}$) was calculated by regression analysis. The results were presented by plotting the activities as a percent of the 15 minute sample on semilogarithmic graph paper.

Results

There was a significant level of anaemia in all the infected calves with a depression of the pack cell volume, haemoglobin and total red cell count. There was not a significant difference in the blood volumes of the infected and control groups. The circulating red blood cell volume in the infected calves was less than that of the controls while the plasma volume in the infected calves was slightly greater (table 10).

The mean "apparent half life" in the infected calves was 76 $\pm$ 8 minutes as compared to 132 $\pm$ 23 minutes in the controls ($p = 0.02$). The daily plasma iron turnover in the infected calves was 1388µg/100ml of plasma (approximately 13.9mg/litre) as compared to a mean of 783µg/100ml of plasma (approximately 7.8mg/litre) in the control calves (table 10).

The maximum iron incorporation into the erythrocytes of infected calves was 94% as compared to 82% in the controls.

Experiment II : The use of ^{59}Fe in the Study of the Sequential Changes in Ferrokinetics in Calves Infected with Subacute T.congolense.

Materials and Methods:

Two groups of calves were used. The first group of calves were the same as those described in Experiment II of the ^{51}Cr experiment. On the day prior to the infection of five of the ten calves each animal was given approximately 100 microcuries of ^{59}Fe (ferric citrate). This procedure was repeated at three-weekly intervals during the course of the infection. Daily blood samples were taken throughout the study period.

A separate group of 8 calves were used in a similar study to investigate plasma iron turnover rates at 0, 3, and 5 weeks post infection in seven calves infected with T.congolense and one control calf. In these animals blood samples were only taken on the day of the plasma iron turnover study.

Haematological and biochemical methods and calculation of results were performed as described in experiment I.

Results

In the regularly bled groups, ten days after infection live trypanosomes could be detected in the jugular blood of all the infected animals and they remained positive

until death. The last infected calf died 8 weeks after infection. All the infected calves developed a severe anaemia and a further fall in the haematocrit was usually associated with impending death. The results of the haematological findings confirmed the presence of a marked progressive normochromic normocytic anaemia in the infected calves. The serum iron levels and iron binding capacity remained similar in both groups. The"apparent halflife" of plasma ^{59}Fe fell steadily with a corresponding steady increase in plasma iron turnover rate. In the control group a similar picture in plasma iron turnover rate was observed (table 11).

In the second group of calves where the calves were bled only three times during the study period, i.e. at 0, 3 and 5 weeks post infection a similar picture was obtained in pack cell volume and plasma iron concentration. The "apparent half life" of plasma iron (^{59}Fe) increased at three weeks but fell to approximately pre-infection levels at 5 weeks in the infected group (table 12). In the control calf there was an increase in the haematocrit value and a corresponding fall in plasma iron turnover rate.

Table 10

Plasma iron turnover in cattle infected with T.congolense

	Animal Identification	PCV	Plasma ^{59}Fe $T\frac{1}{2}$ (minutes)	Serum Iron µg%	Plasma iron turnover µg%/day	Plasma volume ml
INFECTED	12	17	77	124	1334	3609
	37	17	78	187	1136	3937
	18	15	64	109	1445	4030
	20	17	83	160	1635	5733
	mean	16.3	75.5	145.0	1387.5	4327
	s.d.	1.0	8.1	35.2	208.7	1121
CONTROL	131	28	105	147	1186	4076
	132	34	122	120	762	5278
	133	29	161	128	563	3184
	129	26	131	110	620	4074
	mean	29.3	130.3	126.3	782.8	4153
	s.d.	3.4	22.9	15.7	281.6	1034
"t" test		$P < 0.01$	$P < 0.02$	NS	$P < 0.05$	NS

Table 11

^{59}Fe Studies in Calves infected with T.congolense and in Normal Controls (5 per group)

Week Post Inf.	Calves	PCV (%)	Fe (µg %)	^{59}Fe T½ (hr)	PITR (mg/day)	PITR (mg/lit/day)
0	Inf.	30	90	2.4	21.4	6.3
	Con.	28	122	2.3	36.4	8.8
3	Inf.	17	89	2.1	33.7	7.3
	Con.	25	119	2.3	37.5	9.1
6	Inf.	16	95	1.2	68.7	13.8
	Con.	24	99	1.2	61.1	13.4

Table 12

^{59}Fe Studies in Calves Infected with T.congolense and in Normal Controls

Week Post Inf.	Calves	PCV (%)	Fe (µg %)	Fe $T_{\frac{1}{2}}$ (hr)	PITR (mg/day)	PITR (mg/lit/day)	Body Wt. (kg)
0	Inf (7)	32	100	1.6	47.0	10.9	83
	Con (1)	27	100	1.7	63.1	9.8	100
3	Inf (6)	24	114	2.2	46.6	9.0	87
	Con (1)	29	80	1.8	33.7	7.4	106
5	Inf (4)	15	110	1.8	50.8	10.7	95
	Con (1)	30	84	2.3	28.3	6.2	115

Experiment III : Iron absorption studies in calves infected with subacute T.congolense using ^{55}Fe and ^{59}Fe (ferric citrate).

Materials and Methods

Six male zebu calves were used in this experiment of which two were infected with T.congolense and four kept as controls. Each calf was given 25 microcuries of ^{55}Fe orally and approximately 100 microcuries of ^{59}Fe intravenously. Daily blood samples were taken for assay of radioactivity for 30 days.

For assay of radioactivity one ml of blood was treated with one ml of 0.02N NaOH in a counting tube to lyse the red cells. To this was added 10 ml Instagel scintillator (Packard Instruments) and mixed thoroughly. To the mixture was added 2 ml of hydrogen peroxide drop by drop and excessive foaming was controlled by the addition of one ml ethanol drop by drop. The samples were then left in the counting vials for two days for maximum bleaching to take place prior to measurement of radioactivity.

The samples were assayed for radioactivity in a liquid scintillation counter (Packard Instruments) using the automatic external standard for correction of quenching. The resultant counts were plotted as a percentage of the highest activity.

Results

Following simultaneous administration of ^{55}Fe orally and ^{59}Fe intravaneously there was a marked difference in the pattern of utilization between the infected and control calves. The highest blood activity in the infected calves was attained much earlier ie 5 to 7 days post administration of isotope whilst in the control animals maximum activity was not reached until 17 to 19 days. After the maximum radioactivity has been attained in the red cells there was a marked difference in the circulating red cell activity of the two groups (fig 6). In the infected calves the activities of both isotopes fell below 25% of the peak by 13 days post administration. Calf 12 died on the thirteenth day and studies on calf no. 37 were continued up to 30 days. In this calf activities fell below 5% at 24 days and maintained at this level up to the thirtieth day, the last day of this experiment. In the control group the radioactivities were maintained at a level above 50% for the duration of the experiment.

At the beginning of the experiment the haematocrit of infected calf no. 12 was 12% and this gradually fell down to 9% on the ninth day when it died of the trypanosome infection. The haematocrit of calf no. 37 was between 17 and 20% throughout the 30 day experiment.

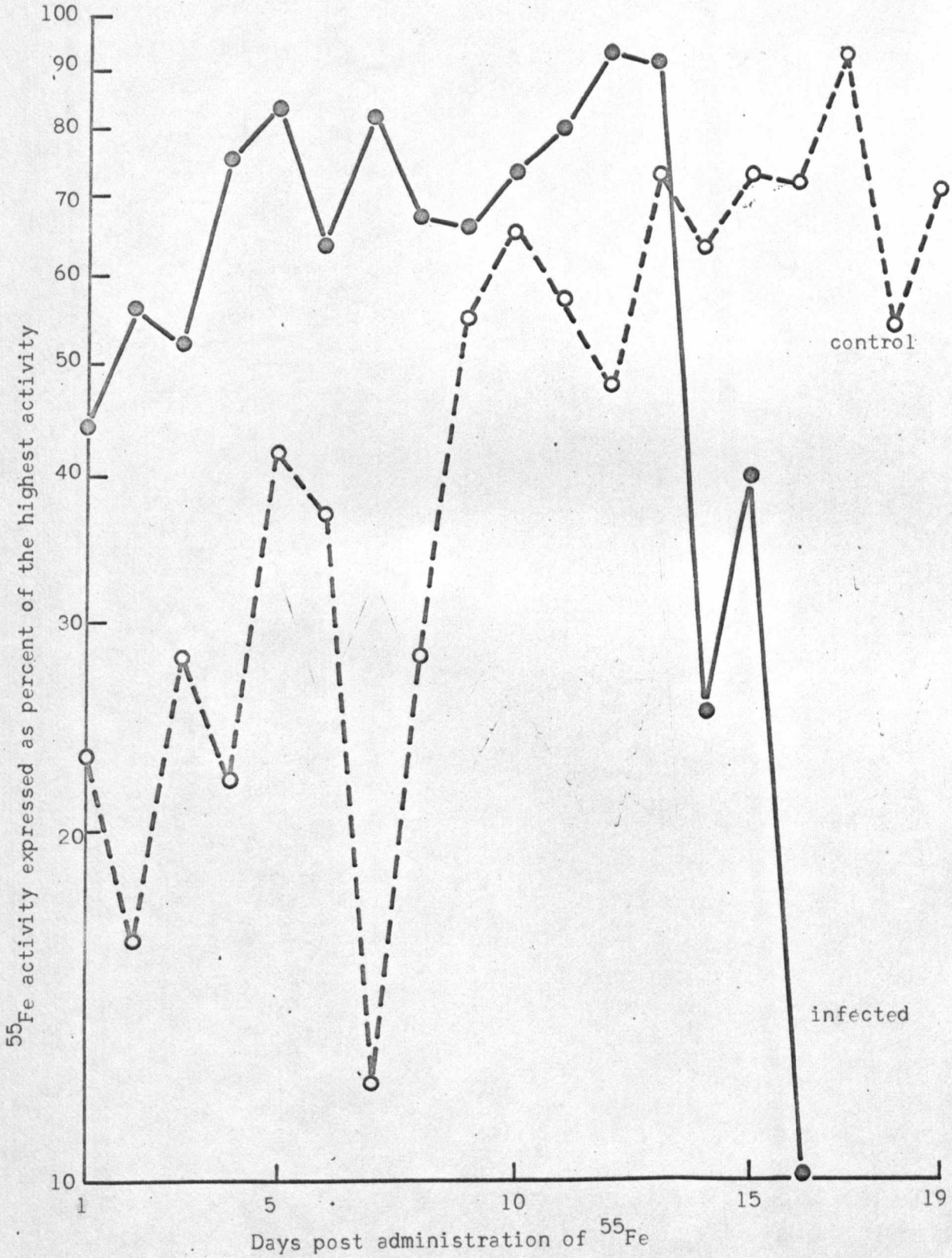


Fig. 6 ^{55}Fe absorption in calves infected with I. congolense and in normal controls.

Discussion (Ferrokinetic experiments I, II, III).

Plasma iron turnover is known to be accelerated in haemolytic anaemias, iron deficiency anaemia, malignancies, polycythemia and leukemia. On the other hand a decelerated plasma iron turnover is generally associated with hypoplastic conditions of the bone marrow. It should be kept in mind that plasma iron turnover is a complex function involving a number of body iron pools. The method used in experiments I and II (Huff et al, 1950) has a number of drawbacks but is simpler to perform and considered adequate for clinical purposes (Finch et al, 1970). It assumes that plasma iron is a homogeneous pool in a steady state. Keeping in mind that non-erythroid turnover of iron is usually a minor fraction of that used in haemoglobin synthesis, the method still maintains its very valuable position as an indicator or erythroid marrow activity. The other criticism against this system is that although it indicates increased turnover of iron it does not differentiate between erythroid and non-erythroid turnover, neither does it distinguish between marrow activity that results in viable red cells and that which does not. Therefore it is possible to find increased incorporation ^{59}Fe into haemoglobin of red cells and the cells not be effective in carrying out their normal functions.

Finch et al (1970) indicated that in haemolytic anaemias the continuous turnover of radioiron through the erythrocyte-reticuloendothelial cell circuit results in a uniform specific activity and utilization will be depressed

in proportion to the ^{59}Fe held in the bone marrow and reticuloendothelial cells.

To sum up, the internal exchange of iron is concerned with haemoglobin iron, iron stored in the reticuloendothelial system and iron stored in the parenchyma of the liver. Upon sequestration of erythrocytes iron split from haemoglobin from the RE cells is either returned to the cell membrane to be taken up by transferrin or incorporated into ferritin and haemosiderin stores. Most of stored iron is not exchangeable and only a minute quantity is exchangeable with the iron from the newly sequestered erythrocytes. Transferrin iron cannot be taken by RE cells and is therefore stored in the hepacytes of the liver. Approximately a third of the body's iron stores are in the muscles (excluding myoglobin) and this iron is normally not measurable by the usual ferrokinetic measurements (Finch et al, 1970).

Radioiron disappearance from the plasma is considered to reflect the turnover of transferrin iron since unbound iron is cleared too rapidly to significantly affect measurement. With anaemia and in the presence of adequate amounts of iron, plasma iron turnover is significantly increased (Crosby and Shelby, 1960; Harker and Finch, 1969).

In trypanosome infections increased deposition of iron in tissues especially in the spleen and the liver has been reported (Murray et al, 1973). Reutilization of storage iron is not as rapid as transferrin iron and reutilization of storage iron decreases proportionately

with an increase in the size of storage iron. From the above discussion it is clear that ferrokinetics is not a simple transfer mechanism but a complex function involving many interdependent factors.

The results of experiments I clearly show that there is a greater level of bone marrow activity in the infected calves than in the control calves. This activity is reflected in an increased plasma iron turnover rate and subsequent increased haemoglobin synthesis rate as a result of increased incorporation of iron.

Anaemia basically may be caused by either a decreased production or an increased loss or both. In this case the shorter red cell life span from the ^{51}Cr experiment shed light on the basic cause of the anaemia as being increased loss. Though the rate of synthesis of haemoglobin and incorporation into red cells is increased the pack cell volume continued to fall indicating the rate of loss is greater than the rate of production. The daily synthesis of haemoglobin and incorporation into red cells, although increased, does not appear to have increased enough to offset the rate of loss.

Fiennes (1954) reported a state of hydraemia in T.congolense infected cattle using the specific gravity of plasma as a basis. Clarkson (1968) and Naylor (1971) used the dye T1824 (Evans blue) to measure the plasma volume of sheep infected with T.vivax and cattle infected with T.congolense respectively and reported an increase in plasma volume of up to 25%. In the present experiment plasma volume was estimated using ferric citrate (^{59}Fe).

An increased plasma volume was indicated, however the observed increase over that of the control calves in experiment I was not significant at 12 to 16 weeks post infection. On the other hand the sequential studies showed that significant increases in plasma volumes of infected animals do occur and these results indicate that such increments do not take place throughout the infection period.

Increased capillary permeability in trypanosome infected animals has been reported by Holdstock et al (1957). This permeability has been attributed to pharmacologically active peptides produced in infected animals thus allowing plasma solids and possibly even red cells (depending in the degree of damage) to leak out of the intravascular space (Goodwin and Richards, 1960; Boreham and Goodwin, 1967; Boreham, 1968). Boreham (1966) showed that kinin production was associated with pathogenicity and that in rats infected with the non-pathogenic T.lewisi release of kinin was not observed even with heavy parasitaemia. It thus might well be that plasma solids escape into the extravascular space and they should also have contributed to an osmotic gradient movement of water into the extravascular space to establish an equilibrium.

Serum iron turnover was significantly greater in the infected calves. Naylor(1971) noted increased bone marrow activity starting at around 14 weeks post infection in cattle infected with T.congolense. However he failed to show any appreciable increase in reticulocyte count or any other red blood cell precursors in the peripheral

circulation although an increased white blood cell production was demonstrated. This finding is not in agreement with those of Fiennes (1954) where increasing numbers of reticulocytes in peripheral blood smears were reported in infected cattle.

In experiments I and II using plasma iron turnover as an index, it was shown that overall erythropoetic activity was greater in the infected calves than in the controls. This is no doubt principally due to increased marrow activity though extramedullary erythropoesis in the liver of cattle infected with T.congolense has been reported by Naylor (1971).

The mean haemoglobin concentration in the infected calves (experiment I) was considerably lower than in the control calves, on the other hand the mean daily haemoglobin synthesis in the infected calves was more than double that in the controls. Using plasma ^{59}Fe turnover rate as a measure, the red cell lifespan in the infected calves was significantly shorter than in the controls. As mentioned above it thus appears that the anaemia in subacute and chronic T.congolense infections of cattle is due to increased loss and not due to decreased production.

The sequential studies of experiment II in this section supported the findings in experiment I and showed that during the course of the infection a severe anaemia developed and that this was reflected in a decreased red cell survival time, indicating that a significant loss of red cells is the primary factor in the aetiology of the anaemia. This increased loss of erythrocytes began very early in the

course of the infection and continued until death.

The results of experiment II tended to show that though erythropoesis was increased in the infected animals during the course of the infection, it was increased in the control animals subjected to the same bleeding of 5 to 7ml per day during the 54 day experiment period. It would appear then that erythropoesis, though increased in the infected animals, is not stimulated to a higher degree than in the control animals which have been bled as frequently as the infected animals. The reason for this may be that erythropoesis in both groups has been stimulated probably to a maximum and a further increase in erythropoesis is probably not possible despite the massive loss of red cells in the infected group. In this respect it is interesting to note that in other studies on the anaemia associated with chronic ovine fascioliasis it was similarly found despite a severe anaemia in infected sheep, erythropoesis could only apparently be increased by approximately three-fold (Holmes, 1969). With regard to the present experiment it should also be borne in mind that both groups of calves were in only moderate condition at the start of the study, that they only received a maintainance ration and that the haematocrit values were relatively low at the beginning of the experiment. The condition of the animals and their haematocrit values were typical of Ethiopian highland cattle at the end of the dry season when this experiment was conducted. This probably explains why erythropoesis was increased in the control calves despite the much lower blood loss (from daily bleeding) compared to the infected calves.

Many factors influence the rate of absorption of iron from the gut, the major one being the rate of erythropoesis and the magnitude of the body stores. Generally increased body stores have been shown to be associated with decreased absorption while in iron deficiency absorption was shown to increase (Bothwell et al, 1958). Increased erythropoesis is associated with increasing absorption and decreased erythropoesis with decreased absorption. Iron deficiency is rare in ruminants unless they were maintained on pastures deficient in iron, cobalt or copper. Iron deficiency of pastures is not common and therefore a straightforward iron deficiency in ruminants does not commonly occur though copper and cobalt deficiency are not uncommon. . Unlike most monogastric animals ruminal microflora in ruminants can synthesize vitamin B_{12} in large quantities which is then stored in the liver and used as needed. Vitamin B_{12} is an essential vitamin in the absorption of iron and cobalt is an essential constituent of the vitamin B_{12} molecule. Copper is an essential catalyst in the incorporation of iron into the haemoglobin molecule.

The absorption of iron is not a simple process of diffusion. Ingested ferric iron is first changed to the ferrous state by the action of hydrochloric acid and the ferrous form is bound to apoferritin within the intestinal cells. It is transported to the plasma in the ferric state by the plasma protein, ferritin (transferrin). Plasma iron saturation or unsaturation was shown to have no effect on the rate of absorption (Weintraub et al, 1965). Excessive loss of iron is probably the major cause of increased absorption in ruminants.

In bovine trypanosomiasis where massive losses of erythrocytes are known to occur due to intravascular haemolysis, increased body stores in the form of haemosiderin deposits in various tissues have been demonstrated. The effect of excessive deposits of haemosiderin on absorption and utilization of iron in the ruminants has however not been described.

Transferrin iron is not exchangeable with iron in the haemoglobin of mature erythrocytes though direct uptake and utilization by circulating reticulocytes can occur (Tingueley and Loeffler, 1956). Once bound to the reticulocyte membrane the iron is no longer exchangeable. The occurrence of significant levels of reticulocytosis in cattle infected with T.congolense has been a subject of controversy. Reticulocytes are rare in normal cattle blood (Schlam, 1965).

In experiments I and II of this section it has been shown that there was an increased plasma iron turnover and incorporation into haemoglobin. Many investigators have shown that iron absorption is mainly controlled by plasma iron turnover rate which in turn is controlled mainly by the rate of erythropoesis. The results of experiment III demonstrated that the time of ^{55}Fe appearance and levels of maximum activity in the infected calves were reached much earlier than in the control calves, and this was most probably brought about by increased plasma iron turnover rate.

Summary

Thirty-two calves were used in the study of various aspects of ferrokinetics in yearling male Ethiopian highland calves infected with subacute T.congolense. The study was in three sections. In the first section ferrokinetic studies were performed using ^{59}Fe in calves in the advanced stages of the disease. In the second experiment ^{59}Fe was used in the study of the sequential changes in ferrokinetics of infected calves from the time of infection to death. In the third experiment ^{55}Fe and ^{59}Fe were used simultaneously to study the iron absorption status of infected animals.

An increased production of red cells was shown by increased plasma iron turnover rates and subsequent higher incorporation into red cells of the infected calves than in the controls. The sequential studies showed that an increased plasma iron turnover rate and subsequent incorporation into erythrocytes started early in the infection and continued throughout the experimental period. Bleeding non-infected control calves (7 ml per day) was also shown to increase plasma iron turnover rate. In the iron absorption studies blood activities of ^{55}Fe and ^{59}Fe rose to peak much earlier in the infected calves than in the control calves.

