STUDIES ON AN ASPECT OF THE ANAEMIA OF BOVINE TRYPANOSOMIASIS *(Trypanosoma vivax)*

BY

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D.V.M., Ahmadu Bello University, 1974
M.S. Kansas State University, 1977

A THESIS

Submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Veterinary Pathology and Microbiology

AHMADU BELLO UNIVERSITY, ZARIA

SEPTEMBER, 1980

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DEDICATION

This piece of work is dedicated to all my well wishers.

ACKNOWLEDGEMENTS

I am extremely grateful to Dr. Daniel I. Saror of the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, who served as my major Adviser. His contributions during the investigation and preparation of the thesis are highly appreciated. Also to Professor C. O. Njoku, Head of Department, Veterinary Pathology and Microbiology for his advice during the investigation.

Gratitude is also extended to Professor Mary Hallaway, former Head of Department of Biochemistry, Ahmadu Bello University, Zaria, for the supply of essential reagents and her critical review and invaluable suggestions for writing the thesis. The exhaustive review and suggestions of Dr. A.A. Ilemobade of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, are highly appreciated. The advice of Dr. C.N. Chineme of the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria during the writing of the thesis is also appreciated.

The technical assistance of Mr. J.E. Nwosu, Chief Technologist, Department of Veterinary Parasitology and Entomology, Mr. S.A. Oyewo, Technologist I, Department of Biochemistry and all the staff of Veterinary Clinical Pathology and Veterinary Protozoology laboratories, as well as the Clerical staff of Veterinary Pathology and Microbiology are highly appreciated.

This investigation was sponsored by the Ahmadu Bello University Board of Research to whom I am very grateful.

INTRODUCTION

Trypanosomiasis has long been recognized as a major constraint for livestock production in Nigeria.

The course of the disease is generally considered to fall into two distinct phases: the initial "acute" phase characterized by a rapidly developing anaemia, and a "chronic" phase during which there is low-grade anaemia, and muscle wasting.

The exact aetiology of the anaemia in trypanosomiasis has been the subject of much controversy. Bone marrow hypoplasia, haemodilution, dyshaemopoiesis, reduced erythrocytes half-life and erythrophagocytosis, among others, have been suggested as mechanisms responsible for the anaemia. While the latter two factors are consistent findings during the acute phase of the disease, the process whereby the erythrocyte half-life is reduced has not been determined

Erythrocytes are known to have sialic acid (neuraminic acid) attached to their surfaces with younger erythrocytes having higher concentrations than older ones. The enzyme, sialidase (neuraminidase) is known to cleave sialic acid off erythrocytes, and such desialylated erythrocytes are rapidly removed from circulation with sequestration by the liver. This may result in reduced erythrocyte counts and hence packed cell volume of the peripheral blood.

For control or eradication of trypanosomiasis, a more complete understanding of the pathogenesis of the disease is essential. If trypanosomes are shown to produce sialidase *in vivo*, it can be postulated that the sialidase produced by trypanosomes during the acute phase of the anaemia when parasitaemia is generally higher, causes removal of sialic acid from the surface of erythrocytes. This could presumably reduce the erythrocytes half-life by altering the erythrocyte membrane surface and make them more susceptible to phagocytosis. Thus, the objectives of this study are to investigate:-

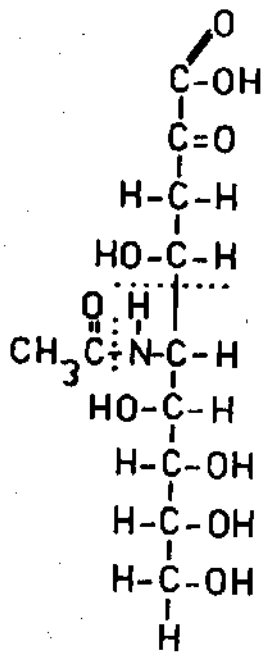
(a) the production of neuraminidase by trypanosomes *in vitro*

(b) the changes in erythrocyte surface sialic acid during acute trypanosomiasis,

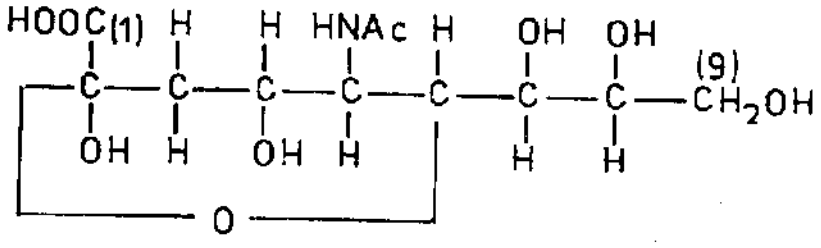
(c) the changes in serum sialic acid levels during acute trypanosomiasis.