From the present study it was concluded that in trypanosome infected cattle erythropoesis is probably maximally increased with no evidence of dyshaemopoesis and impairment of iron absorption. The findings in the present experiments are fully consistent with the results of the experiments using ^{51}Cr described in the previous section, that

a significant and constant loss of erythrocytes from the circulation is the principal cause of the anaemia.

b. FERROKINETIC STUDIES ON ACUTE TRYPANOSOMIASIS IN SPLENECTOMIZED AND INTACT CATTLE INFECTED WITH *T. CONGOLENSE*

Introduction

In the previous sections using ^{51}Cr and ^{59}Fe it was demonstrated that the anaemia in sub-acute trypanosomiasis of cattle was due to a loss of excessive quantities of erythrocytes from the circulation and not due to dyshaemopoesis. It is widely recognized that the spleen enlarged in size during trypanosome infections and the degree of enlargement in the early stages was shown to be in direct relation to the degree of anaemia (Ormerod, 1970). Following trypanosome infections phagocytic activity in the spleen was shown to be considerably increased, and it was suggested that the anaemia in trypanosomiasis was due to haemolysis of the erythrocytes in the sinusoids of the spleen (Delanoe, 1912; Jenkins et al, 1973a).

Erythrophagocytosis by RE cells has been reported as an important factor in the loss of red cells from the circulation (Boycot and Price, 1913; Zuckerman, 1964; Murray et al, 1973). In cattle the spleen normally functions in the sequestration of aged and abnormal erythrocytes, in the storage of normal red cells, and in body defence. Stored red cells from the spleen are immediately released in the general circulation at the time of increased demand by the body.

The role of the spleen in body defence mechanisms is well established. Desowitz and Watson (1953) observed that rabbits developed a greater intensity of parasitaemia and enhanced pathogenic effects from infections of T.simiae

following splenectomy. Jenkins et al (1973a) observed that splenectomized rabbits infected with T.brucei were less anaemic during the infection than non-splenectomized rabbits infected with the same strain although the splenectomized animals succumbed to the "toxic effects" of the trypanosomes more readily than the intact rabbits. Red blood cells from splenectomized rats have been shown to be more resistant to chemical destruction than those from non splenctomized rats (Chanutin, 1953).

As mentioned above splenomegaly is associated with acute trypanosomiasis. Prior (1967) using ^{51}Cr labelled autologous red cells demonstrated that 3 to 38% of the red cell mass may be pooled in the spleen depending on the degree of enlargement. Cells in the splenic pool equilibriate only slowly with those in the circulation and an appreciable part of the marrow output of red cells may be unavailable for physiological function. Generally the severity of anaemia under this condition is in direct correlation to the splenic enlargement. The pooling of cells in the spleen may create repeatedly stagnant conditions in close proximity to an actively multiplying macrophage population and predispose the red cells to phagocytosis. In the absence of the spleen such conditions can be created in the liver though probably not to the same magnitude. The spleen is one of the important iron storage organs of the body. The role of the spleen in the kinetics of iron is not fully understood. It has been shown that in the dog the spleen exercised an inhibitory action on the bone marrow and following splenectomy the bone marrow was shown to release large numbers of reticulocytes (Lorber, 1958).

Information on acute trypanosomiasis in large animals is very scanty, most of the observations having been made on laboratory animals. The strains of T.congolense are differentiated mainly on their mean size and pathogenicity. Generally it has been shown that there is a direct relationship between size and inefectivity of the congolense group of trypanosomes. The main difference between acute and chronic infections in the clinical course of the disease and associated tissue changes. In the chronic infections the clinical course may run for many months and anaemia and tissue degeneration occur quite characteristically. In acute infections on the other hand early and violent response of the host is observed even prior to the appearance of parasites in the peripheral blood smear and anaemia may or may not develop.

It has been suggested that in acute trypanosomiasis suppression of the bone marrow seems to play a more important role in the development of anaemias than excessive red cell loss and tissue destruction observed in subacute and chronic infections (Jubb and Kennedy, 1970). Goodwin and Guy (1973) on the other hand suggested that in T.brucei infections of rabbits renal failure probably due to allergic reactions contributed to the cause of death. This is in agreement with Fienne's (1954) previous findings in acute T.congolense and T.vivax infections of cattle in which vascular dilation and generalized anaphyactic manifestations were shown and death was attributed to anaphylaxis.

In the present experiment ^{59}Fe was used in

ferrokinecic investigations on splenectomized and intact cattle infected with an acute strain of T.congolense.

Materials and Methods

An acute strain of T.congolense (Gambella II) was isolated from cattle in western Ethiopia and maintained in mice.

Nine yearling male zebu calves were used in this experiment. Three of them (group A) were sedated with xylazine hydrochloride (Rompun, Bayer). Paravertebral nerve block was performed on T_{13}, L_1, and L_2 with xylocaine and splenectomy performed through an incision approximately one inch caudal to the last rib in the paralumbar fossa. The spleen was carefully separated from the surrounding connective tissue and the artery ligated with nylon suture. Adrenaline was injected into the spleen to eject as much blood as possible, the vein was then ligated and the whole spleen carefully severed from its vessels and removed. The calves were up and eating within two hours after the operation. The calves were given tetracycline therapy for one week and maintained on a concentrate supplement for two weeks after the splenectomy. A rise in temperature was recorded on the second day after the operation and this fell back to normal on the third and successive days. Following splenectomy there was a small but study increase in the pack cell volumes.

The calves were given a five week recovery period before using them for the infection experiment. At the time of infection the haematocrit values were above presplenectomy values and the temperatures of the calves were normal.

The splenectomized calves and three intact calves (group B) were infected intravenously with 3200 trypanosomes

on the mouse ID_{63} scale. Three normal calves (groupC) were kept as controls. Two days after infection approximately 100 microcuries of ferric citrate (^{59}Fe) was injected intravenously into each of the calves (groups A, B and C). The isotopic and subsequent methods used were the same as those described previously in the ferrokinetic studies of subacute infections.

Results

Because of small numbers in each group and early death of infected calves the conclusions drawn from the results must be treated as tentative.

A significant difference in plasma iron clearance rate between the infected groups of calves (A.& B) and the controls(group C) was shown. The mean "apparent half life" of plasma ^{59}Fe was 63 ± 11 minutes in group C as compared to 105 ± 29 minutes in group A and 93 ± 17 minutes in group B (fig 7). The difference in plasma ^{59}Fe "apparent half life" between the splenectomized calves (group A) and the intact infected calves (group B) was not significant. Plasma iron turnover values in these groups were 1262 µg/kg in group C, 593 µg/kg in group A and 463 µg/kg in group B.

Mean red cell activities in group A was reached at 5 days post administration as compared to 9 days in groups B and C. In the splenectomized group (A) mean red cell activity showed a sharp fall starting at approximately 7 days post administration of isotope (fig 8) and this corresponded with the period of the fall in haematocrit

values of the calves.

During the first 6 days of infection no significant difference in haematological values between the infected groups (A and B) and the normal controls (group C) was observed with the exception of increased MCV in group A. In group B parasites were detected in calf no. 168 at 6 days and in the other two calves at 8 days post infection. There was no significant difference in the MCHC and from the values obtained it was concluded that the anaemia produced was of normocytic normochromic nature.

In group A two calves died prior to the development of parasitaemia in the peripheral blood smear (calf no. 132 at 4 days and no. 102 at 8 days post infection). The third calf (no. 169) developed parasitaemia on the seventh day and died on the eleventh day after infection. By three weeks post infection all infected calves (groups A and B) had died from the infection.

There was no significant difference in the blood, plasma and red cell volumes between the infected calves (groups A and B) and the control calves (group C). Table 13.

Fig. 7 Plasma ^{59}Fe turnover in cattle infected with acute T.congolense (three animals per group)

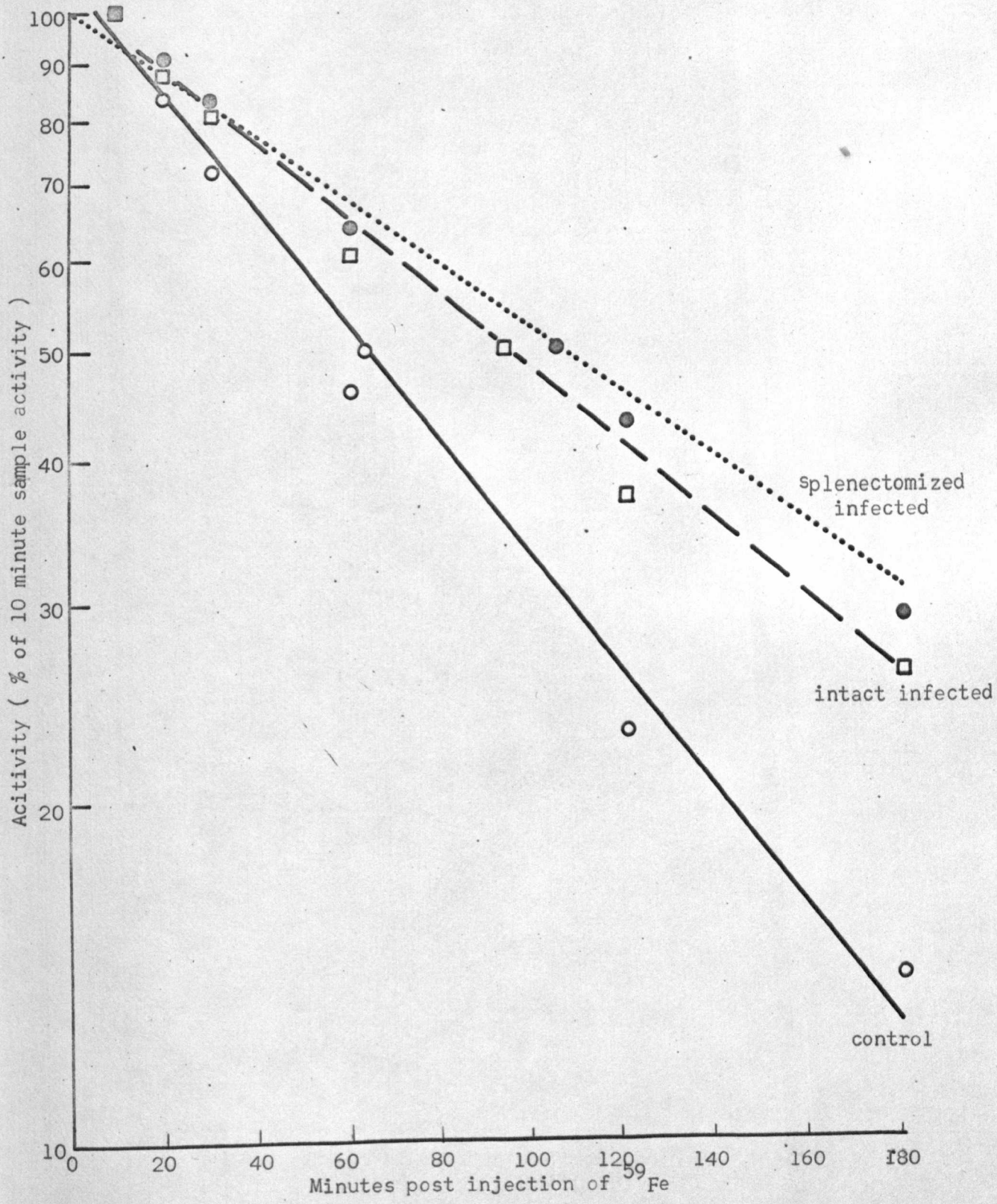


Fig. 8 Incorporation of ^{59}Fe into erythrocytes of cattle infected with an acute strain of T.congolense.

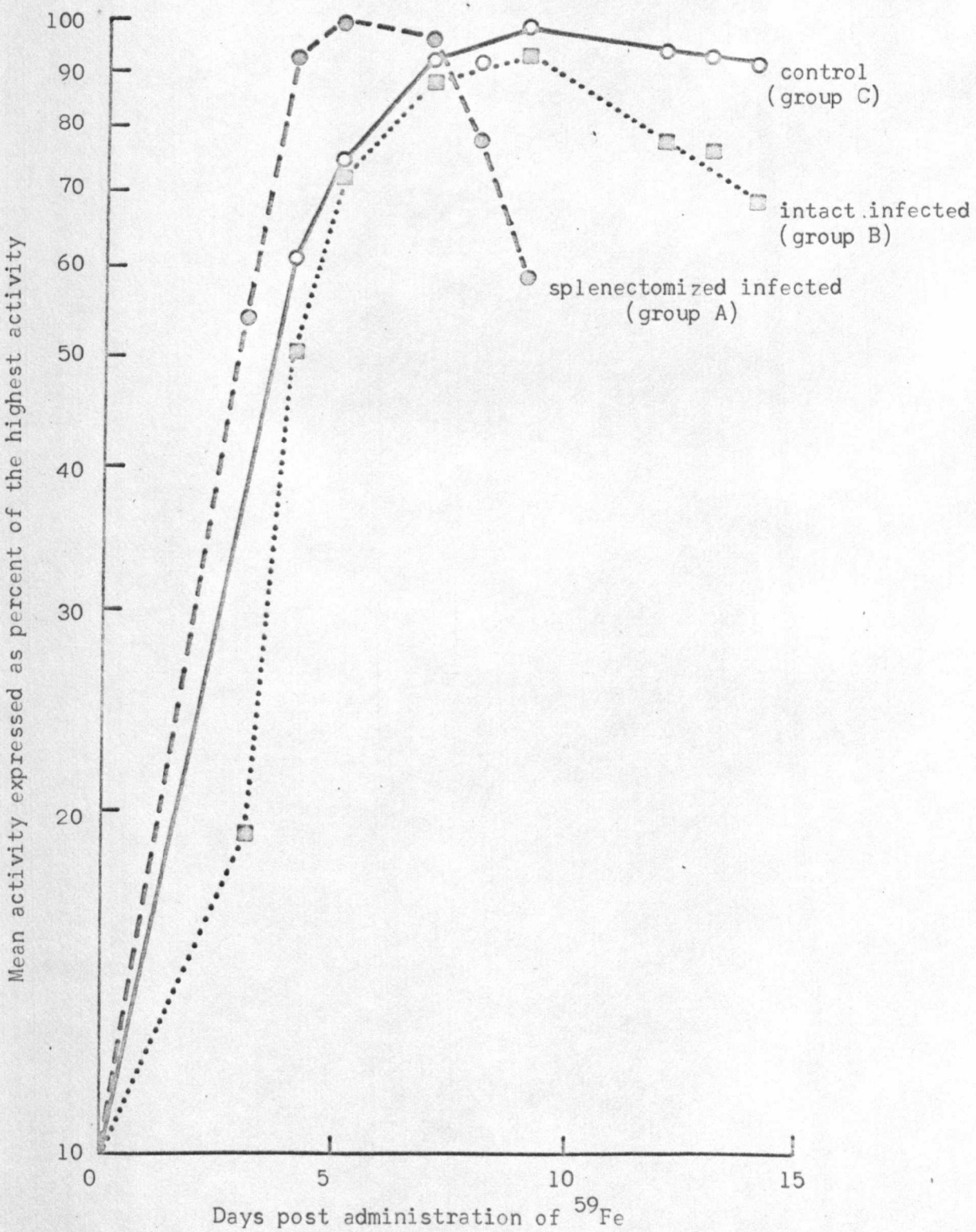


Table 13

Blood volume, plasma volume and circulating red cell volume in splenectomized calves (group A) and intact calves (group B) infected with an acute strain of T.congolense and in normal control calves (group C) The "t" tests shown were performed between group A and C, and B and C.

Group		Blood volume		Plasma volume		Red Cell Volume		PITR
		ml	ml/kg	ml	ml/kg	ml	ml/kg	µg/kg
A	mean	6188	84.0	4448	60.7	1740	20.3	593
	s.d.	1862	11.4	1095	5.1	89	4.7	370
C	mean	7377	85.0	5750	66.7	1623	19.0	1262
	s.d.	1213	7.8	708	4.5	52	4.6	240
"t" test		ns	ns	ns	ns	ns	ns	$P < 0.05$
B	mean	6386	75.3	4705	55.7	1680	19.7	463
	s.d.	70	7.6	22	6.4	53	4.5	177
"t" test		ns	ns	ns	ns	ns	ns	$P < 0.01$

Discussion

The reduced plasma iron (^{59}Fe) disappearance rates in the infected calves of both groups A and B as compared to the control calves (group C) is an interesting finding as it is the reverse of the finding in subacute and chronic in which the rate of disappearance was shown to be more rapid in the infected animals. The same delay in the reappearance of ^{59}Fe in the circulation following incorporation into haemoglobin did not occur. This probably suggests that the erythropoetic capacity of the bone marrow was not adversely affected but that there was some interference in the transport of plasma iron to the haemopoetic tissues. With the evidence available one can offer no better than speculative explanation and such explanation must take into account the surprisingly early occurrence of a change in plasma iron turnover (ie 2 days post infection).

It is perhaps relevant that Fiennes (1954) observed capillary dilatation in cattle infected with acute T.congolense and associated widespread haemorrhage which he ascribed to the capillary dilatation. Richards (1965) showed that in acute T.brucei infections of mice pharmacologically active peptides increased in the blood, urine and tissues. The results of his work showed that during the first three days of infection histamine activity increased progressively even prior to the appearance of parasitaemia and then fell off on the fifth day although still above normal levels. Kinin activity increased on the second day of infection and maintained above normal levels

throughout his experimental period. Kinin extracts administered to rats caused a prolonged fall of arterial pressure. He also observed a progressive significant fall in the plasma kinin precursor, kininogen and this was in agreement with the previous findings of Goodwin and Richards (1960) in mice infected with Babesia rhohaini, and Tella and Magraith (1962) in monkeys infected with Plasmodium knowlesi. Goodwin and Guy (1973; 1973a) suggested that in T.brucei infections of rabbits renal failure probably due to allergic reactions contributed to the cause of death.

In view of these findings it is perhaps justifiable to give a short discussion on the pharmacological effects of kinins on the circulatory system as related to the present experiment. Kallidin and bradykinin are among the most powerful vasodilators known (Breazile, 1971). They are produced within the circulatory system by a reaction of plasma proteins with foreign proteins. Mammalian kininogens, the percursors of kinins, are acidic glycoproteins which in their native states are highly specific substrates for the kallikreins. Webster and Price (1963) and Pierce (1968) showed that plasma kallikrein enzymes release bradykinin from kininogen.

Perhaps the most significant action of the kinins is the dilatation of blood vessels thus lowering the systemic blood pressure and the peripheral resistance through arteriolar dilatation depending on the species and interaction with other vasoactive agents that may be liberated by the host pathologically or physiologically (Trauschold, 1970).

Boreham and Facer (1973; 1973a) showed that in rabbits infected with T.brucei plasma fibrinogen, and fibrin and fibronogen breakdown products increased 3 to 4 fold in the initial stages of the disease although this fell off to below preinfection levels later. They suggested that this might contribute to the erythrocyte sedimentation rate elevation known to occur in trypanosomiasis, thereby changing the flow properties of the blood leading to stasis in the capillary beds.

Boulton et al (1973) observed that during infections of rabbits with T.brucei the plasma factor XII activity increased by more than six-fold and suggested that disseminated intravascular coagulation played an important role in causing haemolysis. Plasma factor XII (Hageman factor) is an initiator of both fibrin formation for coagulation and plasma kinin formation (Eisen and Voigt, 1970). The response of smooth muscle to bradykinin was shown to be increased by breakdown products of fibrinogen (Gladner et al, 1963).

Plasma iron turnover can be affected by the rate of blood flow to the bone marrow (Finch et al, 1970). From the above discussion it was concluded that the retarded plasma iron disappearance rate in the infected calves (groups A and B) could be due to factors contributing to vasodilatation and other factors interfering with capillary blood flow. It appears that the central effect might be the increased plasma factor XII production which is an initiator of both kinin and fibrin formation. The kinins create vasodilatation and decreased flow. The increased levels of plasma fibrin and

fibrinogen and their breakdown products contribute to elevated erythrocyte sedimentation rate and increased blood viscosity. The viscosity of the blood determines the resistance of the blood flow in the capillaries to a much greater degree than in the larger vessels, and in instances in which the blood viscosity is increased, flow of blood within the capillaries may be arrested creating sludging of blood, and finally resulting in cessation of exchange between the capillaries and interstitial fluid (Breazile, 1971).

Incorporation of ^{59}Fe into the erythrocyte and subsequent reappearance in the circulation was apparently more rapid in the splenectomized calves (group A) than in groups B and C. Lorber (1958) showed that the spleen exercised an inhibitory effect on the bone marrow in dogs and that following splenectomy significant increase in blood reticulocyte counts occurred. Similar observations in blockage of iron release from RE cells during inflammation have been made by Freireich et al (1957), Kampschmidt and Arrendo (1963), Hourani et al (1965) and Quastel and Ross (1966).

Splenectomized calves (132 and 102) died prior to the appearance of parasites in the peripheral circulation. This finding is in agreement with the reports of other workers mentioned above. Among the normal functions of the spleen are storage of erythrocytes, sequestration of aged and abnormal erythrocytes and body defence. Spenectomized calves are known to succumb to certain protozoan diseases such as eperythrozoonosis and anaplasmosis

to which they are normally resistant. Participation of the spleen in body defence mechanisms has also been demonstrated by passive transfer of spleen cell mediated immunity from immune hosts in certain protozoan diseases such as plasmodia (Phillips, 1969; Stechschulte, 1969). Wissler (1963) observed that splenectomy suppressed the appearance of circulating antibody in rats.