It is hoped that these studies may help to explain, at least in part, factors contributing to the development of anaemia in the early phase of the disease.

FORMULAE OF SIALIC ACID



N-Acetylneuraminic acid



(Blix, Gottschalk and Klenk, 1957).

PART I

LITERATURE REVIEW

LITERATURE REVIEW

a. The Disease Syndrome:-

Trypanosomiasis has long been recognized as a major constraint to livestock production in tropical Africa. The disease, which is transmitted by Tsetse-flies (Glossina), is endemic in many parts of tropical Africa, including Nigeria, where these flies abound. The economic losses resulting from death of affected animals, stunting, debilitation and poor reproductive performance, have not been quantified but must be staggering.

Trypanosoma congolense, T. vivax and *T. brucei* are the major trypanosomes affecting livestock in Nigeria. Cattle, sheep and goats are most affected but recent reports indicate that dogs, and to a lesser extent, pigs are also affected. Mixed infections are also quite common in the field (Ilemobade, personal communication).

The pathology and pathogenesis of trypanosomiasis have been the subject of much investigation and many excellent reviews are available (Losos and Ikede, 1972; Clarkson, 1976; Jennings, 1976; Murray, 1978; Tabel, 1978). Several factors are known to influence the pathology and pathogenesis of trypanosome infections in animals, and prominent among these, is the ability of these organisms to undergo antigenic variation (Gray, 1965; Seed, 1974; Vickerman and Luckins, 1969; Vickerman, 1978). The latter is the process whereby trypanosomes

sequentially develop different antigens on their surfaces. The host then mounts an immune response which kills the trypanosomes that possess the particular antigen but is usually too late to kill the trypanosomes that have altered their antigenic identity (Murray and Morrison, 1978). The phenomenon of antigenic variation amoung trypanosomes has been actively investigated in recent years. Although much progress has been made, the genetic basis of this phenomenon is still unknown (Barry and Hajduk, 1978; Cross, 1978; Doyle, Hurumi, and De Gee, 1978; Vickerman and Tetley, 1978; Williams, 1978; De Gee, Shah, and Doyle, 1979; Reinwald, Rautenberg and Risee, 1979). Affected animals manifest waves of parasitaemia with each new parasite population carrying new surface antigens. These waves of parasitaemia are usually higher and of shorter duration early in the disease but as the disease progresses, the parasitaemic waves decrease and late in the disease, parasites become difficult to find in the blood stream.

Other factors which are known to influence the course of the disease include the species, the virulence, the numbers of the infecting trypanosomes, and the susceptibility of the host which is further influenced by age, nutrition and intercurrent infections (Losos and Ikede, 1972). Recent studies have shown that the susceptibility of the host is also related to the host's capacity

to limit the intensity of parasitaemia, together with the ability to eliminate the trypanosomes from the body (Murray and Morrison, 1978). That the presence of live trypanosomes in the host is essential to the pathogenesis of trypanosomiasis is supported by the finding that following treatment with trypanocidal drugs, the haematological picture generally rapidly returns to normal (Holmes and Jennings, 1976; Mackenzie and Cruickshank, 1973).

The course of the disease is generally acute, subacute, or chronic. Some animals die during the acute phase, while others survive into the chronic stage before death. Other animals may develop the chronic disease but survive for long periods in a wasted, stunted condition, while others may recover spontaneously.

Anaemia has long been recognized as a significant pathological feature of animal trypanosomiasis. The onset of anaemia in experimental trypanosomiasis for example, tends to coincide with the patency of parasitaemia. The development of the anaemia has been viewed in phases, based on the rate and the presence or absence of trypanosomes in the circulation, and the rapidity of the development of anaemia is influenced by several host and parasite factors.