All infected calves (groups A and B) had died in less than four weeks after infection and death was possibly due to anaphylactic and associated events described above.

Summary

Ferrokinetic studies were performed on nine male zebu calves, six of them infected with an acute strain of T.congolense. Three of the infected calves had been splenectomized approximately five weeks prior to the beginning of ferrokinetic studies. ^{59}Fe was administered intravenously two days after infection with trypanosomes.

The results showed that early in the infection plasma ^{59}Fe disappearance was significantly delayed in the infected calves (groups A and B) over the control group (C). There was no significant difference in subsequent ^{59}Fe utilization between groups A, B and C though apparently faster in the splenectomized calves. It was concluded that the initial delay in plasma iron turnover was possibly due to the effect of biologically active substances such as kinins that are liberated early in the infection.

SECTION II

IMMUNOLOGICAL ASPECTS OF *T.CONGOLENSE* INFECTIONS IN CATTLE

A. STUDIES ON ACTIVE IMMUNIZATION OF CATTLE USING RADIATION-ATTENUATED ORGANISMS

B. IMMUNOSUPPRESSION OF CATTLE INFECTED WITH *T.CONGOLENSE*

A. STUDIES ON ACTIVE IMMUNIZATION OF CATTLE USING RADIATION-ATTENUATED T.CONGOLENSE

Introduction

A major drawback to understanding the immune mechanisms in trypanosomiasis has been and continues to be the complexity and seemingly unlimited variation of the trypanosome antigens. The response of host is equally complex and not well understood. Trypanosome antigens are of two types. The stable antigen is common to all variants of a species and even to some of the other species. The variant antigens mark the identity of the different populations of trypanosomes within a given species, which develop in succession in the same infected host. The capability of a given salivarian trypanosome to produce antigenically different populations is almost unlimited (Gray, 1965).

The artificial induction of immunity to salivarian trypanosomes appears to depend on the variant antigen and there is no cross protection against heterologous antigenic types. This fact tends to discourage attempts at active immunization though Soltys (1964) pointed out that although a chemotherapeutic armamentarium has become available to combat trypanosomiasis more effectively than ever before, the appearance of drug resistance strains and the relatively great administrative difficulties in giving the drug to large numbers of animals at frequent intervals have made it virtually impossible to control the disease by

chemotherapeutic means. This is especially true in dealing with the nomadic tribes. Therefore the possibility of immunization to control the disease would be most welcome, since by this method other infections have been controlled without necessitating drastic changes in the socio-environmental order.

Gray (1967) and Weitz (1970) reviewed some of the principles on the immunology of trypanosomiasis. Immunity to trypanosomiasis can be innate or acquired. Innate or natural resistance to trypanosomiasis may take the form of a total refractory state to trypanosome species and this may be exemplified by the baboon, or immunity against specific species of trypanosomes and this may be exemplified by the pig which is resistant to T.vivax but highly susceptible to T.simiae. Innate immunity could also be observed in specific individuals, strains, or breeds within a susceptible species and this may be exemplified by the N'dama and Muturu cattle of west Africa which can be easily infected but throw off the infection readily (Stewart, 1951; Chandler, 1952; Desowitz, 1959; Stephen, 1966).

Artificial immunity may be induced actively or passively. The difficulties of active immunization are related to a number of factors such as multiplicity of the trypanosome species, variations in strain virulence, the instability of the trypanosome antigens, and the extreme variant specificity of the protective antitrypanosome antibody. Various methods including premunition (Ehlrich and Shiga, 1904), intentional infection with virulent parasites followed by drug cure (Fiennes, 1950; Cunningham, 1967),

and use of live attenuated parasites have been attempted with variable degrees of success. Among the methods used for the attenuation of parasites, ionizing radiation has been found to be most promising.

Most of the radiation-attenuated vaccines so far successfully produced are those against helminth parasites. Among these works are those of Jarret *et al* (1958) against cattle lungworm, Mulligan *et al* (1961) on *Haemonchus contortus* and *Trichostrongylus colubriformis* of sheep, Sokolic *et al* (1963) on sheep lungworms, Urquart (1961) on *Taenia saginata* and Bitakaramire (1973) on *Fasciola gigantica* of cattle. Successful reports of radiation-attenuated anti-protozoal vaccines include those of Ceithmal and Evans (1946) against *Plasmodium gallinacium* of chickens, Sanders and Wallace (1966) against *T.lewisi* in mice, Duxbury and Sadun (1967) against *T.rhodesiense* in mice and Duxbury *et al* (1973) on *T.congolense* in cattle.

The successful report of Duxbury *et al* (1973) was against an extremely mild strain of *T.congolense* and they pointed out that even in nonprotected animals the effects of the disease were not severe. In view of this finding in mild infections, experiments were carried out in the present section to observe whether immunization against a virulent strain of *T.congolense* using radiation-attenuated organisms held any promise. Pathophysiological studies were carried out on these cattle as an additional index of the degree of immunity produced.

Materials and Methods

Nine yearling male calves were used in this experiment. They were divided into three groups (A,B,C) of three calves per group.

An acute strain of T.congolense (Gambella II) was used for infection. Infectivity titration of the trypanosomes was performed in mice according to the method described by Lumsden et al (1963).

The Fricke dosimeter method of irradiating ferrous sulphate and spectrophotometrically determining the level of ferric ions produced was used for the calibration of the ^{60}Co radiation source (Bhabha Atomic Energy, Bombay) prior to irradiation of parasites.

Blood from a heavily infected mouse was collected in a heparinized syringe and immediately diluted 25 fold with normal saline at 38°C. The suspension was transferred to 2ml tubes and the tubes placed in the peripheral positions of a circular rack (fig 10). The rack was filled with warm water (38°C to approximately 1cm below the rims of the rubes and placed in the ^{60}Co radiation source for irradiation. A dose of 60Kr was delivered to the trypanosome suspension.

Each calf in group A was injected with approximately 13×10^6 irradiated trypanosomes intravenously. Two weeks later a booster dose of approximately 34×10^6 irradiated trypanosomes were injected into each of the same calves. Six weeks after the first immunizing dose each calf in group A and a non-vaccinated group of three calves (group B) was challenged with ID_{63} 3200 virulent homologous trypanosomes.

Three calves (group C) were used as normal controls.

Two days after infection with virulent trypanosomes approximately 100 microcuries of ^{59}Fe (ferric citrate) was administered to each calf in group A, B and C. Administration of isotope, sampling, assay of radioactivity and calculation and presentation of results was according to the method described earlier in the section on ferrokinetic studies in subacute infections.

Results

Haematology

No significant difference between group A and C was observed. An apparent fall in the mean PCV and haemoglobin concentration was observed in group A. From the results it was concluded that a mild normocytic normachromic anaemia developed in the vaccinated calves (group A). As compared with group A calves, group B calves showed a significant drop in the mean PCV and haemoglobin concentration during the third week of infection ($P < 0.05$). Alterations in MCV and MCHC in both groups A and B were not significant. The haematological findings are presented in table 14.

Ferrokinetic studies

The mean "apparent half life" of plasma ^{59}Fe in the control calves (group C) was 63 ± 11 minutes as compared to 80 ± 19.5 minutes in group A and 93 ± 17 minutes in group B calves (fig 9). The difference in the mean values of

Table 14

Haematological indices in calves vaccinated with irradiated T.congolense and challenged (group A), challenged controls (group B) and normal controls unchallenged (group C). The "t" tests indicated are between groups A and C, and B and C.

Group		WEEKS POST CHALLENGE											
		0 - 1 week				1 - 2 weeks				2 - 3 weeks			
		PCV %	Hb gm %	MCV μ^3	MCHC %	PCV %	Hb gm %	MCV μ^3	MCHC %	PCV %	Hb gm%	MCV μ^3	MCHC %
A	mean	28.0	8.7	48.4	31.7	25.0	9.2	46.0	31.9	23.2	7.6	43.4	36.5
	s.d.	1.4	0.3	4.3	2.4	4.2	6.8	10.0	1.4	1.1	0.4	6.2	5.6
C	mean	28.0	9.2	45.0	33.0	25.3	8.5	53.1	32.0	28.0	9.0	42.8	32.8
	s.d.	5.7	1.6	10.3	0.6	5.5	1.9	1.3	1.3	3.9	1.4	3.1	1.2
"t" test		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B	mean	32.0	9.8	43.3	31.0	28.7	8.2	53.3	31.5	21.0	6.7	47.1	33.6
	s.d.	11.0	3.5	0.3	0.3	5.0	1.1	3.1	3.6	2.7	0.6	5.5	1.3
"t" test		NS	NS	NS	NS	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$	NS	NS

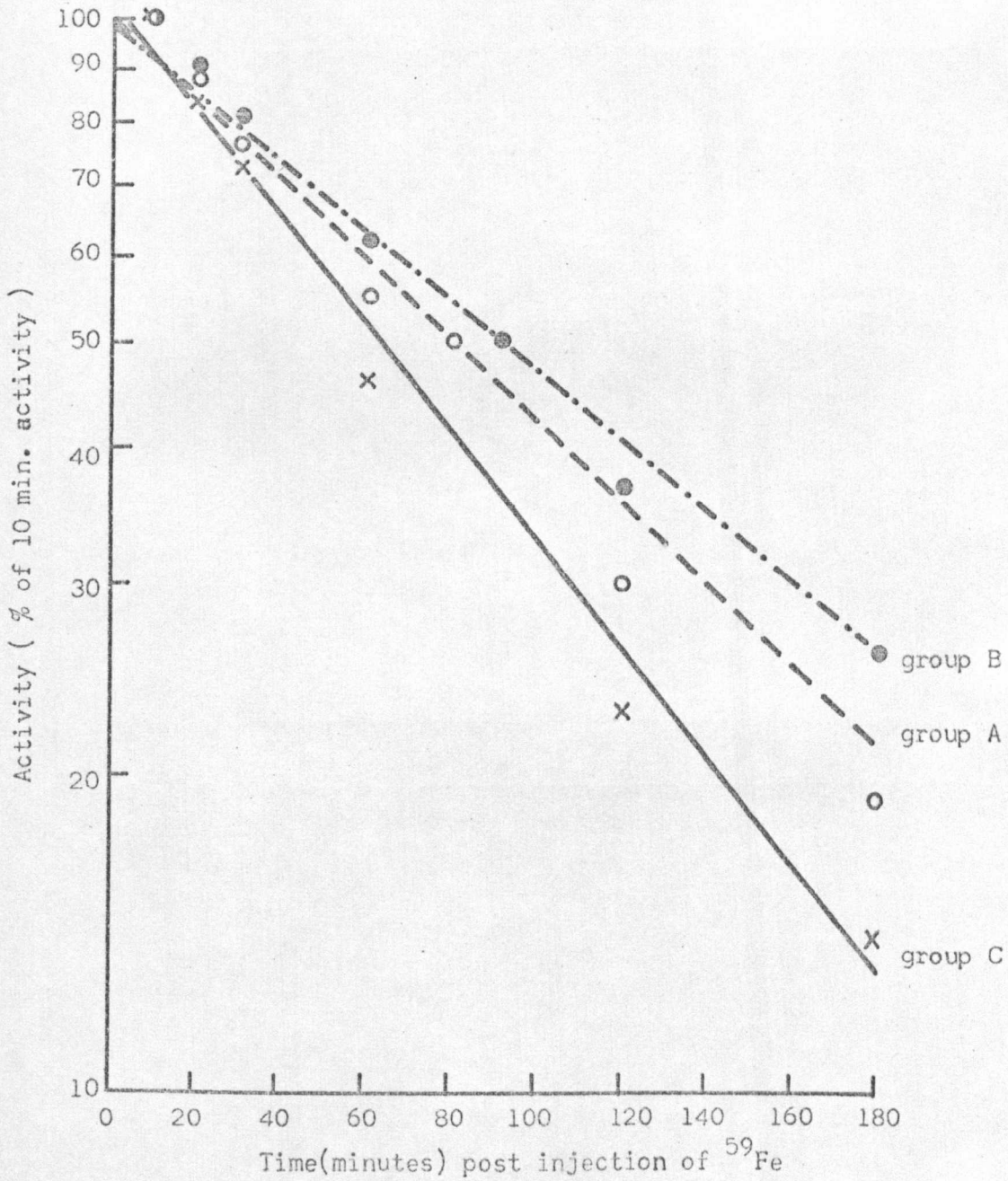
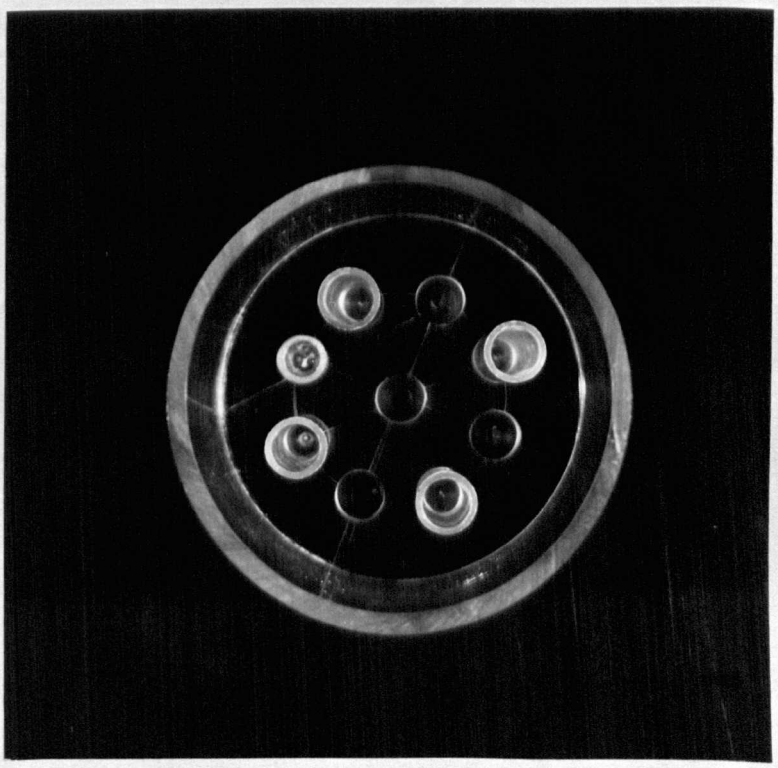


Fig. 9 Plasma ^{59}Fe disappearance in vaccinated(group A) and challenge control cattle(group B) infected with T. congolense and in normal controls.

Fig. 10 Perspex irradiation rack with four tubes in peripheral positions



groups A and C was shown to be statistically not significant while that between group B and C was significant ($P < 0.05$). The corresponding plasma iron turnover values in the three groups are 767 ± 228 µg/Kg in group A, 463 ± 177 µg/Kg in group B and 1262 ± 240 µg/Kg in group C.

Discussion

It has been shown that the effect of radiation on parasites is to interfere with the normal physiological processes. A dose of 12Kr delivered to T.rhodeniense interfered with normal division and infectivity and between 100 and 600 Kr was shown to be lethal (Halberstaedter, 1938) though Duxbury and Sadun (1967) indicated that trypanosomes to which up to 100Kr was delivered maintained their immunogenicity. Advantage is taken of the big difference between the dose necessary to arrest multiplication and infectivity, and that which produces lethal effects, to induce live attenuated organisms.

As mentioned earlier the same degree of success as in the small laboratory animals has not been produced in the large domestic animals; and even in the small laboratory animals the success story is limited to homologous strains only. There is no doubt this is a significant step forward but it is well to keep in mind that there are many obstacles to be overcome in producing an effective immunizing agent and in assessing the degree of such immunity.

Resistance to a challenge dose, ie resistance of a host to a measured and defined infective dose of trypanosomes

may be measured on the basis of a number of factors depending on a significant alteration of the course of the infection in the immune host as compared to normal susceptible animals. Among the more commonly used factors are the clinical effects developing as a result of the disease, the level of parasitaemia developing following infection, and the prepatent period is the time interval between the first introduction of parasites into the system and the first appearance of parasites in the peripheral circulation.

Deviation from the typical haematological picture or alterations in the constitution of the serum proteins, both of which may be considered as clinical effects produced as a result of infection, are considered objective and measurable factors for the assay of immunity (Weitz, 1970). In the present experiment haematological factors and plasma iron turnover rate were used for the measurement of the level of immunity. This probably is the first time plasma iron turnover has been used as a measure of immunity.

The challenge dose has in many past works been based solely on the number of trypanosomes in the inoculum, however this method of measuring the challenge dose fails to take into account the great variability between trypanosome strains and even the differences in the virulence of the strain under different experimental conditions. The ID_{63} method of Lumsden *et al* (1963) used in the present experiment overcomes these problems by measuring statistically the infective dose of several dilutions of a suspension of trypanosomes for mice using the first appearance of

trypanosomes in the peripheral circulation of the mice receiving the highest dilution as a basis. This method is considered the most accurate method for the determination of infectivity of a trypanosome strain over periods for challenge of test animals as well as measurement of the different effects such as pathophysiological effects of infection (Weitz, 1970).

Haematological indices and ferrokinetic studies were used in the present experiment for the assessment of the degree of immunity. The plasma ^{59}Fe disappearance rate and subsequent reappearance in the general circulation following incorporation into haemoglobin was not significantly different in the vaccinated calves from the non infected control calves as compared to a significantly delayed plasma iron turnover in the non vaccinated calves. This may be considered a positive demonstration of immunity and as mentioned earlier this may be the first time ferrokinetic measurements were used in the assessment of the immune status of a host to a challenge infection. The haematological indices, PCV and MCV were not significantly altered in the vaccinated calves following infection as compared to a significant alteration in the challenged non immunized calves and this may be considered another indication of the development of immunity in the inoculated calves.

Although some evidence of protection is given by the above measurements virtually no protection was given using death due to the disease as a measure. The reasons for the sudden death of vaccinated calves following challenge are not known. The size of the immunizing dose may have

been too little and conversely the size of the challenge dose may have been too large for the level of protection produced. An antigenic variation of the strain T.congolense used between the time of vaccination and the time of challenge may have occurred. The possible effects of immunosuppression are discussed in the next section. On the other hand the factor or factors involved in the cause of death may have little or no influence on the haematological indices or ferrokinetic mechanisms. Obviously many other factors may be included in the list of possibles and it is also possible that those listed above either singly or in combination resulted in a fatal outcome. With the evidence available it is believed that a degree of resistance had been conferred on the calves by prior exposure to the irradiated trypanosomes but was overcome either due to an inadequate immunizing dose, too strong a challenge dose or antigenic variation of the trypanosome strain used.

It is obvious that much more work is required in this field of investigation to make meaningful use of the above results as well as to understand the immune protection mechanisms in trypanosome infected hosts more fully.

B. IMMUNOSUPPRESSION IN CATTLE INFECTED WITH T.CONGOLENSE.

Introduction

Immunosuppression of trypanosome infected hosts has recently added a new dimension to the complexity of the immunology of trypanosomiasis. It is perhaps more accurate to speak of a revival of interest in immunosuppression as Castellani (1903) and Low and Castellani (1903) have observed earlier the increased susceptibility of trypanosome infected human patients to secondary infection by bacteria and viruses. MacGregor and Barr (1962) observed that standard doses of the same batch of tetanus toxoid failed to induce an antitoxin response in significantly more Gambian children with malarial parasitaemia than their counterparts which had been kept free of malaria from birth by continuous prophylaxis and this gave rise to the suggestion that malarial infection may have suppressed the response to tetanus toxoid by antigenic competition. This finding was later confirmed by Greenwood *et al* (1972) that children with acute malaria showed a diminished antibody response to the "O" antigen of *Salmonella typhi* and to tetanus toxoid. It is interesting that they also noted antibody response to the "H" antigen of *S.typhi* and the cellular immune response were not affected suggesting that the suppression of antibody formation applies only to specific antigens.

Immunosuppression in trypanosomiasis was demonstrated by Goodwin *et al* (1972) who showed that mice and rabbits infected with chronic *T.brucei* showed suppressed formation of haemaglutinins against injected sheep red blood cells. Urquhart *et al* (1973) used superinfection of trypanosome infected rats with *Nippostrongylus braziliensis* to demonstrate immunosuppression and observed failure of immune expulsion of the worms which was shown to occur under normal circumstances (see review by Jarret *et al*, 1968). They also demonstrated impaired production of IgG and IgE. Freeman *et al* (1973) demonstrated that early in the infection of mice with *T.brucei* the number of cells producing IgM antisheep red cell response was generally not affected while the IgG antisheep red cell producing cells were reduced by 80% and later in the infection they showed that both IgG and IgM producing cells were reduced to less than 2% of the control group.

Greenwood *et al* (1973) observed that the response of human patients with trypanosomiasis to foreign antigens was less than in control subjects and the expression of cell mediated immunity, induction of cell mediated immunity and expression of humoral immunity were all impaired in these patients. The observation was also made that the level of immunosuppression may have a direct relationship to the level of circulating parasitaemia developing; as in trypanosomiasis caused by *T.rhodesiense* (high parasitaemia) there appears to be a greater level of immunosuppression than in *T.gambiense* (low parasitaemia) infection.