Recent evidence indicates that the severity of the anaemia is closely related to the intensity of parasitaemia such that animals that are able to limit the magnitude of parasitaemia tend to develop anaemia at a slower rate while those animals that are unable to limit the parasitaemia develop acute trypanosomiasis. Although anaemia is a well recognized finding in bovine trypanosomiasis (Hornby, 1921; Fiennes, 1954; 1970; Naylor, 1971b; Wellde, Lotzsch, Deindl, Sadun, Williams, and Warui, 1974; Mamo and Holmes, 1975; Saror, 1975; 1979; Maxie, Lasos, and Tabel, 1976; 1979; Kobayashi, Tizard and Woo, 1976; Valli, Forsberg, and McSherry, 1978; Dargie, Murray, Murray and McIntyre, 1979a; Dargie, Murray, Murray, Grimshaw, and McIntyre, 1979b) the cause or causes of the anaemia have not been established. However, a number of hypotheses have been advanced by various workers to explain the mechanisms of anaemia of animal trypanosomiasis. Prominent among these hypotheses are haemodilution, defective trace mineral metabolism, haemolysis, reduced erythrocyte life-span and bone marrow depression (dyshaemopoiesis).

(b) Haemodilution in Trypanosomiasis

Haemodilution has long been suggested as a contributory factor in the anaemia of animal trypanosomiasis (Fiennes, 1954; Clarkson, 1968; Naylor, 1971b; Mamo and Holmes, 1975; Anosa and Isoun, 1976; Holmes and Jennings, 1976; Maxie and Valli, 1978;

Valli, Forsberg, and McSherry, 1978). By applying the vital red to stain the plasma, Fiennes (1954) observed haemodilution in the hyperacute and acute phases of bovine trypanosomiasis; however, one ox was a case of chronic hydraemia (haemodilution) during the study. Haemodilution was also observed in sheep infected with *Trypanosoma vivax* (Clarkson, 1968) using the Evan's blue dilution technique. While an actual increase in plasma volume was observed, there was no change in total blood volume. It was suggested that the increased gamma globulin, IgM may act as a plasma expander, or the resultant increased osmotic pressure might be responsible for the increase in plasma volume (Clarkson, 1968). The Evan's blue dilution technique, as measurement of haemodilution was also applied in cattle infected with *Trypanosoma congolense* (Naylor, 1971b). Although no measurement was made for blood volume, it was observed that about one-third increase in plasma volume occurred between the 2nd and 6th weeks of infection (Naylor, 1971b). The results of Mamo and Holmes, (1975) showed an absolute fall in the circulating red cell mass and an over-compensatory increase in the plasma volume of cattle chronically infected with *Trypanosoma congolense*. It was then suggested that haemodilution was of secondary importance in the development of anaemia (Mamo and Holmes, 1975). Further proof of haemodilution, as a contributory factor in the anaemia of animal trypanosomiasis was reported by Anosa and Isoun

(1976). The latter workers measured plasma volumes with ^{131}I-albumin and red cell volumes with ^{51}Cr-labelled erythrocytes in sheep and goats infected with *Trypanosoma vivax*. Their results showed a fall in red cell mass, and increases in both plasma and blood volumes indicating that the anaemia was in part due to haemodilution and partly as a result of fall in circulating red cell mass. The results of Holmes and Jennings (1976) showed that the phenomenon of haemodilution also occurred in rabbits infected with *Trypanosoma congolense* and *Trypanosoma brucei*. Red cell mass was measured by ^{51}Cr-labelled homologous red cells, while plasma volume was measured with ^{59}Fe labelled rabbit transferrin. It was observed that red cell mass decreased and plasma volumes increased in both infections. These changes were observed as from one week post infection. However, some species differences were observed. The plasma volume was markedly elevated in the *T. brucei* infected rabbits while the circulating red cell volume showed no significant reduction; whereas the *T. congolense* infected rabbits showed a less marked rise in plasma volume, the circulating red cell volume in the latter group showed a pronounced fall (Holmes and Jennings, 1976). After a subsequent ^{125}I-albumin labelling investigation (Holmes and Jennings, 1976) it was concluded that the increased plasma volume was associated with increased peripheral vasodilation.

Further evidence of haemodilution in bovine trypanosomiasis has been reported (Maxie and Valli, 1978; Valli *et al.*, 1978). The results obtained by using ^{51}Cr-labelled red cell mass and hence total blood volume of calves infected with *Trypanosoma congolense* indicated there was a relative increase in plasma volume which became significantly different from control calves (95% or greater) at weeks 6, 11, 13 and 18 after infection (Maxie and Valli, 1978; Valli *et al.*, 1978). The results showed that haemodilution was partly responsible for the anaemia. Subsequent studies with ^{59}Fe-labelled serum to measure plasma volume (Valli *et al.*, 1978) showed that infected calves had 8.37 litres of plasma while controls had 6.48 litres and these were significant at the 99% level. The dilution effect in bovine trypanosomiasis has also been related to the histological findings of widespread microvascular dilation (Valli and Forsberg, 1979) a feature also reported in *T. brucei* and *T. congolense* infected rabbits (Holmes and Jennings, 1976).

The contribution of haemodilution to the development of anaemia in trypanosomiasis has been called into question by some workers (Jennings, Murray, Murray, and Urquhart, 1974; Van den Ingh, Zwart, Van Miert, and Schotman, 1976; Dargie, 1978 Dargie *et al.*, 1979ab). Plasma volume changes in the anaemia induced by the

derivative of *T. brucei* TREU 667 infection in rats and mice were not determined, but in another strain, *T. brucei* TREU 226 which invariably produces anaemia within 3 days, accompanied by massive parasitaemia, no detectable increase in plasma volume, as measured by ^{125}I-labelled albumin, was found (Jennings *et al.*, 1974). They concluded that it was unlikely that plasma volume changes play a significant part in the anaemias induced by *T. brucei* TREU 667 infections in rats and mice. Van den Ingh *et al.*, (1976) observed decreases in some parameters e.g. packed cell volume and haemoglobin concentration in cattle infected with *T. vivax* while other parameters e.g. blood urea level, did not change substantially; it was then concluded that the contribution of haemodilution to the development of anaemia in animal trypanosomiasis was unlikely (Van den Ingh *et al.*, 1976). This view has been supported by Dargie, 1978, and Dargie *et al.*, 1979ab, who investigated the role of haemodilution in the anaemia of cattle infected with *T. brucei* and *T. congolense*. This was done in groups of adult Ndama and Zebu cattle using ^{51}Cr-labelled red cells, ^{125}I-labelled albumin and ^{59}Fe-labelled transterrin (Dargie, 1978; Dargie *et al.*, 1979ab). The concurrent measurements during the study provided a comprehensive insight into the dynamics of the host-parasite interaction and thereby a better understanding of the disease process (Dargie *et al.*,

1979b). They observed that the plasma volumes of both infected groups were significantly higher ($P < 0.02$ Ndama; $P < 0.002$ Zebu) and circulating red cell volumes significantly lower ($P < 0.01$ Ndama; $P < 0.002$ Zebu) than the corresponding control values. Total blood volumes were not altered by the infections (Dargie, 1978; Dargie *et al.*, 1979ab). It was concluded that the phenomenon of haemodilution is not a feature in the anaemia of animal trypanosomiasis (Dargie, 1978; Dargie *et al.*, 1979ab). They also advanced reasons why previous studies could not be accepted. It was reported that ^{59}Fe was rapidly transfered from the plasma (Dargie *et al.*, 1979b) and as such ^{59}Fe used to measure plasma volume (Valli *et al.*, 1978) did not equilibrate with the total plasma space and hence seriously over-estimated the plasma volume of anaemic animals.

In view of new experimental evidence, it seems reasonable to conclude that haemodilution may not play a significant role in the development of the anaemia in animal trypanosomiasis.

(c) Trace elements in Trypanosomiasis.