It should be borne in mind that the phenomenon of immunosuppression is not limited to trypanosome and malarial infections. Clinton *et al* (1969) and Strickland *et al* (1973) pointed out the existence of such a phenomenon in other protozoal infections, Mcgregor (1972) in viral infections, Rees and Waters (1972) in bacterial infections, and Kakalamanis *et al* (1972) in mycoplasma infections.

Factors other than infections above described may have a significant effect on the response of the host. It is widely recognised that animals on a poor nutritional status succumb to the effects of a disease more than in well nourished animals. The size of the immunizing dose, the route of immunization, the immunological maturity or age of the host, and the virulense of the infecting organism, are a few of the more important factors that have to be considered.

Most of the experiments performed both on immunization and immunosuppression of trypanosomiasis are in laboratory rodents. These studies are extremely useful in understanding the underlying mechanisms operating in this disease. However, in the final analysis the application of these findings to the large domestic animals to which trypanosomiasis is of major economic importance remains the ultimate goal; and the results of the findings in laboratory animals cannot be directly extrapolated to the large domestic animals.

The demonstration of immunosuppression in the large domestic animals is bound to have far reaching implications on the livestock industry in the trypanosome

belts of the African continent. There are major national and international campaigns such as the O.A.U. JP-15 campaign currently involved in the eradication of rinderpest and bovine pleuropneumonia through mass vaccination. It would therefore not be surprising if trypanosomiasis "sabotaged" the efforts and large sums of money invested in these campaigns.

A case in point that may have relevance to the present discussion is the rinderpest vaccination campaign of 1967 and 1968 in Ethiopia. In many areas of southern and western Ethiopia there were reports of large losses of cattle by farmers due to a "break" in the rinderpest vaccination though this has never been officially acknowledged. At the time such varied reasons as too virulent a vaccine and sabotage have been claimed by individuals. In light of the demonstration of immunosuppression by trypanosome infections it is possible that this factor played an important role. There is no concrete evidence for this view except that the majority of the animals that died were those from trypanosome infested regions. Losses due to trypanosomiasis could therefore be shown to occur directly or indirectly through a direct effect of the infection and suppression of the immune response to vaccines as well as predisposition of the animals to secondary infections.

In the present study the effect of experimental infection with T.congolense on the antitoxin response to a commercial trivalent clostridial vaccine was determined in zebu yearling cattle.

Materials and Methods

Twenty-one male yearling Ethiopian cattle were purchased from a trypanosome-free area adjacent to Addis Ababa on the Ethiopian highland plateau. They were initially treated with anthelmintics to remove gastrointestinal nematodes* and liver flukes**, and then observed over several weeks to confirm the absence of clinical disease. The animals were maintained in pens and fed a concentrate ration along with hay and water ad lib.

All the calves were vaccinated against Rinderpest and Foot and Mouth Disease (types A, O & C) prior to the commencement of the experiment. The twenty-one animals were divided into three groups; one of five animals (group A) one of ten animals (group B) and one of six animals (group C).

The experimental design is presented in table 15 The experiment was divided into three parts. In the initial part the Ilnological response to a primary vaccination with a polyvalent clostridal vaccine*** after initial infection of group A was compared with that of normal controls (group B). In the second part of the study a group of calves which had been infected after a primary vaccination (group C) were compared with the normal controls (group B). In the third part group B was divided into two groups each of five animals (B_1 and B_2) and group B_2 infected prior to a third vaccination of both groups B_1 and B_2.

Throughout the studies heperinized blood samples were taken thrice weekly for routine haematology and

* Thibenzole - Merck, Sharp and Dohne, U.K.
** Zanil - Imperial Chemical Industries, U.K.
*** Tribovax - Wellcome Research Laboratories, U.K.

parasitaemia checks. Serum samples for subsequent antitoxin analysis were taken regularly during the experiments and up to two weeks after the final vaccination of each group.

Results

In all the infected groups of calves positive parasitaemias were apparent by ten days post infection and this was followed by the development of moderate to severe anaemia (table 16). Weight losses in the infected animals were not significant and appetites remained good despite an unthrifty appearance.

The sera were examined for antitoxin response to the various components of the vaccine (tetanus, septicum and oedimatiens alpha) by the Wellcome Research Laboratories at Beckenham.

In each part of the experiment an immunosuppressive effect was found following infection. The results of the first part of the study involving the response to a primary vaccination three weeks after infection with T.congolense (group A) and in normal controls (group B) are given in figures 11 - 13. The pattern of response is seen to be similar for each component though the differences at week 6 were only significant ($p < 0.05$) between group A and B for the tetanus component (fig. 13).

In the second part of the experiment it is readily apparent from figures 14 - 16 that the responses to the primary vaccination are similar between group B and C but that following infection of group C there was a marked

Table 15

Experimental design on immunosuppression studies in cattle infected with T.congolense

Week	Group A (5 calves)	Group B (10 calves)		Group C (6 calves)
0	Infected	-	-	-
3	1st vaccination	1st vaccination		1st vaccination
6	terminated	-	-	infected
9		2nd vaccination		2nd vaccination
11		-	-	terminated
		B_1	B_2	
14		-	infected	
17		3rd vaccination	3rd vaccination	
19		terminated	terminated	

Table 16

Haematocrit values of cattle following infection with T.congolense and in normal controls (mean ± s.d.)

Week	Group A	Group B1 & 2		Group C
		B1	B2	
0	27.8 ± 4.0≠	26.7 ± 2.6		-
1	21.6 ± 1.5	24.9 ± 3.2		-
2	19.0 ± 2.2	24.1 ± 3.7		-
3	17.0 ± 2.4	23.6 ± 2.9		-
4	16.3 ± 3.5	25.0 ± 3.0		-
5	16.0 ± 2.8	28.0 ± 3.3		-
6	14.6 ± 3.8	29.0 ± 5.1		31.3 ± 4.2 ≠
7	-	28.7 ± 4.0		29.5 ± 5.9
8	-	28.9 ± 4.3		24.8 ± 3.2
9	-	31.5 ± 4.2		18.7 ± 1.2
10	-	30.2 ± 4.5		17.5 ± 2.3
11	-	31.1 ± 4.6		14.2 ± 3.5
14		31.4 ± 1.1	37.2 ± 6.7 ≠	
15		30.6 ± 2.6	30.8 ± 6.3	
16		33.0 ± 1.4	27.2 ± 4.5	
17		33.0 ± 116	24.8 ± 2.9	
18		29.6 ± 1.5	21.4 ± 3.6	
19		33.6 ± 1.5	17.4 ± 4.9	

≠ - infection

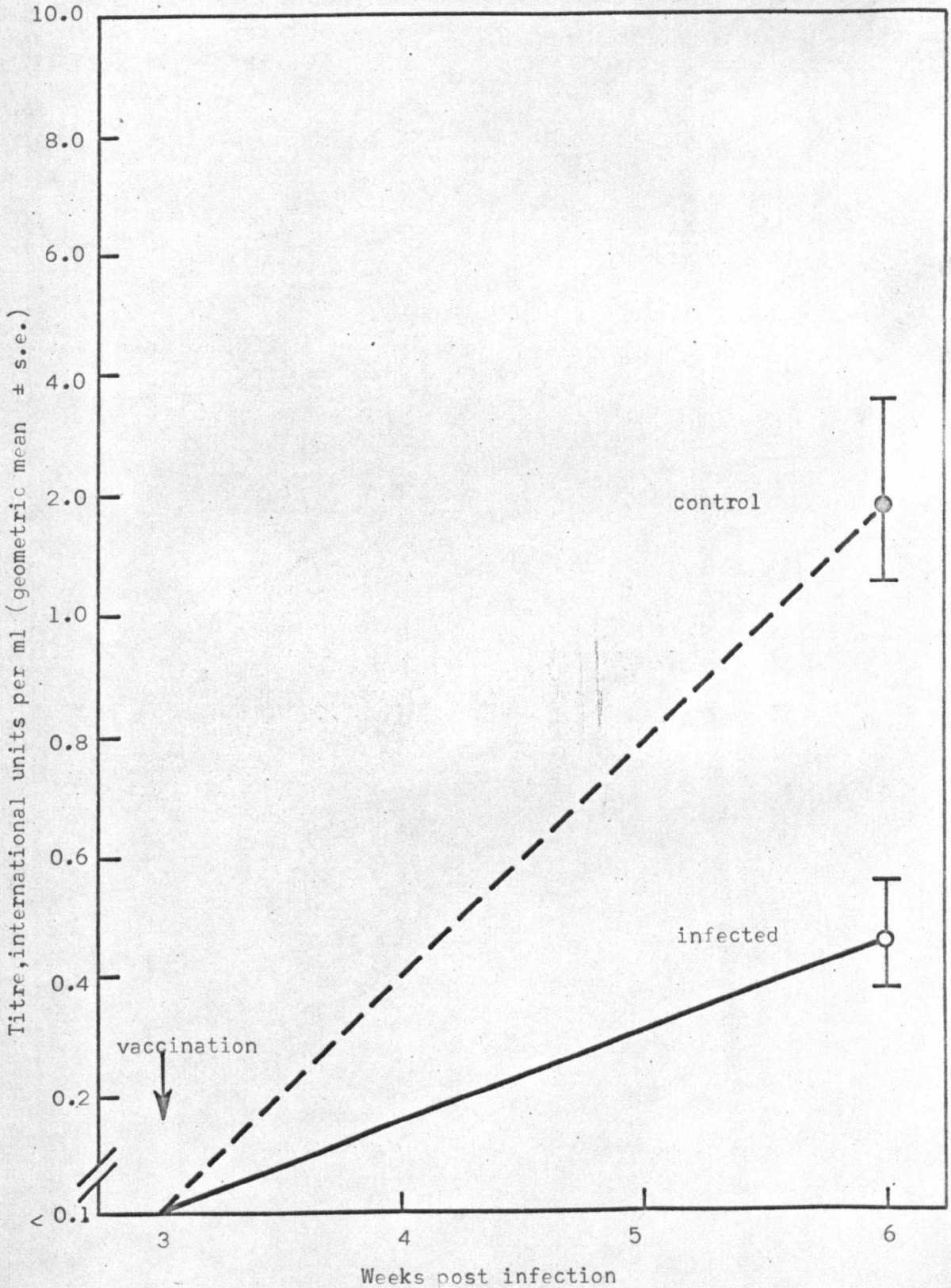


Fig. 11 Immunosuppressive effect of T. congolense infection in cattle on the primary response to the oedimatiens alpha component of Tribovax vaccine.

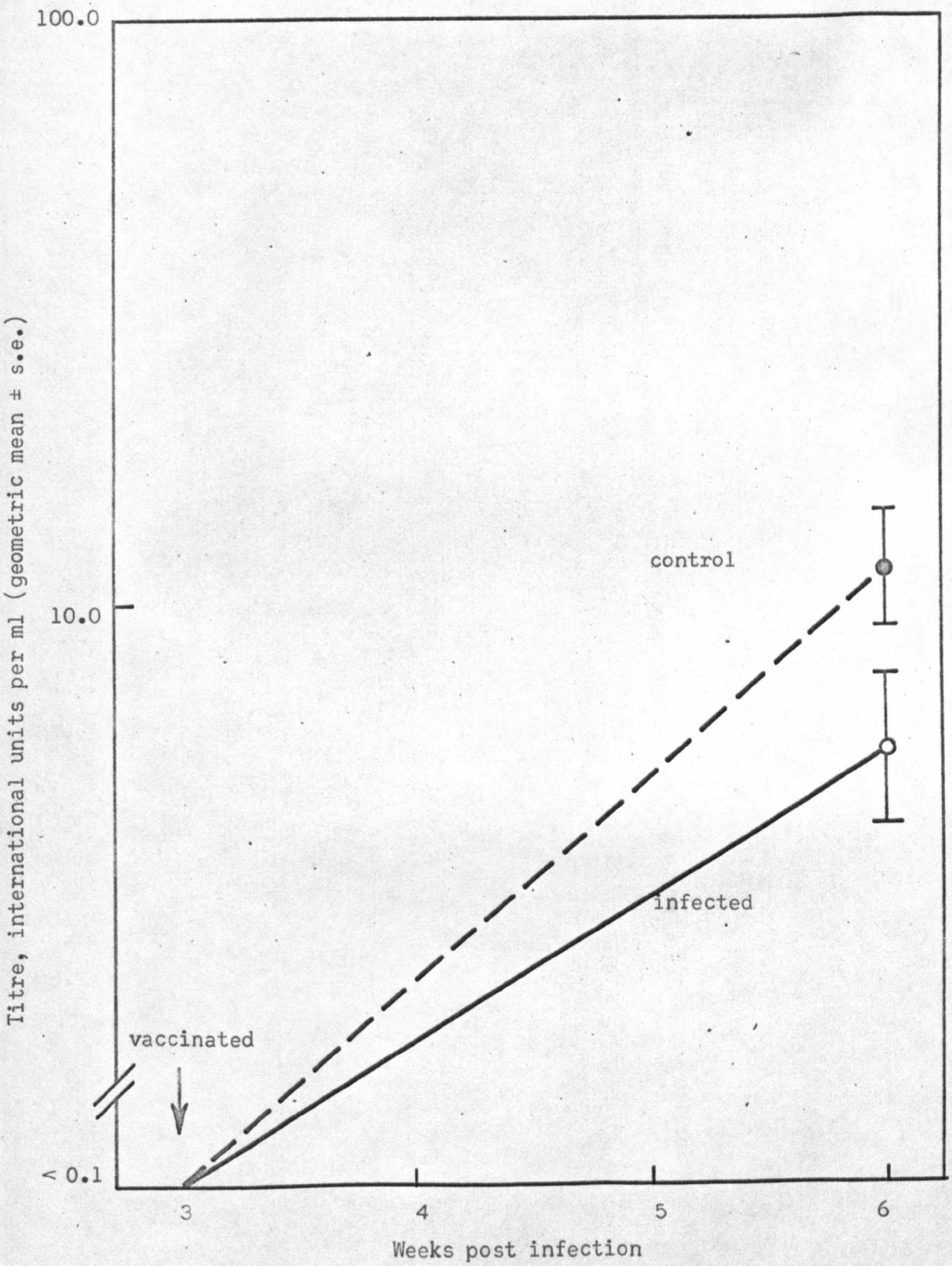


Fig. 12 Immunosuppressive effect of I. congolense infection in cattle on the primary response to the septicum component of Tribovax vaccine.

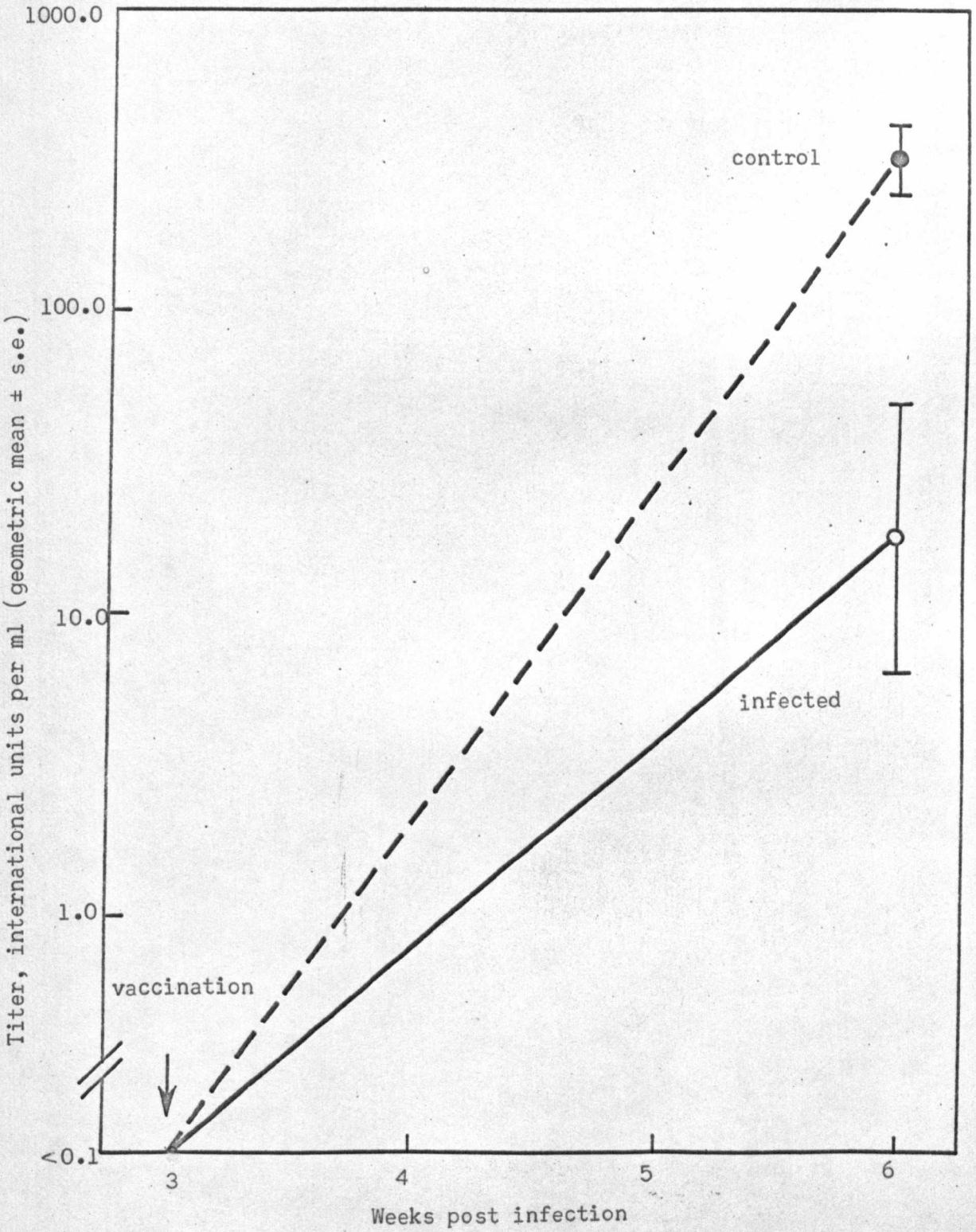


Fig. 13 Immunosuppressive effect of T. congolense infection in cattle on the primary immune response to the tetanus component of Tribovax vaccine.

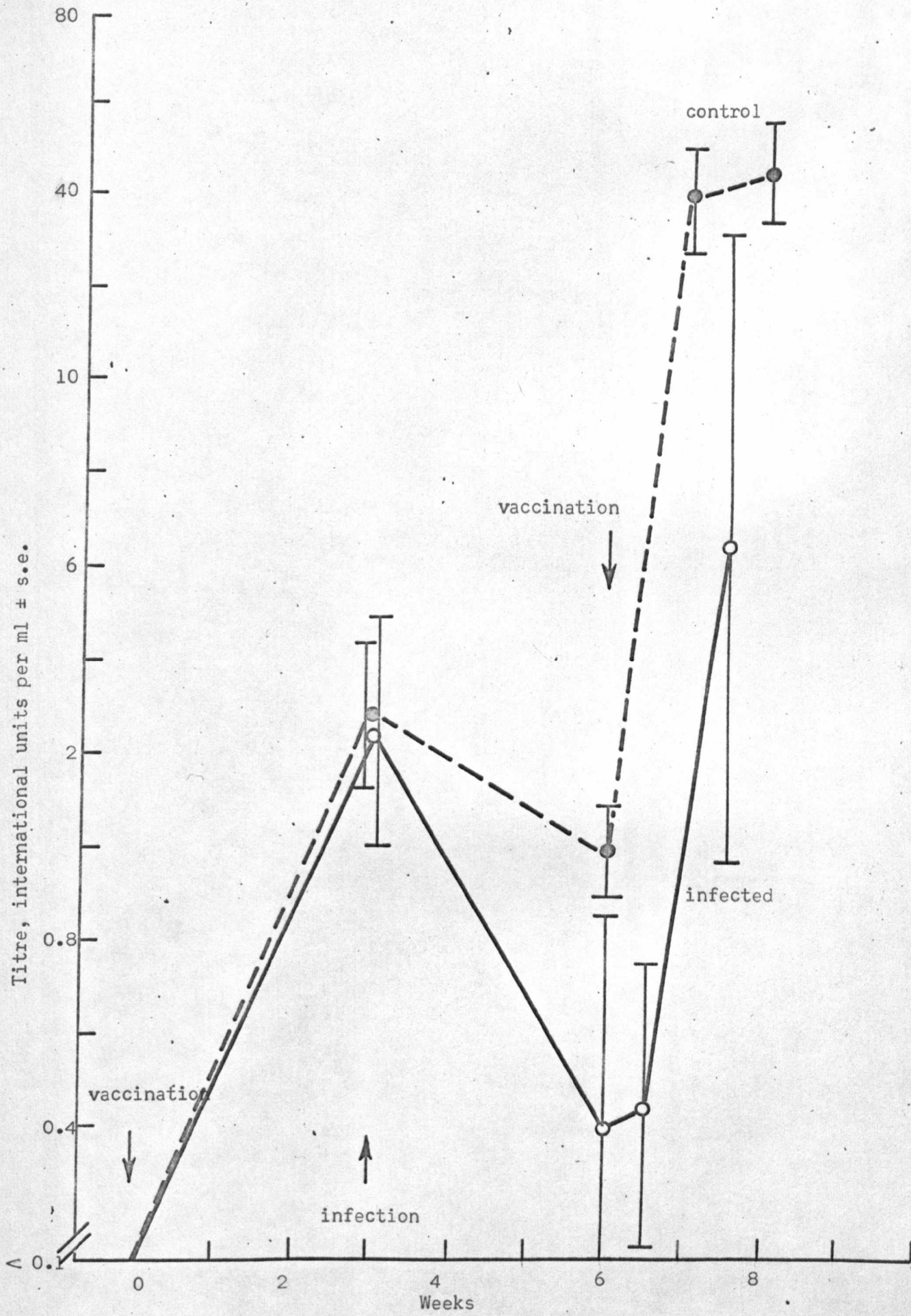


Fig. 14 Immunosuppressive effect of I.congolense on the oedimatiens alpha component of Tribovax in cattle.

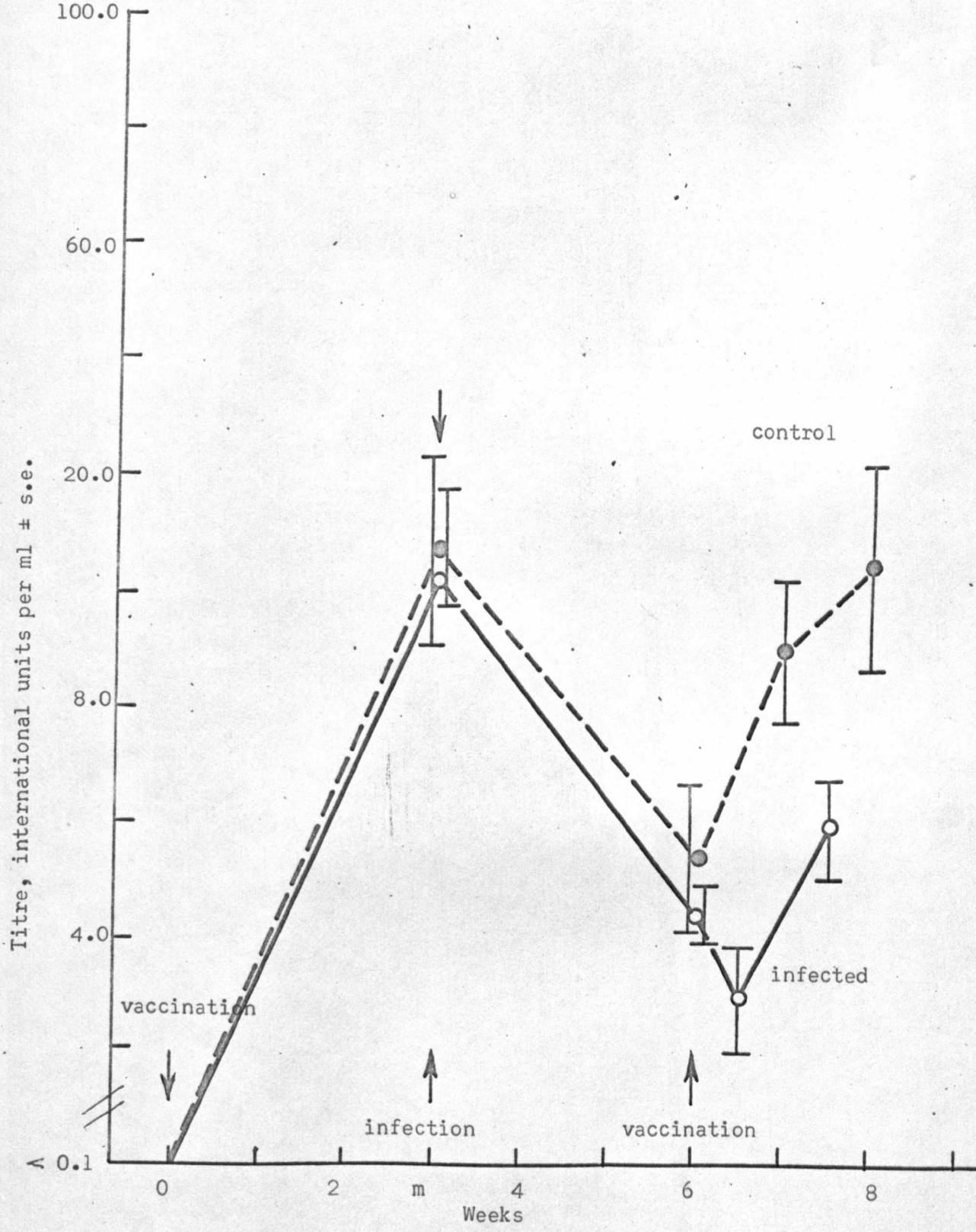


Fig. 15 Immunosuppressive effect of T. congolense on the septicum component of Tribovax in cattle.

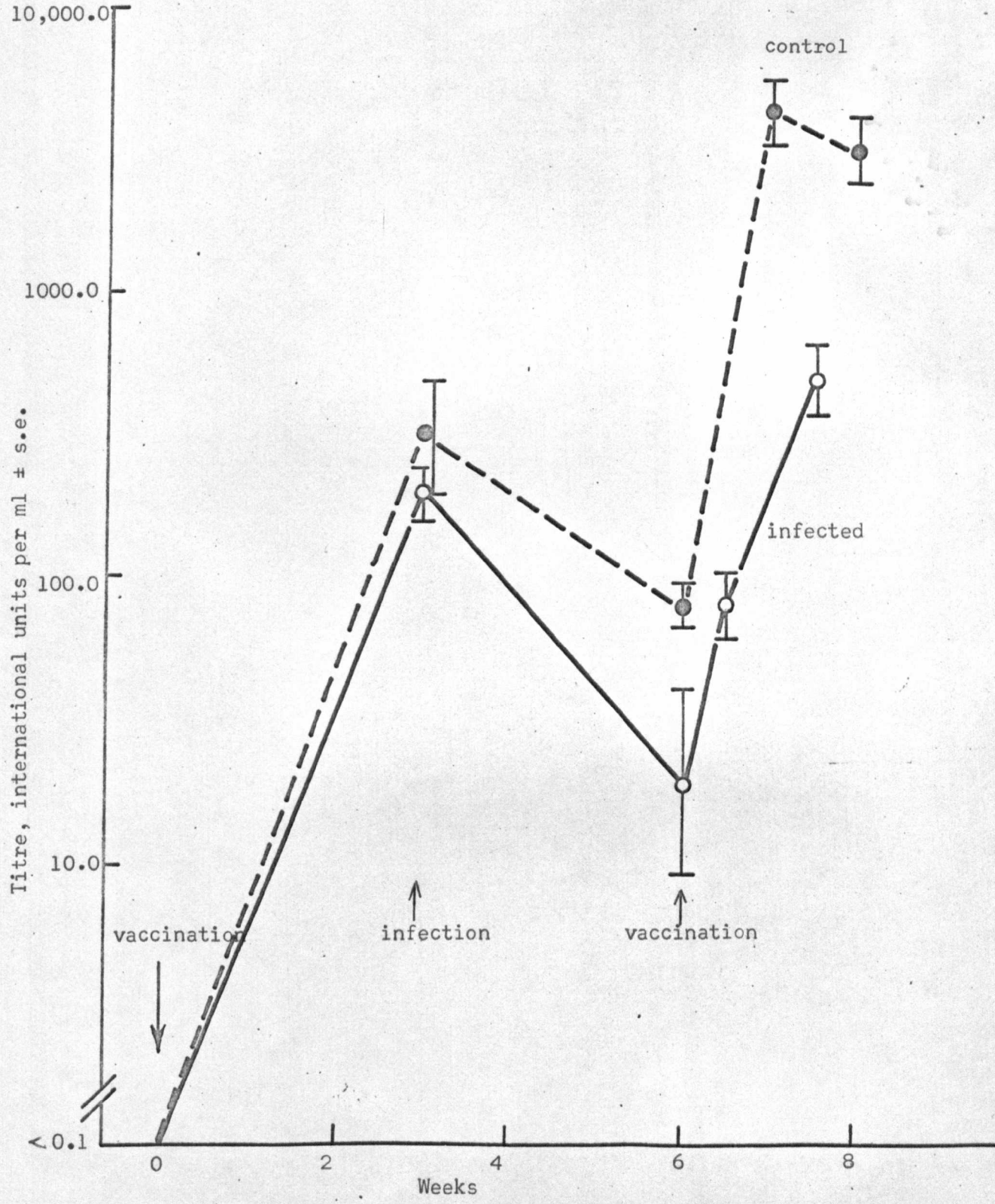


Fig. 16 The effect on the response to the tetanus component in cattle vaccinated with Tribovax and infected with T. congolense.

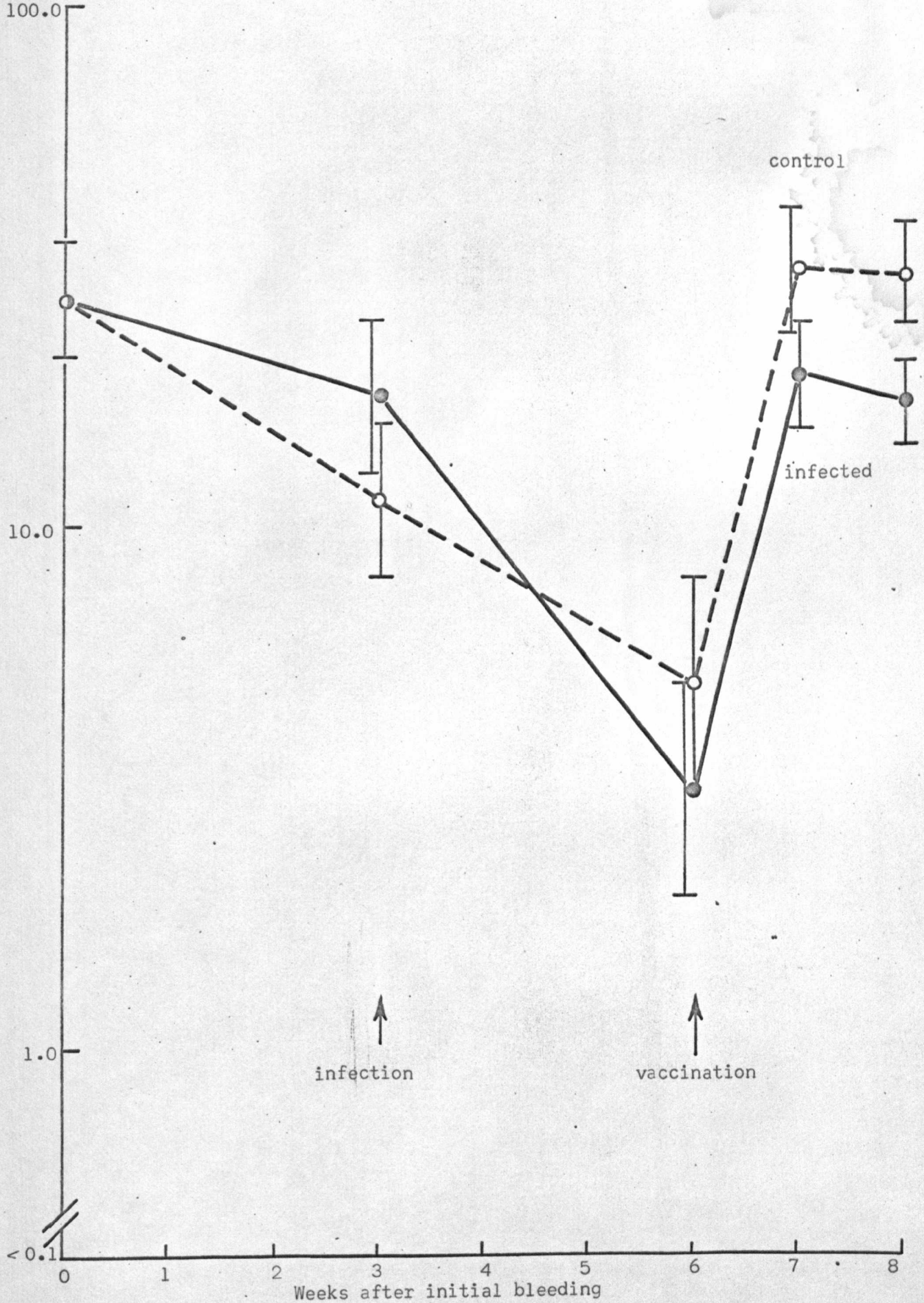


Fig. 17 The effect on the response to the oedimatiens alpha component of T. congolense infection in cattle previously vaccinated with Tribovax.

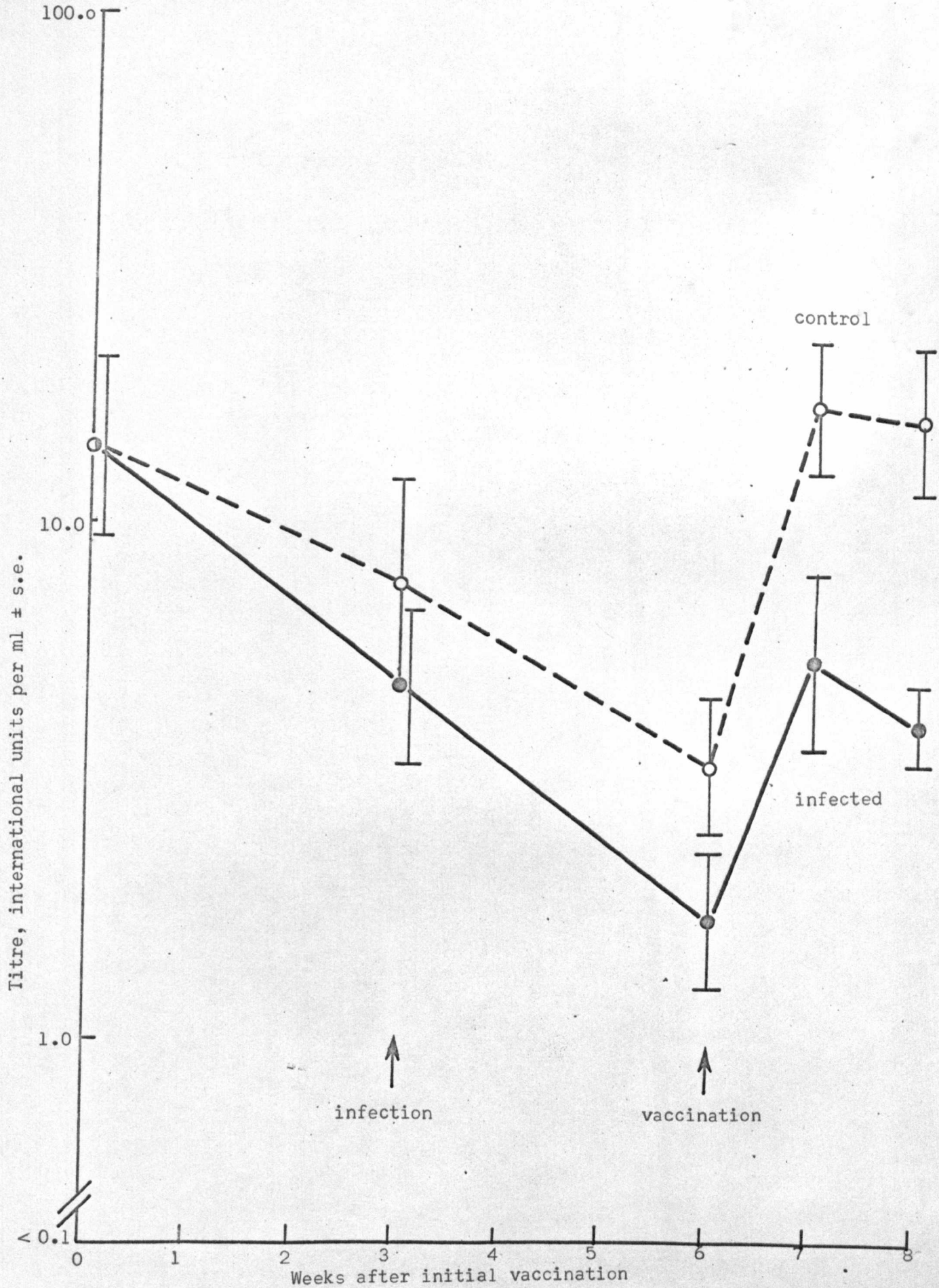


Fig. 18 The effect on the response to the septicum component of I.congolense infection in cattle previously vaccinated with Tribovax.

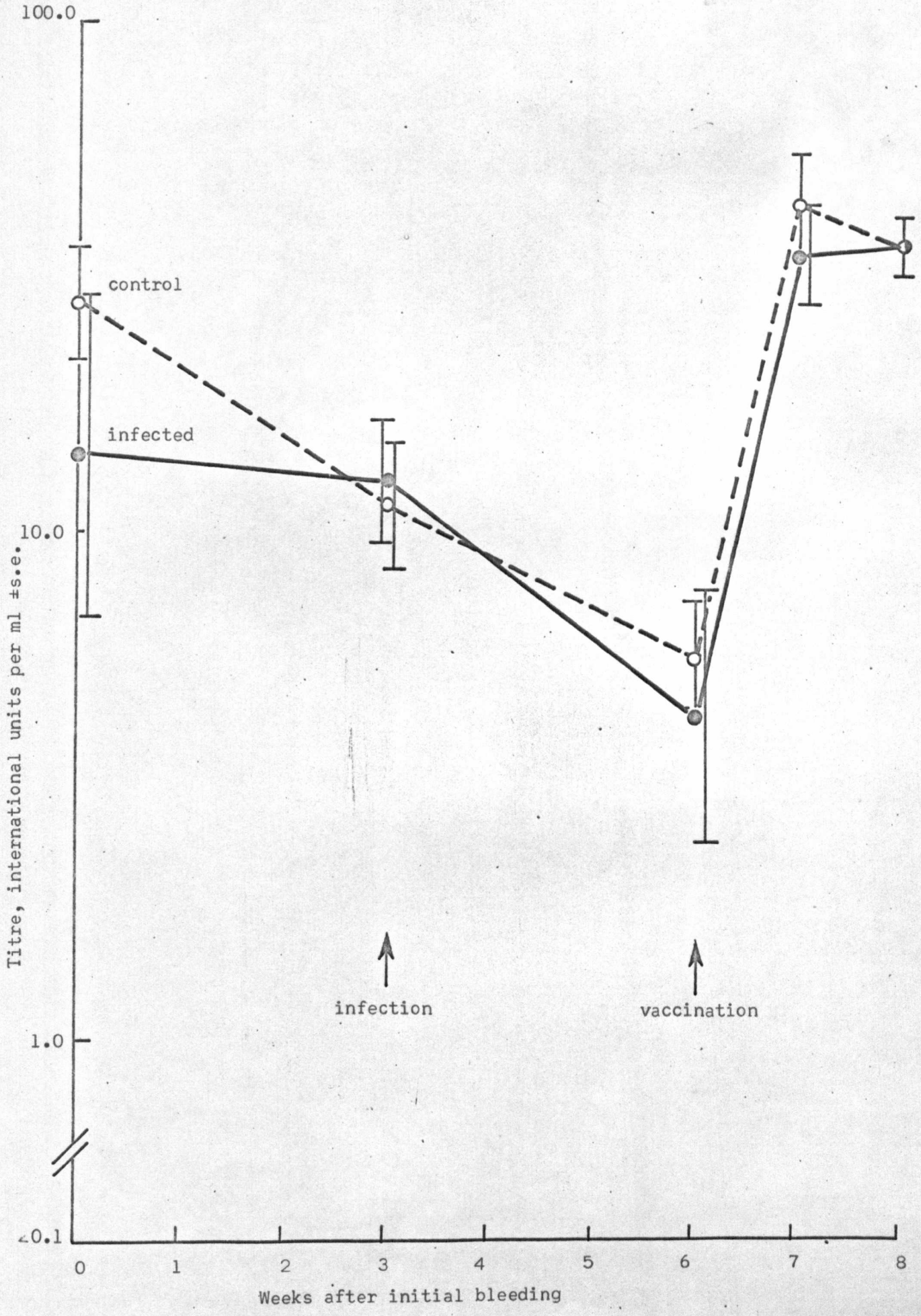


Fig. 19 The effect on the response to the tetanus component of T. congolense infected cattle previously vaccinated with Tribovax.

difference in the secondary response between the two groups for each component of the vaccine viz. (oedimatiens $p < 0.01$, septicum $p < 0.02$, tetanus $p < 0.001$) 10-14 days after the second vaccination.

The results of the third part are given in figures 17-19. In this part of the experiment a depression effect was noted in the oedimatiens and septicum components but not for the tetanus component. The response to the septicum component reached statistical significance ($p < 0.01$) and a reversal of the difference in response between groups B1 and B2 to the oedimatiens component is particularly noteworthy (fig. 17).

Discussion

The results of the previous experiment clearly provide evidence that trypanosome infections can have an immunosuppressive effect in cattle as in small laboratory animals.

Greenwood et al (1972) demonstrated that the antibody response to the O antigen of S.typhi in malaria infected children was suppressed and not the H antigen. It has for some time been recognized that there was an increased globulin response in trypanosome infections of the large domestic animals (French, 1935). Desowitz (1959, 1960) showed that most of this increase was in the beta globulin IgM and based on this finding it was suggested that the antibody response in trypanosome infections was concentrated in this fraction. However, in view of the

fact that increased macroglobulin response is a nonspecific reaction in many infections and the absence of specific evidence on the biological activity of macroglobulins, it could not be categorically stated that the IgM response is solely due to antibody production. Jennings et al (1973) demonstrated increased catabolism of serum globulin in T.brucei infected mice and suggested that this may be a contributory factor in the marked immunosuppressive effects associated with trypanosomiasis.