Iron and copper deficiencies have been causally associated with anaemia in man and animals (Jubb and Kennedy, 1970). Although many parasitic diseases of cattle are accompanied by disorders of iron

metabolism associated with anaemia (Jennings *et al.*, 1956; Hansard, Foote, and Dimopoullos, 1959; Campbell and Campbell-Gardiner, 1960) there have been few studies on the role of iron and/ or copper in the anaemia of bovine trypanosomiasis. Fiennes (1970) observed that in chronically infected cattle, the anaemia becomes microcytic and the spleen atrophied, indicating the possibility of iron deficiency or bone narrow failure. Tartour and Idris (1973) reported on metabolism of iron in Zebu cattle with *T. congolense* infection. They observed decreases in both serum iron (SI) and unbound iron binding capacity (UIBC) during the first week of infection. A slight increase in SI was observed in the 3rd week of infection. As the disease advanced , an irregular pattern of increased and decreased SI was observed. While the slight decreases of these parameters early in the infection wererelated to dilution effect, the periodic increases in SI were attributed to an accerated rate of erythrocyte destruction during haemolytic crises. Thus iron deficiency is not a feature of the anaemia in cattle caused by *T. congolense* (Tartour and Idris, 1973).

Saror (1975) investigated the behaviour of serum iron in Zebu cattle with *T. vivax* and *T. congolense* infections. Serum iron levels were decreased early in the infections in both *T. vivax* and *T. congolense* infected cattle. The initial drop in SI tended to coincide with the initial peak of pyrexia and parasitaemia.

Serum iron values remained below pre-infection levels for 2 to 3 weeks post infection. Thereafter, there were periodic increases in SI values as the disease advanced. It was concluded that iron deficiency was not a significant factor in the development of anaemia of bovine trypanosomiasis, at least for the first 9 weeks post infection (Saror, 1975). The role of serum copper in bovine trypanosomal anaemia has also been investigated (Saror, 1976). There were no significant differences in serum copper levels between the infected and control calves and it was concluded that copper deficiency was not a contributory factor in the development of trypanosomal anaemia (Saror, 1976).

Serum iron determinations in cattle infected with *T. congolense* (Valli *et al.*, 1978) showed no significant differences between the test and control; this was due to a great variation in the results from infected calves. However, in more severely affected calves, both serum iron and total iron-binding capacity on terminal serum samples were decreased. In less severely affected calves, both serum iron and total iron-binding capacity were elevated although not significantly different from control calves. It was suggested that the early marrow erythroid shift and increase in mean corpuscular volume were as a result of shortened uptake time of iron into the marrow (Valli *et al.*, 1978). In a subsequent group of calves

the radio iron half-time in infected calves was 1.7 hours and 3.6 hours in controls. However, serum iron would be expected to drop slightly with increased uptake by the marrow, early in the infection and then rise terminally as erythropoiesis becomes ineffective. Also iron-binding capacity could be expected to drop late in the disease, similar to the drop in albumin in chronic disease (Valli *et al.*, 1978). The results of Maxie *et al.*, (1979) from cattle infected with *T. vivax* and *T. congolense*, showed that serum iron concentration was significantly decreased in the *T. vivax* group on days 2, 6, and 9 and in the *T. congolense* group on day 9. The serum iron concentration was significantly increased in the *T. congolense* animals on days 23 and 40 post infection. Changes in the latent iron-binding capacity or percentage transferrin saturation were not significant in any group. It was suggested that the early hypoferremia seen in both infected groups might indicate increased erythropoiesis (Maxie *et al.*, 1979).

Ferrokinetic measurements were also undertaken in cattle infected with *T. congolense* and *T. brucei* (Dargie, 1978; Dargie *et al.*, 1979ab). Serum iron levels of the infected Zebu cattle were generally higher than normal, although 2 animals in their study had abnormally low values, e.g. less than 100ug/100ul (Dargie, 1978; Dargie *et al.*,

1979b). No such trend was observed in the Ndama but the values for this breed were lower than for the Zebu and significantly so ($P < 0.05$) in the case of the infected groups (Dargie *et al.*, 1979b). As a result of these differences, latent iron-binding capacity levels were 30% lower than normal in the infected Zebu, and only 17% lower in the Ndama. In both groups, but particularly in the Zebu, there were clear trends towards hypotransferrinaemia and increased transferrin saturation. These results would indicate a defective iron reutilization and the changes more marked in the Zebu cattle (Dargie *et al.*, 1979ab). It was concluded therefore that the anaemia of bovine trypanosomiasis was ultimately complicated by some degree of marrow dysfunction the basis of which was probably reticulo-endothelial iron blockage (Dargie, 1978; Dargie *et al.*, 1979ab). It does appear that from available experimental evidence, iron and/or copper deficiency is not significant in acute trypanosomiasis, but may have a role in the chronic phase of the disease.

(d) Haemolysis in trypanosomiasis

There is a concensus of opinion that the anaemia of animal trypanosomasis is haemolytic in origin (Fiennes, 1954; Naylor, 1971b; Jennings *et al.*, 1974; Mamo and Holmes, 1975; Mamo *et al.*, 1977; Saror, 1975; Van den Ingh *et al.*, 1976; Kobayashi *et al.*, 1976; Valli *et al.*, 1978). The mechanism(s) involved in the haemolysis has been the subject of much investigation. It is generally agreed

that the haemolysis is extra-vascular (Naylor, 1971b; Jennings 1974; Saror, 1975), although few studies had indicated that the haemolysis could be intravascular (Fiennes, 1954; Mamo *et al.*, 1977). Among the underlying mechanisms which conceivably could account for the haemolytic crisis are the release of a haemolytic factor, erythrophagocytosis, and an immunologic basis of cellular destruction.

(i) Haemolytic factor:-

It was first observed by Fiennes (1954) that if cells from normal bovine blood were incubated with trypanosome infected plasma the cells were haemolysed. The existence of a haemolytic factor was supported by the appearance of red blood cells in smears made at the time of crisis, by the positive indirect van den Bergh reaction, by the presence of urobilin in the urine of an extreme case, and by the improvement of reticulocyte counts following treatment (Fiennes, 1954). The haemolytic factor was also always present terminally, although during the course of the disease it was found to be present only intermittently and at its highest concentration at the time of trypanolysis. Fiennes (1954) advised that while haemolysis might be associated with the acute course of the disease or complicated in the fatal termination of the disease the haemolytic factor was not to be regarded as the underlying cause of the progressive anaemia.

The role of a haemolytic factor in the development of the anaemia of animal trypanosomiasis has further been investigated in

recent times. It was reported that *Trypanosoma brucei* produced a small molecular weight protein (10,000 daltons) which if incubated with normal red cells was capable of causing lysis (Nguyen Huan, Webb, Lambert, and Miescher, 1975). The haemolytic factor has since been characterized and purified and was observed to coincide with major haemolytic changes during the course of experimental *Trypanosoma brucei* infection in mice (Nguyen Huan *et al.*, 1975). The haemolytic factor is obviously significant in the development of anaemia in mice. It has been shown to be produced by other trypanosomes e.g. *T. vivax* affecting cattle, but its role in the development of anaemia has not been ascertained (Murray *et al.*, 1977). It has also been shown that *Trypanosoma congolense* generates, on autolysis, materials which are potently cytotoxic and haemolytic (Tizard and Holmes, 1976). This material is lipid in nature. Further studies have shown that the haemolytic activity might be related to the presence of free fatty acids generated by the action of phospholipase A on endogenous phosphatidyl choline. Some lysolecithin also contributes to the lytic activity. It was also noted that the absence of this phospholipase A in *Trypanosoma lewisi* probably makes the latter non-haemolytic (Tizard, Holmes, York, and Mellors, 1977; Tizard, Nielsen, Mellors, and Assoku, 1978a). A second class of haemolysins has also been characterized for *Trypanosoma brucei*. The latter haemolysin required more time (12 to 18 fold) than that of *Trypanosoma congolense* to lyse

equal volumes of sheep erythrocytes *in vitro*. This difference in the rates of haemolysis of sheep erythrocytes was found to be due to differences in the amounts of phosholipase A and hence, free fatty acids generated (Tizard, Sheppard and Nielsen, 1978b). It was concluded that a similar mechanism for the release of free fatty acids from the activity of phospholipase A on endogenous phosphatidyl choline exists in both *T. congolense* and *T. brucei* (Tizard *et al.*, 1978b) infections. These workers suggested that the haemolytic factor reported in *T. brucei* (Nguyen Huan *et al.*, 1975) might be a phospholipase.