Many investigators have performed various experiments in an attempt to elucidate the mechanisms of immunosuppression. Advances in the field of immunology have shown that the immune response of a host is not only humoral but also cell-mediated. Immune response is associated with two types of lymphocytes ie. the thymus derived"T"lymphocytes and the non thymic origin"B"lymphocytes. It has been shown that the introduction of an antigen activates the "T" cells specifically and "T" cells can cooperate with "B" cells, which are the elaborators of humoral antibody, to allow an enhanced antibody response (Mitchell and Miller, 1968). The "T" cells and not antisera, have been shown to possess immunological memory following prior exposure to specifix antigens. Gowers and Uhr (1966) demonstrated adoptive secondary antibody response by injecting suspensions of small lymphocytes and this provided evidence that the lymphocytes carried memory. Agents which interfere with the mitotic cycle, eg. radiation, were shown to abolish the establishment of IgG antibody responses (Miller, 1973).

Good (1972) reviewed the structure/function relationship of the lymphoid system. Thymus derived lymphocytes tend to be distributed selectively in certain regions of the peripheral lymphoid tissue. In the lymph nodes they occupy preferentially the deep cortical regions, and the parafollicular and perivascular sites of the Malphigian white matter of the spleen. The "T" cells circulate in blood or lymph as small and medium sized lymphocytes, percolate through the lymphoid tissues, and recirculate. "B" cells (plasma cells) are antibody producing cells and exhibit evidence of allelic exclusion; a single cell and its clone produce only one class of immunoglobulin and antibody of considerable homogeneity. There are many heterogenous cells in the "B" cell system and cells capable of selectively synthesizing and secreting IgA, IgD, IgE, IgG and IgM immunoglobulins can be defined. The final reactivity of an animal to a specific antigen can be defined the resultant of its cell-mediated immunity and the effect of the response of B lymphocytes to stimulation by soluble antigen (Turk, 1973).

Luckins (1972) observed that zebu cattle in enzootic trypanosome areas developed pronounced macroglobulinaemia. Later (1973) he performed experimental studies of T.congolense and T.vivax infections in zebu cattle and showed increased production of IgM within two weeks of infection and this coincided with the appearance of parasites in the peripheral circulation. He also observed little change in the IgG response during infection. Freeman et al (1973) on the other hand observed that early in the infection

of mice with T.brucei numbers of cells producing IgM antisheep red cell response were not significantly altered whereas the numbers of IgG antisheep red cell producing cells were reduced by 80%. Later in the infection both IgM and IgG producing cells were severely reduced. These apparently conflicting findings between different species of trypanosomes as well as between laboratory and large domestic animals underlines the dangers in extrapolating the findings in one group or species to another.

Recently Murray et al (1974) studied the role of the macrophages and lymphocytes in the immunosuppressive mechanism of T.brucei infections in mice and observed that experienced macrophages transferred to naive hosts were capable of immunological memory and therefore initiation of active antibody response. They also observed that when normal macrophages were transferred to trypanosome infected hosts antibody production was not stimulated. In T.brucei infections of mice extensive multiplication of the lymphoid cells and production of plasma cells was observed in the spleen and lymphoid tissues starting early in the infection and extending throughout the infection period. It is perhaps possible that the increased macroglobulinaemia observed in trypanosome infected bovines was probably due to such increase in the immunoglobulin producing cells and in view of increased clearance of ^{51}Cr labelled red cells in trypanosome infected cattle described in previous sections of this thesis and the demonstration of erythrophagocytosis in T.congolense infected bovines by Mackenzie and Cruickshank (1973) defective macrophages could probably be ruled out as

a cause of immunosuppression in the bovine.

Various theories have been advanced to explain the phenomenon of immunosuppression in trypanosomiasis. Urquart et al (1973) postulated a mitogenic substance elaborated by the trypanosomes resulted in a nonspecific stimulation of the B cells to produce immunoglobulins and that this probably led to inhibition of subsequent immune response. Another similar mitogen theory has been advanced by Rich and Pierce (1972) but they postulated the mitogen might act through activation of a population of "T" suppressor cells. Goodwin (1970) suggested that successive waves of trypanosome variant antigens competing for the same sites caused the suppression of immune response to new antigens. Failure between the cellular components resulting from "B" cell stimulation and antibody response to cooperate in specific activities has been advanced by Terry et al (1973). Murray et al (1974a) supported the possibilities of all the above findings and added the suggestion that the presence of living trypanosomes, rather than the development of a progressive lesion initiated by the infection, is the basic cause of immunosuppression. On the other hand Targett et al (1973) on their studies on the survival of T.musculi in mice showed that parasites persisted in the kidneys of recovered mice for a long time and hosts were able to withstand strong challenges probably due to continuous stimulation of antibody by the persisting parasites.

Erythrophagocytosis is now established as a major cause of the anaemia in trypanosomiasis of the bovine

and autoimmune reactions have been proposed as one of the possible causes (Mackenzie and Cruickshank, 1973). Immunosuppression would therefore presumably reduce the effects of damage occurring due to autoimmune reactions. Allt *et al* (1971) observed that infection of rabbits with *T.brucei* protected them from experimental allergic encephalomyelitis. Earlier a similar study was performed in Chagas myocarditis, the lesion of which is ascribed to autoimmune reactions, by Kumar *et al* (1970). They found that the extent and rate of myocardial damage as well as mortality rate was actually increased in mice immunosuppressed with cyclophosphamide showing that protective effects are not necessarily conferred.

Immunosuppression in trypanosome infections in the laboratory animals and more so in the large domestic animals is a relatively new subject and not fully understood. From the above discussions it is clear that there are many factors difficult to reconcile with one another. "It is somewhat difficult to comprehend the co-existence of a state of immunosuppression of humoral responses to a variety of antigens with the well demonstrated sequential development of antibody to a succession of trypanosome antigenic variants throughout the course of a prolonged infection (Gray, 1970). Perhaps as suggested earlier, the immunosuppressed state is due to antigenic competition of these variants; alternatively, and possibly more likely in natural infections, immunosuppression may occur only during relatively acute parasitaemic phases of the infedtion or operate at a relative rather than an absolute level" (Urquart *et al*, 1973).

The practical implications of immunosuppression to the African livestock industry has already been mentioned. It is estimated that four million square miles of the continent are infested with the tsetse fly and productive cattle cannot be kept due to the threat of trypanosomiasis (Buxton, 1955). According to Willet (1970) it is debatable whether animal trypanosomiasis, through the vast extent of malnutrition and protein shortage for which it is largely to blame, is not now a greater problem, even on purely medical grounds, than human trypanosomiasis itself.

With the addition of immunosuppression two distinct possibilities other than the direct pathological effects due to the infection are to be expected. In the first place increased susceptibility to other natural non trypanosome infections is a constant threat. The second point is the increased uncertainty of the effect of vaccinations against the many other diseases still rampant on the continent. Further experiments on immunosuppression in trypanosome infected cattle are underway using other vaccines for some of the important tropical animal diseases. It is hoped that these additional experiments will increase the scope of knowledge on the occurrence of this important phenomenon in the bovine.

SECTION III

SOME ASPECTS OF THE PATHOLOGY OF *T. CONGOLENSE* INFECTIONS IN CATTLE

Introduction

It is generally accepted that T.congolense is the most important of the trypanosome species affecting cattle in Africa. For obvious reasons most of the current pathological descriptions on trypanosomiasis have been made on laboratory animal models. T.congolense, especially, has received very little attention even in the laboratory animal models primarily because it was shown to a difficult species in which to get consistent laboratory animal infections.

Pathological descriptions of chronic T.congolense infections in cattle have been presented by Fiennes et al (1946) and Fiennes (1953, 1970). Naylor (1971a, 1971b) described some aspects of the pathology of chronic T.congolense infections in cattle and emphasized that there is very little information on the pathology of the trypanosomiasis in the large domestic animals. Losos and Ikede (1972) have presented a fairly detailed review of the pathology of the trypanosomiasis in animals.

A short description of the major pathological lesions observed in the cattle used in the present experiment is useful from many standpoints. Firstly the effects produced by T.congolense vary in intensity depending on the strain of both the trypanosomes and the cattle hosts. The descriptions presented by the above mentioned investigators are on the chronic disease running over many months. The strain used in the present experiment produced a subacute disease running a course of approximately 5 to 8

weeks. Perhaps the description given in the present experiment is the first account of the effect of an Ethiopian strain of T.congolense on Ethiopian cattle. Secondly the sequential changes in the development of lesions are presented based on a simultaneous infection of a number of cattle and serial slaughter of individual infected animals at weekly intervals. Most of the previous works on large animals concentrated on the terminal pathological changes. Thirdly it gives a connected account and helps present a better overall picture of the disease in cattle when presented along with the other pathophysiological findings described earlier in this thesis.

Materials and Methods

Seven yearling male cattle were inoculated with approximately 4.3×10^6 T.congolense each. One infected animal was slaughtered per week starting the first week after infection and the gross pathological lesions recorded. Tissue samples from various organs were fixed in 10% formol-saline, dehydrated and cleared in alcohol-amyl acetate-chloroform series and embedded in paraffin wax. Sections were stained routinely with haematoxylin and eosin and also with Perl's Prussian Blue. The general design of the experiment is shown below (note that death in some calves occurred prior to the scheduled date of slaughter).

Weeks post infection	Calf identification							
	infected							control
	23	24	25	26	27	28	29	31
1	killed							
2		killed						
3			killed					
4				killed				
5					killed			
6						killed		
7							killed	killed

Results

Haematological findings

A progressive development of significant levels of anaemia starting at approximately 2 weeks post infection was shown (tables 17, 18, 19).

Parasitaemia

Parasites in the peripheral circulation were detectable starting at 5 days post infection and all animals were positive by 8 days (table 20).

Body temperature

There were no dramatic changes in body temperature during the period of infection. No consistent correlation between body temperature and levels of parasitaemia in the peripheral circulation could be demonstrated (table 21).

Pathological findings

A short account of the major gross pathological observations following portmortem examination and the histological results are presented below.

Gross pathological findings

0 - 4 weeks post infection

During the first four weeks of infection no obvious lesions were found (apart from evidence of previous infection with *Fasciola spp* in two animals) and the animals were in good bodily condition.

Table 17

PCV(%) changes in cattle infected with T.congolense

	Infected							Control
Calf	23	24	25	26	27	28	29	31
Date								
7/9	32.5	27.5	36.0	36.5	32.0	30.0	31.0	33.5
8/9	31.0	28.0	33.5	34.0	33.0	32.0	30.0	30.0
12/9	32.0	31.0	33.0	41.0	36.0	34.0	34.0	34.0
13/9	31.0	30.0	32.0	39.0	33.0	31.0	33.0	33.0
15/9	-	28.0	27.0	38.0	28.0	33.0	29.0	33.0
18/9	-	25.0	24.5	34.5	27.0	28.0	25.0	29.0
20/9	-	23.0	24.5	30.0	24.0	23.5	26.0	32.0
22/9	-	-	24.0	34.0	26.0	21.0	28.0	28.5
25/9		-	24.5	28.0	26.5	21.0	24.0	31.0
27/9		-	19.0	22.0	19.0	17.0	19.0	29.0
29/9			-	22.0	19.0	17.0	19.0	29.0
2/10			-	18.0	19.0	17.5	19.0	31.5
4/10				18.5	17.0	16.5	18.0	30.0
6/10				17.0	16.5	15.0	18.5	30.0
9/10				16.0	15.0	14.0	14.0	29.0
11/10				14.0	13.5	13.0	14.0	30.0
13/10				13.5	12.0	-	11.0	29.5

Table 18

Haemoglobin (gm %) values in calves infected with *T.congolense*

	Infected							control
Calf	23	24	25	26	27	28	29	31
Date								
7/9	11.8	9.6	12.5	12.9	11.4	11.0	10.5	11.2
13/9	12.5	10.8	12.0	9.5	11.6	11.6	12.5	12.5
20/9	-	9.4	10.6	11.2	10.1	9.2	11.4	12.3
28/9	-	-	6.4	7.3	6.3	5.4	6.3	9.9
4/10			-	6.3	5.6	4.9	6.4	10.4
6/10				5.3	5.9	4.8	5.7	9.2
11/10				4.9	4.8	4.3	4.5	11.2

Table 19

Total red cell counts (10^6) in cattle infected with T.congolense

	Infected							Control
Calf	23	24	25	26	27	28	29	31
Date								
28/8	7.30	5.62	6.89	8.08	8.55	-	-	-
7/9	10.00	6.50	7.33	10.02	6.87	6.37	6.97	6.78
13/9	9.10	6.80	8.11	-	6.36	8.32	7.92	6.58
20/9	-	4.42	5.68	6.64	5.28	5.46	4.96	5.80
28/9	-	-	5.31	6.12	4.89	4.27	6.87	7.99
4/10	-	-	-	4.32	3.19	3.26	3.75	7.85
11/10	-	-	-	1.78	2.05	1.99	0.95	6.46

Table 20

The development of Parasitaemia in cattle infected with T.congolense (the level of parasitaemia shown is according to the method used by Desowitz and Watson (1953) described earlier)

	Infected							Control
	23	24	25	26	27	28	29	31
Date								
7/9	-	-	-	-	-	-	-	-
8/9	-	-	-	-	-	-	-	-
12/9	+	++	+	-	-	-	+	-
13/9	+	+	+	-	-	-	-	-
15/9	killed 14/9	+	++	+	+	+	+	-
18/9		++	+	+	+++	+++	+	-
20/9		+	++	+	+++	++	++	-
22/9		killed	+	+	++	++	++	-
25/9			+	+	+	+	++	-
27/9			++	++	++	+	+	-
29/9			+	++	+++	+	+	-
2/10			killed	+	++	++	+	-
4/10				+	++	++	+	-
6/10				+	+	+	+	-
9/10				+	++	++	+	-
11/10				+	++	+	+	-
13/10				+	++	12/10	++	-
18/10				dead	killed 16/10		dead 15/10	-

Table 21

Body temperature changes (°C) in cattle infected with T.congolense

	Infected							Control
Calf	23	24	25	26	27	28	29	31
Date								
8/9	39.9	39.6	40.1	39.8	40.2	39.1	39.6	40.3
12/9	38.3	37.9	38.7	37.5	36.6	36.4	37.1	36.1
13/9	40.0	39.2	39.2	38.6	40.2	39.0	38.6	38.2
14/9	39.9	38.9	39.4	37.2	39.2	38.0	38.2	37.7
15/9	-	38.4	40.0	39.2	39.1	37.7	38.9	37.4
18/9		39.2	39.2	38.9	38.2	39.0	39.7	38.1
19/9		37.8	38.7	38.7	38,7	37.8	38.1	37.3
20/9		37.5	39.0	38.4	39.3	38.7	37.8	38.0
21/9		37.9	38.8	37.9	38.2	39.0	38.5	37.4
22/9		-	38.4	37.9	38.8	37.9	38.0	37.0
25/9		-	37.2	37.1	37.4	36.4	37.4	37.7
26/9			38.7	39.1	39.5	36.9	39.8	37.9
28/9			37.6	39.4	37.7	38.9	39.1	38.9
29/9			-	38.8	38.7	38.3	38.9	37.1
2/10				37.9	37.7	38.2	38.0	36.3
3/10				39.4	38.6	38.3	38.6	37.2
4/10				39.0	39.1	39.0	39.3	38.1
5/10				39.2	38.9	39.6	39.6	37.6
6/10				39.0	39.7	39.3	39.8	38.3
9/10				39.5	39.5	39.2	39.0	38.6
10/10				39.4	39.3	39.3	39.4	38.2
11/10				39.0	39.3	39.3	39.2	38.2
12/10				39.3	39.2	37.9	38.3	37.5
13/10				39.1	39.1	-	38.2	36.6
16/10				40.1	35.3	-	-	38.8

4 - 6 weeks post infection

By five weeks a marked deterioration was noted in all the infected calves. There was a marked loss of body condition and some animals were comatose at slaughter. Marked gross pathological lesions also became apparent.

General

All the animals were in poor bodily condition at slaughter and showed general enlargment of lymph nodes. Subcutaneous gelatinous fluid was a constant finding.

Thorax

The heart was flabby with petachial haemorrhages along major vessels. The pericardial fat was gelatinous and hydropericardium (20ml) was found in one calf (5.5 weeks infected).

The lungs were grossly oedematous and straw coloured fluid dripped from the cut surfaces and the interlobular septae were very prominent.

Abdomen

There was a general absence of adipose tissue in the abdomen. Gelatinous fluid was frequently found in the omental folds and other sites of normal fat deposition. There was general lymphnode enlargement throughout. Hepatomegally was also a constant finding. The spleen was very markedly enlarged and showed bulging on cut surfaces. The perirenal fat was gelatinous whilst the kidneys appeared normal.

Musculature

There was general "wet" appearance of the musculature and gelatinous fluid was especially obvious along fascial divisions.

Brain

The drain showed no gross lesions

Histological Findings

The Mononuclear Phagocytic System (van Furth et al, 1972).

There was a marked increase in numbers and activity ofthe mononuclear macrophages in lymph nodes (fig 21) spleen and bone marrow. In addition the Kuppfer cells of the liver were obviously increased in number.

Immunological Apparatus

The lymph nodes in addition to showing a marked macrophage response had broad medullary cords packed with plasma cells (fig 22). Germinal centres were present in the cortex and usually appeared very active (fig 23) although in a few instances they were small and inactive and the cortex appeared reduced in width. The spleen showed marked cellular activity both in the red and white pulp. The white pulp was increased in volume with a development of germinal centres (fig 23) and in some cases was populated with large lymphoid cells. Large lymphoid cells and plasma cells were occasionally found encircling the follicular artery; an area usually recognised in the bovine as the thymic-dependent area. In the red pulp, there was

a marked increase in plasma cells; there was evidence of extra-medullary erythropoiesis and numerous macrophages were present. An obvious feature in all spleens was that they were packed with red blood cells which in some cases were obviously being phagocytosed by macrophages (fig 24); in addition, extensive deposits of laemosiderin were found (fig 25).

Organ and Tissue Damage

Throughout the body it was possible to identify trypanosomes in the microcirculation of all tissues and organs (fig 26) but never definitely in the tissues. In addition these vessels usually contained numerous mononuclear cells including lymphocytes, large lymphoid cells, monocytes and macrophages in some cases, especially in sinosoids, even plasma cells being obvious. These cells migrated from the circulation and produced a striking feature which was common to most tissues and organs, namely perivascular cuffing with lymphocytes, lymphoblasts, plasma cells and macrophages. The organs most obviously affected were the brain, heart, skeletal muscle, fascial tissues everywhere and kidneys. It should be noted that in most tissues and organs it was possible to identify severe tissue oedema. This latter feature was also noted as being marked at post-mortem examination.

In the brain perivascular cuffing and oedema was found in both the cerebrum (fig 27) and cerebellum. In addition there were occasional foci of gliosis. In some areas the vessel's walls appeared thickened,

possibly the result of a degenerative change or due to oedematous swelling of the vessel wall. It is worth noting that in the heart and in the skeletal musculature numerous sarcocysts were present. In the heart these sarcocysts were also found in Purkinje fibres. At no point was any cellular reaction found in association with the sarcocysts. In addition to marked interfibre oedema, the main lesion present in the heart was an interstitial myocarditis which in some cases was very severe (fig 28). The blood vessels in these areas were cuffed with lymphocytes, lymphoblasts, plasma cells and macrophages and this cellular infiltrate was spreading to involve the myocardial fibre tissue which was degenerate and dying in some locations. Similar changes were seen in skeletal musculature. In the kidneys of all cases, focal areas of mononuclear cells of the type previously described were found in the cortex and in the medulla. A striking feature of this change was that these focal interstitial accumulations were always located around blood vessels. This may be an important diagnostic point in trypanosomiasis. In the liver, in addition to the marked Kuppfer cell activity mononuclear cells were numerous in blood vessels and in the sinusoids.