Of the free fatty acids, it was shown that linoleic acid was probably the major contributor to the haemolytic activity. The mechanism of erythrocyte lysis is probably due to the detergent effect of linoleic acid, but it may also be due in part to the activities of its oxidized products (Tizard *et al.*, 1978a; Tizard, Holmes and Nielsen, 1978c). The significance of free fatty acids in the development of trypanosomal anaemia is questionable since free fatty acids are rapidly bound by serum albumin (Goodman, 1958 - cited by Tizard *et al.*, 1978bc). Thus, serum levels of free fatty acids have to be enormous in order to both exceed the binding capacity of the albumin and also achieve haemolytic levels (Tizard,

et al., 1978bc). This situation may however be possible in *T. congolense* infections where the organisms normally accumulate in large numbers in the capillaries (Losos and Ikede, 1972) and local destruction of these organisms occurs in association with local ischemia (Tizard *et al.*, 1978c). Nevertheless, calculations based on the amount of free fatty acids required to cause haemolysis *in vivo* indicated that this mechanism of erythrocyte destruction is probably unimportant in bovine trypanosomiasis (Tizard *et al.*, 1978c). There is thus little experimental evidence to support the suggestion that intravascular hemolysis is a significant factor in the development of anaemia in cattle with Trypanosomiasis (Fiennes, 1970).

While the hypothesis of a haemolytic factor in the pathogenesis of anaemia of animal trypanosomiasis appears attractive, it is probably only applicable at periods of high parasitaemias. The role of a haemolytic factor may, therefore, not be significant during the periods when trypanosomes are difficult to find in the bloodstream.

(ii) Erythrophagocytosis:-

Erythrophagocytosis has long been found to occur in the spleen of cattle suffering from naturally acquired trypanosomiasis

(Connal, 1911 - cited by Mackenzie and Cruickshank, 1973). Abundant phagocytosis of red cells has also been reported in rabbits infected with *T. brucei* (Boycott and Price-Jones, 1913).

Fiennes (1954) had observed erythrophagocytosis in the spleen, lymph nodes and haemolymph nodes of cattle experimentally infected with *T. congolense*, and had concluded that the cause of the anaemia in the acute stage of the disease had consisted, in part of the removal of red cells by erythrophagocytosis. Further observations on erythrophagocytosis as a contributory factor in the induction of anaemia in cattle infected with *T. congolense* have variously been reported (Naylor, 1971a; Losos, Paris, Wilson, and Dar, 1973; Valli and Forsberg, 1979).

Mackenzie and Cruickshank (1973) found varying degree of erythrophagocytosis in the spleen, lymph nodes and haemolymphs of naturally *T. congolense* infected sheep dying after acute haematological crisis. Erythrophagocytosis was not observed in sheep dying after chronic progressive disease. Also in sheep protected by trypanocidal drugs, which ultimately served as controls, the reticulo-endothelial system was found to be normal apart from a mild splenic haemosiderin deposition. It was concluded that the anaemia was due to a more rapid removal of erythrocytes from the circulation and that erythrophagocytosis of red cells was of substantial importance in the development of anaemia of animal

trypanosomiasis.

Erythophagocytosis has also been observed in bone marrow smears of *T. vivax* infected goats (Saror, 1980).

The phenomenon of erythrophagocytosis also occurs in the spleen of rats and mice infected with *T. brucei* (Murray, Murray, Jennings, Fisher, and Urquhart, 1974; Jennings *et al.*, 1974).

The observations of erythrophagocytosis, coupled with the general finding of haemosiderin deposits in the reticulo-endothelial system, have indicated that the haemolytic anaemia is extravascular in origin.

The causes of erythrophagocytosis have been reviewed by Jennings (1976) and these include the attachment of trypanosome antigen to the surface of red cells, their subsequent combination with anti-trypanosome antibody in the circulation, and eventual sequestration by erythrophagocytosis. Mackenzie, Boyt, Nesham, and Pirie (1978) also demonstrated the presence of trypanosomal material on sheep erythrocytes by the indirect flourescent antibody test and concluded that the phagocytosis of erythrocytes in sheep infected with *T. congolense* was due to the coating of the red blood cells surface with trypanosomal antigen. However, other alterations in the surface of the red cells, other than coating by trypanosomal antigen, might also make the red cells susceptible to phagocytosis (e.g. removal of sialic acid from the surface of erythrocytes).

(iii) Immunological basis of the anaemia of animal trypanosomiasis:-

There are reported instances of acute trypanosomal infections in which the onset of anaemia is related to the decline of the first wave of parasitaemia and the concurrent rise in serum antibody levels (Balber, 1974; Assoku, 1975; Kobayashi *et al.*, 1976).