Haemopoietic System

The spleens of infected animals were packed with red blood cells and marked extramedullary erythropoiesis was often obvious. While erythrophagocytosis was seen,

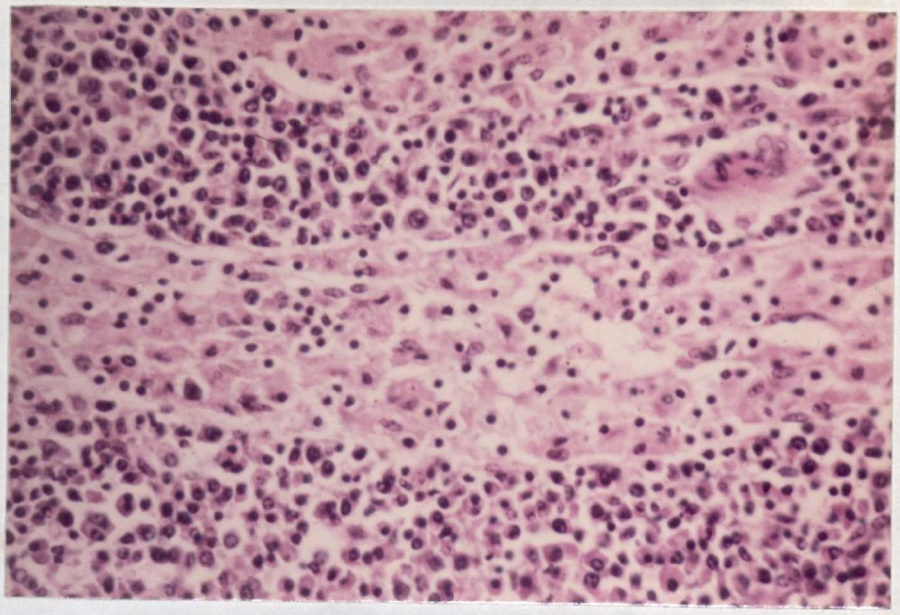


Fig. 21 Medulla of bovine lymph node five weeks post infection. Medullary sinuses are packed with macrophages and medullary cords are expanded with plasma cells. H.E. x250

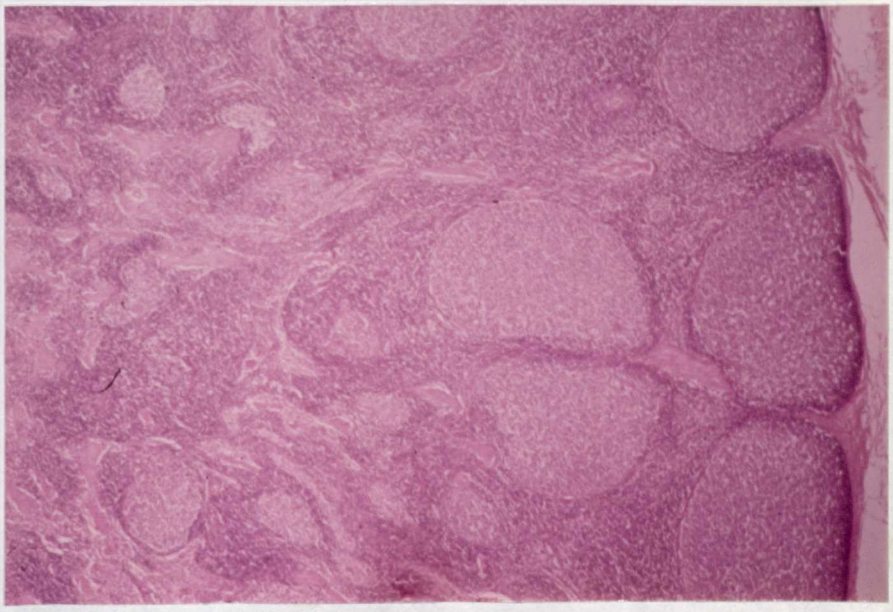


Fig. 22 Lymph node of bovine five weeks post infection. The lymph node is packed with numerous lymphocytic follicles containing large active germinal centers. H.E. x30

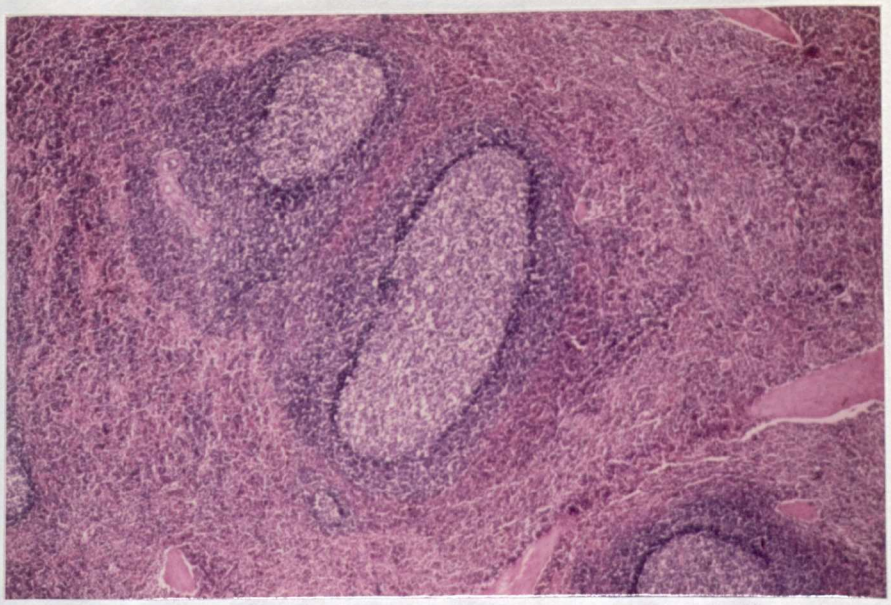


Fig. 23 White pulp of bovine spleen five weeks post infection. The white pulp is expanded with active germinal centers. H.E. x30

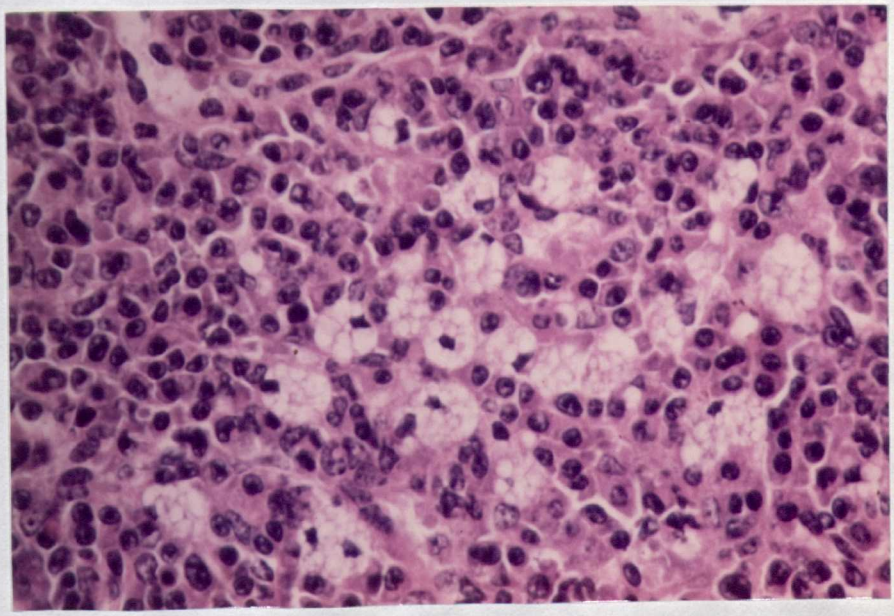


Fig. 24 Red pulp of bovine spleen five weeks post infection. The numerous macrophages present are packed with phagocytosed red cells. Note also the presence of numerous plasma cells. H.E. x500

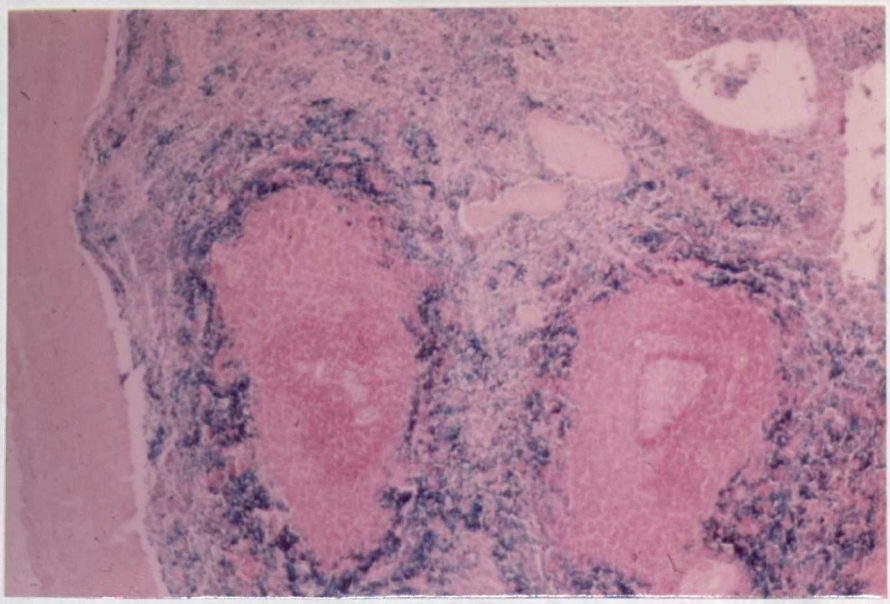


Fig. 25 Spleen of bovine five weeks post infection packed with haemosiderin deposits. Perl's Prussian Blue, x30.

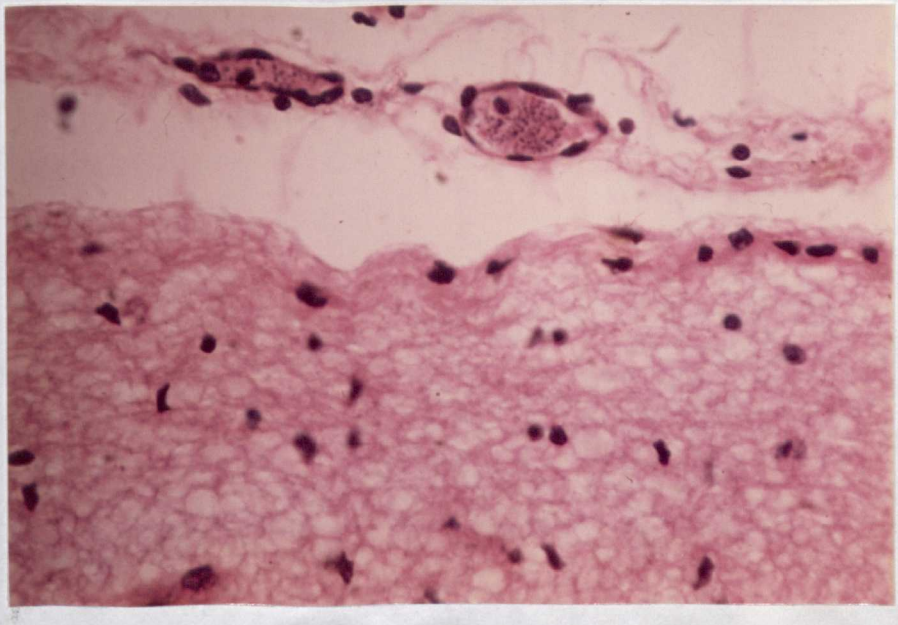


Fig. 26 Post capillary venules in cerebrum of bovines five weeks post infection. The blood vessels are packed with trypanosomes. H.E. x500 .

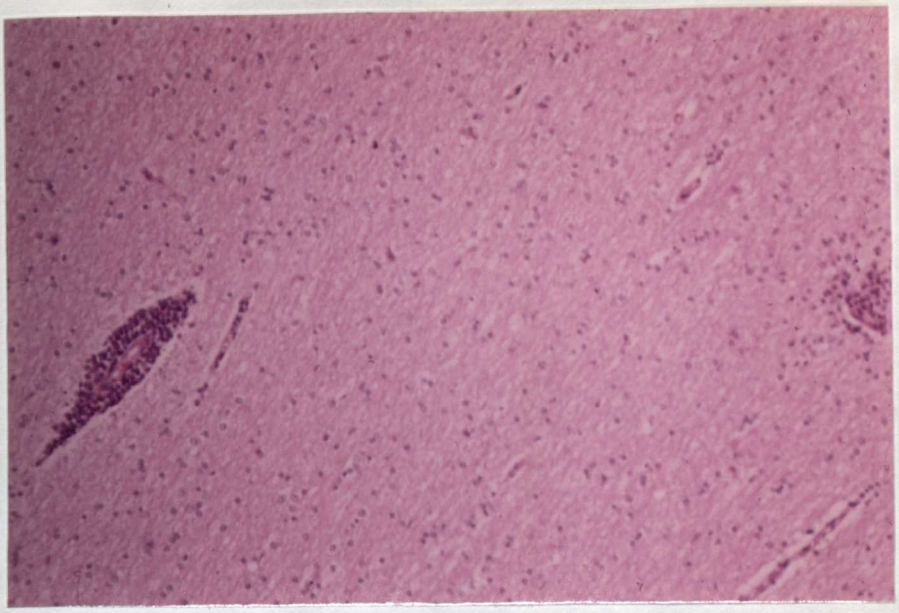


Fig. 27 Perivascular cuffing and gliosis in white matter of cerebrum in the bovine. H.E. x110

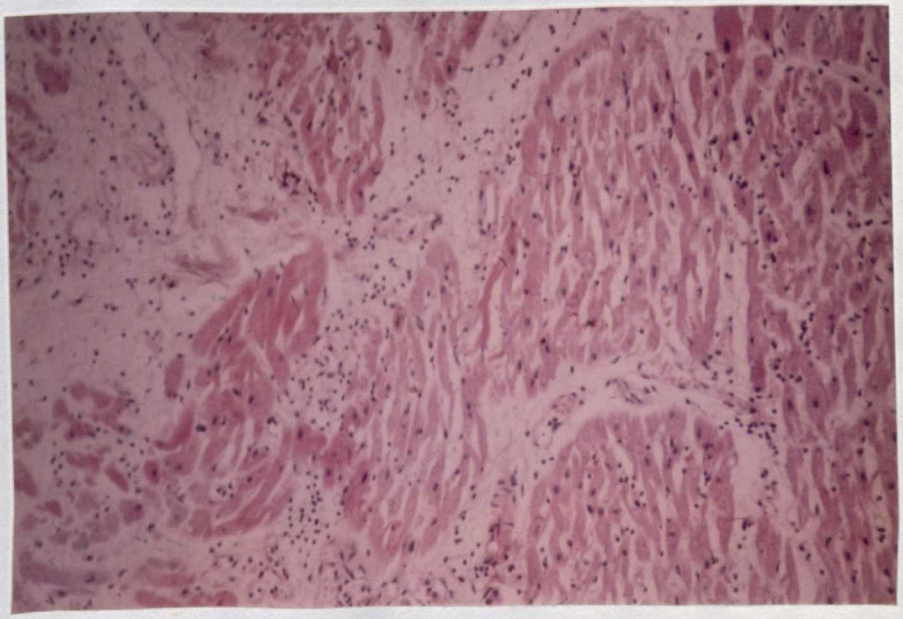


Fig. 28 Cardiac muscle. Severe myocarditis in the bovine five weeks post infection. Note marked interfibre oedema and extensive infiltration with mononuclear cells. H.E. x110

it was difficult to assess the extent of this. The degree of haemosiderin deposits was increased in some cases.

Discussion

The pathological findings show that anaemia develops early in the infection and also strongly suggests this to be the most important cause of death. Consistent gross lesions were the development of muscle tissue wasting, oedema of the fascia and oedema and degeneration of the adipose tissue. Miscroscopically oedema and perivascular cuffing were shown to be very common and trypanosomes and mononuclear cells were abundant in the microcirculation of all tissues and organs examined.

From the results of the present experiment it could be observed that the gross pathological lesions start to become obvious after four weeks of infection and death may follow soon after. Obviously the pathological effects would vary depending on the virulence of the strain and the resistence of the host. For example Naylor (1971) failed to show any brain lesions in cattle infected chronically with T.congolense. In the present study brain lesions in the form gliosis, perivascular cuffing of the microcirculation, thickened blood vessel walls, and oedema of both the cerebrum and cerebellum were shown. This most probably is a demonstration of differences in the virulence of the two strains of trypanosomes. Macroscopically he observed only slight morbid changes with only some of the cattle showing

emaciation and slight oedema and this is in contrast with the findings in the present experiment.

A marked increase in the mononuclear phagocytic cells of the infected cattle was a common feature. The number and activity of macrophages in the spleen and lymph nodes as well as the Kuppfer cells of the liver were shown to be markedly increased. The spleens were shown to be packed with red blood cells in all cases and in some cases phagocytized red cells were shown in the splenic macrophages and this finding is in agreement with those of Mackenzie and Cruickshank (1973) in T.congolense infected sheep.

In experiment I of the red cell survival studies using ^{51}Cr it was shown that a significant proportion of the ^{51}Cr activity lost daily from the circulation was not recovered in the faeces and urine but retained in the body. It is more than coincidence that the macrophagic cells and red cell concentrations increased significantly in the spleens of the infected cattle in the present experiment and retention of ^{51}Cr in infected cattle described in section I. The most probable explanation for this, based on evidence presented, is that large numbers of red cells were removed from the circulation and concentrated in the spleen where they were phagocytized in due course. Theoretical considerations on some of the possibilities involved in erythrophagocytosis have been discussed in the previous section on isotopic studies on the anaemia of trypanosomiasis.

The pathological findings in the present experiment indicate a strong similarity with the descriptions given by Murray et al (1974) on T.brucei infections in rats. It is

thought that the fundamental pathological processes in all forms of animal trypanosomiasis are the same (Fiennes, 1970) though Losos *et al* (1973) challenged this and agreed with the view that *T.congolense* is strictly a blood parasite. Based on their studies on the sequential pathological changes in *T.brucei* infected rats Murray *et al* observed that there are three processes operating in the pathology of trypanosomiasis. First, damage to the immunological apparatus is believed to lead to immunosuppression and increased susceptibility to secondary infections; this has been discussed in the immunosuppression studies earlier in this thesis. Secondly, specific organ damage particularly to the myocardium is thought to be severe enough to cause death in some of the animals. Increased protein catabolism in infected animals (Jennings *et al*, 1973) is believed to be the cause of muscle wasting away. The reasons for the extensive damage to the adipose tissues are not clear although Roberts (1973) showed a marked reduction in the levels of serum lipids in *T.congolense* infected sheep. The third important factor is the development of anaemia which in some cases is severe enough to be the cause of death.

The overall pathological findings can be summed up by the pathologist's final comments. "Most of the organs and tissues were damaged by the presence of *T.congolense* and striking histological changes can be seen in these, however it was considered that the cause of death in the animals in this study was due mainly to the development of anaemia and also myocarditis, which in some cases was very severe".

GENERAL SUMMARY AND CONCLUSIONS

I The anaemia in trypanosomiasis

A. Haematological and biochemical studies

The haematological findings are in general agreement with those of Fiennes (1954, 1970) and Naylor (1971). In essence the infections produced a normochronic normocytic anaemia which in most cases was very severe in the advanced stages.

The biochemical tests performed were mostly on serum proteins and serum iron and iron binding capacity. Serum total protein concentrations showed a fall in all three forms of the disease (acute, subacute and chronic) with the most rapid decline occurring in the acute infections. In the chronic form there occurred a fall in the early parts of the disease with a subsequent rise to preinfection levels in the later parts of the disease. The increase in total protein was primarily due to the globulin fraction while albumin values remained low.

Serum iron levels and iron binding capacity in chronic infections showed fluctuations as the disease progressed but generally fell very low as death approached. This is in marked contrast to the findings of Tartour *et al* (1973) who associated excessively high serum iron levels with premortal collapse.

B. Isotopic studies on the anaemia

Erythrokinetic studies were performed using ^{51}Cr labelled red cells. This probably is the first application of this isotope in the study of the anaemia of bovine trypanosomiasis. Its use was considered necessary to establish some of the important kinetic changes involved in the loss of red cells and the fate of these cells. The significant findings in the erythrokinetic studies are summarized below.

1. There was a significantly greater loss of red cells from the circulation of the infected animals as compared to the controls. The "apparent half life" of ^{51}Cr labelled autologous red cells in the infected animals was 3.7 ± 0.7 days as compared to 6.1 ± 0.8 days in the controls.
2. The amount of ^{51}Cr recovered in the faeces and urine was only approximately 51% of that cleared from the circulation.
3. On evaluating the sequential changes in erythrokinetics a significant and progressive fall in the "apparent half life" of ^{51}Cr labelled red cells with the advancing disease was shown.
4. A significant increase in the absolute blood volume was observed as the disease progressed (a 21% increase in the infected animals as compared to 6% in the controls).

Ferrokinetic studies were performed using intravenous injections of ferric citrate (^{59}Fe) simultaneously with ^{51}Cr into the same calves described

significantly increased in trypanosomiasis and this finding refutes the suggestion by some previous investigators that haemopoesis may be suppressed. Absorption of ^{55}Fe increased in rate considerably as would have been expected from the increased plasma iron turnover rate and this ruled out malabsorption of iron as a contributory cause to the falling plasma iron levels and the developing anaemia.

The main reason for the anaemia appears to be due to a considerably greater loss of red cells from the circulation than to a significantly depressed rate of production.

The discrepancy in the amount of ^{51}Cr lost from the circulation and that cleared in the faeces and urine is probably due to retention of most of the cells cleared from the circulation and this will be mentioned later on with the pathological findings.

These findings strongly suggest that intravascular haemolysis per se is not the most important reason for the anaemia developing in bovine trypanosomiasis as has been suggested by many investigators.

There is a major difference between the acute disease and subacute and chronic in plasma ^{59}Fe turnover rate. In the subacute and chronic there is an increased rate of uptake as compared to a delayed uptake in the acute. The depressed rate of disappearance of isotopic iron from the circulation of acutely infected animals has been ascribed to biologically active substances elaborated by the host as a result of the infection and

these were shown to be elaborated very early in trypanosome-infected experimental animals; as early as two days post infection.

II Immunological aspects of trypanosomiasis

A. Studies on the protective value of radiation attenuated T.congolense and pathophysiological observations on challenge animals

In this experiment the following main observations were made.

1. Plasma ^{59}Fe turnover rate and haematological indices were used as a measure of protection and these indicated that some small degree of protection has been afforded by radiation attenuated organisms. The use of ferrokinetics represents a deviation from the normally common use of serological methods for the assessment of the degree of protection.
2. The vaccinated animals died at approximately the same time as the infected controls.

The deductions made out of the above findings are that a protection in terms of reduced pathogenity has been afforded but no real protection in terms of longevity over non vaccinated animals has been attained.

B. Immunosuppression in bovines infected with trypanosomiasis

A trivalent clostridial vaccine was used in these experiments and the findings are summarized below.

1. In animals infected prior to vaccination a significantly

lower immune titer developed as compared to the controls.

2. In those vaccinated and then infected after the titer has risen normally, the immune response to secondary vaccinations was significantly depressed as compared to the controls.

3. The serological response to booster vaccinations in the infected animals was less than in normal controls.

These experiments mark the first demonstration of depression of the immune response in the bovine as a result of infection with trypanosomes though this phenomenon has been demonstrated by other workers in the laboratory animals. Discussions on the theories of the basic mechanisms as well as the possible repercussions to the African livestock industry are presented in the text.

III Pathological findings

Gross and histological observations were made on a group of experimental cattle and the major findings are summarized.

1. Splenomegally, degeneration of muscular, adipose and connective tissues, petechial and echymotic haemorrhages especially on the cardiac musculature are among the major gross findings.

2. Histologically it was shown that a marked increase in the mononuclear lymphoreticular cells occurred. The spleen was packed with red cells some of which were shown to be phagocytized. Extramedullary haemopoesis was shown in the spleen. Myocarditis was common and in some cases very severe. Severe oedema

of the fascial connective tissue in all the tissues examined was demonstrated. Lesions of the C.N.S. in the form of gliosis and perivascular cuffing of the microcirculation were observed though the brain appeared macroscopically normal

From these findings and the pathophysiological results the following deductions were made.

1. There occurred excessive removal of red cells from the peripheral circulation concentrating them in the spleen and that some of these cells were phagocytized by the macrophages that were shown to be markedly increased in the spleen. Some intravascular haemolysis occurs but this probably is not the major factor in the development of the anaemia.
2. Some loss of red cells from the peripheral circulation does occur through haemorrhages though the magnitude of such loss could not be established.
3. The above two factors, especially the first, are likely to be responsible for most of the significant loss of ^{51}Cr from the circulation of infected animals described earlier. Most of the lost cells have probably been concentrated in the spleen creating a discrepancy between the amount of ^{51}Cr lost from the circulation per unit of time and the amount recovered in the urine and faeces.
4. The extramedullary haemopoesis observed is possibly important as an indicator of bone marrow incompetence in meeting the body's demands, otherwise

it is probably of limited usefulness as compared to the active bone marrow.

5. Based on the pathological findings the cause of death in the experimental animals was attributed to anaemia and myocarditis.

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A P P E N D I X

Table I

Pack cell volume (PCV), Haemoglobin (HB), Mean corpuscular haemoglobin concentration (MCHC), and Mean corpuscular volume (MCV) values in cattle infected with acute T.congolense and in controls.

	Animal identification	Weeks post infection											
		0				1				2			
		PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %
INFECTED	161	24	7.4	43.5	31.3	24	8.1	56.3	33.7	16	5.6	46	35
	168	40	12.3	43.1	30.8	34	7.1	50.1	27.3	25	8.5	44	32
	181	26	7.9	53.8	32.0	28	9.4	53.1	33.4	23	7.7	51	34
	mean	30	9.2	46.8	31.4	28.7	8.2	53.3	31.5	21	7.1	47	34
	s.d.	8.7	2.6	6.0	0.6	5.0	1.1	3.1	3.6	4.7	1.3	3.5	1.3
CONTROL	129	23	6.6	55.8	32.0	20	6.4	52.8	32.3	22	7.5	46	34
	177	32	10.3	37.7	32.7	31	10.3	52.3	33.2	31	9.5	41	34
	178	24	8.1	52.3	33.7	25	8.6	54.0	30.6	30	10.1	41	34
	mean	26	8.3	48.6	32.8	25.3	8.5	53.1	32.0	28	9.0	43	33
	s.d.	4.9	1.8	9.6	0.6	5.5	1.9	1.2	1.3	4.9	1.4	3.1	1.9
	"t" test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table II

Haematological indices in splenectomized cattle infected with acute T.congolense and in controls.

Weeks post infection

	Calf	0				1				3			
		PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %	PCV %	HB gm%	MCV μ^3	MCHC %
INFECTED	102	25	7.7	45.0	30.9	21	6.4	64.6	30.7	died	-	-	-
	132	35	11.2	48.6	32.1	died	-	-	-	-	-	-	-
	169	17	4.9	67.7	30.8	23.7	7.0	58.7	30.4	9	3.7	60	41.1
	mean	26	7.9	53.8	31.3	22	6.7	61.7	30.5	9	3.7	60	41.1
	s.d.	9.0	3.2	12.2	0.7	1.4	0.4	4.1	0.2	-	-	-	-
CONTROL	129	23	6.6	55.8	32.0	20	6.4	52.8	32.2	22	7.5	46.2	34.1
	177	32	10.3	37.7	32.7	31	10.3	52.3	33.2	31	9.5	41.6	30.6
	178	24	8.1	52.3	33.7	25	8.6	54.0	30.6	30	10.1	40.5	33.7
	mean	26	8.3	48.6	32.8	25	8.4	53.0	32.0	28	9.0	42.8	32.8
	s.d.	4.9	1.9	9.6	0.8	5.5	1.9	0.9	1.3	4.9	1.4	3.0	1.9
"t" test		ns	ns	ns	ns	ns	ns	$P < 0.05$	ns	-	-	-	-

Table III

A study of blood, plasma and circulating red cell volumes in calves infected with acute T.congolense (GMB II) two days post infection

	Calf	Weight Kg	Blood volume		Plasma volume		Circulating rbc volume	
			Total(ml)	ml/Kg	Total(ml)	ml/Kg	Total(ml)	ml/Kg
INFECTED	161	89	5954	67	4585	51	1369	15
	168	94	7191	77	4962	53	2229	24
	181	73	6012	82	4569	63	1443	20
	mean	85.3	6385.6	75.3	4705.3	55.7	1680.3	19.7
	s.d.	11.0	69.8	7.6	22.2	6.4		4.5
CONTROL	129	80	6487	81	5320	67	1167	15
	177	93	8756	94	6567	71	2189	24
	178	86	6877	80	5369	62	1513	18
	mean	86.3	7373.3	85.0	5750.3	66.7	1623.0	19.0
	s.d.	6.5	1213.2	7.8	707.6	4.5	519.8	4.6
	"t" test	NS	NS	NS	NS	NS	NS	NS

Table IV

Studies of blood volume, plasma volume and circulating red cell volume in splenectomized calves infected with acute T.congolense (two days post infection)

	Calf identification	Weight	Blood volume		Plasma volume		Circulating rbc volume	
		Kg	Total(ml)	ml/Kg	Total(ml)	ml/kg	Total(ml)	ml/Kg
INFECTED	102	86	8310	97	5568	65	2742	22
	132	61	4827	79	3379	55	1448	24
	169	71	5428	76	4397	62	1031	15
	mean	72.7	6188.3	84.0	448.0	60.7	1740.3	20.3
	s.d.	12.6	1861.8	11.4	1095.4	5.1	89.2	4.7
CONTROL	129	80	6487	81	5320	67	1167	15
	177	93	8756	94	6567	71	2189	24
	178	86	6877	80	5369	62	1513	18
	mean	86.3	7373.3	85.0	5750.3	66.7	1623.0	19.0
	s.d.	6.5	1213.2	7.8	707.6	4.5	52.0	4.6
"t" test		NS	NS	NS	NS	NS	NS	NS

Table V

Pack Cell Volume (%): A study of sequential changes in calves infected with subacute T.congolense

	Calf identification	Days Post Infection							
		0	4	11	18	25	32	39	46
INFECTED	86	29	33	25	20	15	17	15	14
	87	33	31	31	17	18	14	-	-
	88	29	30	25	18	16	14	12	-
	89	27	27	24	-	-	-	-	-
	90	32	31	30	24	18	17	14	-
	mean	30	30	27	19.8	17	16	14	14
	s.d.	2.4	2.2	3.2	3.1	1.5	1.7	1.5	
CONTROL	79	24	26	26	24	24	24	24	21
	80	25	28	19	25	26	24	27	29
	82	30	31	28	25	23	21	23	24
	84	31	32	36	25	24		19	23
	85	30	30	27	27	23	24	25	26
	mean	28	29	27	25	24	21	24	25
	s.d.	3.2	2.4	6.1	1.1	1.2	4.3	3.0	3.0
	"t" test	NS	NS	NS	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.01$	

Table VI

Blood Haemoglobin concentration (gm %): Studies of sequential changes in calves infected with subacute T.congolense

		Days Post Infection								
		-3	4	11	18	25	32	39	46	53
INFECTED	86	10.5	10.1	8.5	5.5	5.6	5.0	4.8	4.4	2.9
	87	13.6	9.3	8.1	5.1	4.8	4.2	D		-
	88	11.8	9.7	7.6	5.8	5.2	4.5	4.6	D	-
	89	8.1	8.3	6.6	D	-	-	-	-	-
	90	10.3	9.3	8.5	6.8	5.4	4.7	4.4	-	-
mean ± s.d.		10.9 ±2.02	9.3 ±0.67	7.9 ±0.79	5.8 ±0.72	5.3 ±0.33	4.60 ±0.33	4.6 ±0.2		
CONTROL	79	7.7	8.1	8.6	7.5	7.1	7.3	6.8	8.3	9.0
	80	8.8	8.8	7.0	6.6	7.0	7.2	7.9	7.9	9.2
	82	10.3	10.2	8.5	7.0	6.8	6.6	7.4	8.3	10.3
	84	11.0	10.1	10.3	6.9	5.5	4.3	7.5	7.1	8.5
	85	9.7	9.3	9.6	7.4	7.2	7.0	8.0	9.0	-
mean ± s.d.		9.5 ±1.28	9.3 ±0.88	8.8 ±1.24	7.0 ±0.36	6.7 ±0.69	6.5 ±1.24	7.5 ±0.46	8.1 ±0.7	8.3 ±0.8
P		NS	NS	NS	< 0.01	< 0.01	< 0.05	< 0.001		

Table VII

Mean corpuscular volume (μ^3) : A study of sequential changes in calves infected with subacute T.congolense

		0	4	11	18	25	32	39
INFECTED	86	35.5	44.1	40.9	32.4	37.5	35.4	40.9
	87	41.3	42.5	52.3	33.9	45.2	38.8	-
	88	36.3	44.1	38.5	34.9	41.6	32.2	37.2
	89	33.7	33.7	31.8	-	-	-	-
	90	41.0	39.6	37.2	35.9	30.2	34.6	30.0
	mean	37.6	40.8	40.1	34.3	38.6	35.3	36.0
	s.d.	3.4	4.4	7.6	1.5	6.4	2.7	5.5
CONTROL	79	29.7	48.4	46.3	45.8	44.9	39.6	40.1
	80	38.8	38.0	36.2	42.4	45.0	40.6	44.4
	82	41.1	41.3	34.8	34.0	29.6	30.7	41.3
	84	41.9	40.8	49.8	30.5	35.5	-	37.5
	85	42.3	45.9	34.3	37.2	39.2	40.1	45.4
	mean	38.8	42.9	40.3	38.0	38.8	37.8	41.7
	s.d.	5.2	4.2	7.2	6.2	6.0	4.7	3.2
	P	NS	NS	NS	NS	NS	NS	NS

Table VIIT

Mean corpuscular haemoglobin concentration : sequential changes in calves infected with subacute T.congolense.

		0	4	11	18	25	32	39
INFECTED	86	36.2	30.6	34.0	27.5	37.3	29.4	32.0
	87	41.2	30.0	26.1	30.0	26.7	30.0	-
	88	40.7	32.3	30.4	32.3	32.5	32.1	38.3
	89	30.0	30.7	27.5	-	-	-	-
	90	32.2	30.0	28.3	28.3	30.0	27.6	31.4
	mean	36.1	30.7	29.3	29.5	31.6	29.8	33.9
	s.d.	5.0	0.9	3.1	2.1	4.5	1.9	3.8
CONTROL	79	32.1	31.2	33.1	31.3	29.6	30.4	28.3
	80	35.2	31.4	36.8	26.4	26.9	30.0	27.2
	82	34.3	32.9	30.4	28.0	29.6	31.4	30.8
	84	35.5	31.6	28.6	27.6	22.9	30.7	39.5
	85	32.3	31.0	35.6	27.4	31.3	29.2	32.0
	mean	33.9	31.4	32.9	28.1	28.1	30.3	31.6
	s.d.	1.2	2.6	3.4	3.2	3.3	0.8	4.8
	P	NS	NS	NS	NS	NS	NS	NS

Table IX

Total protein (gm %) : sequential changes in calves infected with subacute T.congolense

	Calf identification	Days post infection						
		0	5	12	19	26	33	40
INFECTED	86	6.3	6.8	5.8	5.5	5.5	5.0	6.0
	87	6.0	6.8	5.6	died	-	-	-
	88	5.8	5.8	6.0	6.0	5.5	5.6	5.9
	89	5.5	6.0	6.0	5.6	5.0	5.3	died
	90	6.4	6.5	6.8	5.8	5.0	5.1	died
	mean	6.0	6.4	6.0	5.7	5.3	5.3	5.95
	s.d.	0.36	0.5	0.5	0.2	0.3	0.3	0.1
CONTROL	79	6.8	6.5	6.8	6.8	6.8	6.8	6.5
	80	8.0	7.5	7.5	7.5	7.3	6.8	8.0
	82	6.3	6.9	6.8	7.0	6.5	6.5	7.5
	84	7.0	7.5	7.1	7.5	7.3	7.4	8.8
	85	6.9	7.3	7.3	7.0	6.9	6.3	7.7
	mean	7.0	7.1	7.1	7.2	7.0	6.8	7.7
	s.d.	0.6	0.4	0.3	0.3	0.3	0.4	0.8
	P	< 0.01	< 0.05	< 0.01	< 0.001	< 0.001	< 0.001	< 0.05

Table X

Serum albumen (gm %) : Sequential changes in calves infected with subacute T.congolense

	Calf identification	Days post infection					
		0	5	12	19	26	33
INFECTED	86	2.4	2.6	2.2	2.1	2.1	1.7
	87	2.7	3.0	2.3	2.4	2.1	2.2
	88	2.8	2.9	2.7	2.5	2.2	1.9
	89	2.0	3.3	2.0	-	-	-
	90	2.6	2.6	2.3	2.3	1.9	1.6
	mean	2.5	2.7	2.3	2.3	2.1	1.9
	s.d.	0.3	0.3	0.2	0.2	0.1	0.3
CONTROL	79	2.7	2.4	2.6	2.6	2.7	2.2
	80	2.6	2.8	2.6	2.7	2.2	2.3
	82	2.8	2.9	2.7	2.7	2.4	2.3
	84	2.6	3.1	2.7	3.0	2.5	2.3
	85	2.7	3.2	3.1	3.0	2.9	2.4
	mean	2.68	2.9	2.7	2.8	2.5	2.3
	s.d.	0.0	0.3	0.2	0.2	0.3	0.0
	P	NS	NS	< 0.02	< 0.01	< 0.02	< 0.01

Table XI

Sequential serum iron (µg %) determination in calves infected with subacute *T. congolense*

	Calf identification	Days post infection				
		0	12	26	40	54
INFECTED	86	80	120	104	100	200
	87	90	110	110	100	-
	88	80	70	70	90	-
	89	80	90	-	-	-
	90	110	150	70	70	90
	mean	88.0	112.0	88.5	90.0	145.0
	s.d.	13.0	31.1	21.5	14.1	77.8
CONTROL	79	100	100	96	70	100
	80	170	190	160	130	130
	82	110	145	120	130	40
	84	120	150	90	60	90
	85	110	170	130	106	-
	mean	122.0	151.0	119.2	99.2	90.0
	s.d.	27.7	33.6	28.2	32.9	37.4
	P	< 0.05	NS	NS	NS	NS

Table XII

Total iron binding capacity : sequential studies in calves infected with subacute *T.congolense*

	Calf identification	Days post infection				
		0	12	26	40	54
INFECTED	86	480	478	300	420	372
	87	360	426	240	432	-
	88	480	360	240	300	-
	89	300	408	-	-	-
	90	480	660	270	348	-
	mean	420.0	446.4	262.5	375.0	372
	s.d.	84.9	122.1	28.7	62.3	-
CONTROL	79	600	420	408	450	468
	80	450	540	372	390	312
	82	360	420	348	360	300
	84	510	432	378	360	450
	85	480	408	450	366	-
	mean	480.0	444.0	391.2	385.2	382.5
	s.d.	87.5	54.3	39.2	38.3	88.8
	P	NS	NS	< 0.001	NS	NS

Table XIII

Pack cell volume - sequential changes in calves infected with chronic T.congolense

Calf No.	Weeks post infection										
Infected	0	2	3	4	6	7	8	9	10	11	12
18	28.0	31.0	21.0	18.3	20.0	16.5	16.0	16.5	15.5	16.0	15.0
19	28.5	24.3	20.5	19.0	20.0	16.5	18.0	17.5	16.0	18.0	17.0
20	29.8	25.3	20.5	20.0	16.0	15.0	16.0	16.0	15.0	17.5	17.0
mean	28.8	26.9	20.7	19.1	18.7	16.0	16.7	16.7	15.5	17.2	16.3
s.d.		3.6	0.3	0.9	2.3	0.9	1.2	0.8	0.5	1.0	1.2
Control											
17	40.0	41.3	40.8	34.8	33.8	39.0	38.0	35.5	35.0	35.5	-
21	33.5	31.0	36.0	32.5	29.5	29.0	34.0	28.5	26.0	30.0	29.0
22	27.8	28.3	30.1	26.0	34.5	30.5	29.0	34.0	35.0	38.0	34.0
mean	33.8	33.5	35.6	31.1	32.6	32.0	33.7	32.7	32.0	34.5	31.5
s.d.	8.9	6.9	5.4	4.6	2.7	5.4	4.5	3.7	5.2	4.1	3.5
"t" test	NS	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Table XIV

Weekly index Haemaglobin (gm %) in calves infected with the Gemu Gefa strain (chronic) of T.congolense

	Calf No.	Weeks post infection						
		0	2	3	4	6	7	8
INFECTED	18	8.8	5.5	6.6	6.1	5.1	6.1	4.6
	19	9.4	7.4	9.2	7.5	6.1	5.4	4.9
	20	8.7	6.8	6.9	6.9	4.6	5.0	4.2
	mean	9.0	6.6	7.5	6.8	5.3	5.5	4.6
	s.d.	0.4	0.7	1.2	0.7	0.8	0.6	0.4
CONTROL	17	11.9	11.0	13.6	13.7	10.0	1115	11.4
	21	10.3	10.1	10.5	10.5	8.0	5.9	7.9
	22	8.4	8.9	10.9	10.6	9.6	6.3	8.5
	mean	10.2	10.3	11.6	11.6	9.2	7.9	9.3
	s.d.	0.9	0.6	1.8	1.8	1.1	3.1	1.9
"t" test		NS	$P < 0.05$	$P < 0.02$	$P < 0.01$	$P < 0.01$	NS	$P < 0.01$

Table XV

Weekly Index mean corpuscular volume (μ^3) in calves infected with Gemu Gefa (Chronic) strain of T.congolense and normal controls

	Calf No	Weeks post infection						
		1	2	3	4	6	7	8
INFECTED	18	52.3	62.1	67.9	52.8	53.4	49.7	55.0
	19	47.9	48.8	53.5	50.0	50.3	54.8	63.8
	20	40.2	43.1	56.4	52.6	54.4	42.1	61.3
	mean	46.8	51.3	59.3	51.8	52.7	48.9	60.0
	s.d.	7.8	9.8	7.2	1.6	2.1	6.4	4.5
CONTROL	17	46.2	44.8	44.7	46.5	44.1	41.2	43.2
	21	41.8	42.5	66.5	52.5	46.1	46.3	53.6
	22	38.1	37.2	46.4	37.9	43.1	49.9	51.4
	mean	40.9	41.5	52.5	45.6	44.4	45.8	49.4
	s.d.	7.1	3.9	12.1	7.3	1.5	4.4	5.5
	P	NS	NS	NS	NS	<0.01	NS	NS

Table XVI

Weekly index Mean Corpuscular Haemoglobin Concentration (%) in calves infected with the Gemu Gefa strain of T.congolense (Chronic).

	Calf No.	Weeks post infection						
		0	2	3	4	6	7	8
INFECTED	18	30.4	30.7	30.1	36.8	31.2	39.6	28.7
	19	39.7	40.6	41.8	26.3	30.3	32.8	27.0
	20	37.7	32.4	35.3	39.7	28.7	33.6	26.4
	mean	35.9	34.6	35.7	34.3	30.1	35.3	27.6
	s.d.	4.9	5.3	5.9	7.1	1.3	3.7	1.2
CONTROL	17	35.6	35.3	37.6	35.1	28.3	32.8	32.1
	21	30.0	27.3	29.9	-	27.2	20.3	23.3
	22	32.6	34.2	33.9	40.6	27.7	20.5	29.4
	mean	32.7	32.3	33.8	37.9	27.7	24.5	28.3
	s.d.	2.8	4.3	3.9	3.9	0.5	7.2	4.5
	"t" test	NS	NS	NS	NS	NS	NS	NS