Studies by Balber (1974) on the anaemia in mice infected with *T. brucei* indicated that the development of a severe anaemia was associated with the ability of the host to restrict the parasite growth during infection. Since restriction of the parasite growth was primarily mediated by immunologic mechanisms, it seemed likely that the immune response elicited by trypanosomes was playing an important role in the aetiology of the anaemia (Balber, 1974).

Assoku (1975) had also observed that rats infected with *T. evansi* showed significant immune responses to the infecting trypanosomes, with peak agglutinin titres occurring 10 - 12 days after infections, coincidentally with maximum destruction of erythrocytes. Circulating or adsorbed erythrocyte auto-antibodies were not observed during the investigation, but it was shown that repeated intravenous injections into rats of more than 100ug per 100gm body weight of soluble *T. evansi* antigen resulted in moderately severe, probably antibody mediated, haemolytic anaemia (Assoku, 1975). It was

therefore hypothesised that the development of the anaemia accompanying *T. evansi* infection might be immunologically-mediated (Assoku, 1975).

This view is also supported by Kobayashi *et al.*, (1976) who observed trypanosome antigen-antibody-complement complexes on the surfaces of erythrocytes of calves infected with *T. congolense*. They speculated that these immune-complexes caused the elimination of affected erythrocytes, leading to the clinical anaemia. Immunoglobulins bound to erythrocytes of 13 infected calves were detected by a direct antiglobulin test from 7 to 10 days post infection, the reaction being strongest between 3 and 9 weeks post infection. Erythrocytes reacting positively to this test were then lysed and the immunoglobulins eluted. Of the 74 eluates, 16 contained lgM and lgG. Antibody activity of these eluates against *T. congolense* was then demonstrated by means of the complement fixation, the indirect haemagglutination, and the indirect antiglobulin tests. It was postulated that the anaemia in mammalian trypanosomiasis, and specially in *T. congolense* infected calves, was of immunological origin (Kobayashi *et al.*, 1976).

Antibodies of the lgG and lgM classes had also been separated from the sera of mice infected with *Trypanosoma gambiense* (Takayanagi *et al.*, 1973). Both fractions were found to be

associated with the agglutination reaction, the formation of variant serotypes, and protection against infection.

Further circumstancial evidence for the immunolgical basis of the anaemia of animal trypanosomiasis had included the observed elevation of IgM immunoglobulin during animal trypanosomiasis (Luckins and Mehlitz, 1978; Clarkson, 1968; Clarkson *et al.*, 1975; Kobayashi and Tizard, 1976; Moulton and Sollod, 1976; Tabel, Rurangirwa, and Losos, 1978) and the presence of complement fragments on cell surfaces (Kobayashi and Tizard, 1976; Nielsen *et al.*, 1978abc). Antibodies to certain complement components, e.g. C_3 (immunoconglutinin) which are largely of the IgM class, (Eisen, 1974) were also elevated during infections with pathogenic African trypanosomes (Ingram and Soltys, 1960; Kobayashi *et al.*, 1976). However, Takayanagi *et al.*, (1974) have shown that complement was not involved in the attachment and ingestion of *Trypanosoma gambiense* to rat macrophages and it is therefore reasonable to suggest that the role of complement in the pathogenosis of the anaemia might be restricted only to the haemolysis of erythrocytes. It has also been suggested though, that the mechanisms of red cell destruction in trypanasome infections are complex and may vary with the species of trypanosmes,

the host and the stage of infection (Ikede, Lule, and Terry, 1977). For example, the anaemia was found to be more severe in mice infected with *T. congolense* than those infected with *T. brucei*, and antiglobulin test was only rarely positive in the late stages of *T. brucei* infection (Ikede *et al.*, 1977).

After observing anaemia within 4 days in some of the mice innoculated with *T. brucei*, Jennings, *et al.*, (1974) suggested that the rapid onset of the anaemia might have been due to damage to the erythrocytes as a direct or indirect result of trypanosome infection. An immunological mechanism might have been significant in the development of the anaemia only after day 7 of infection, a period when an immune response might have been mounted by the host. The cause of the anaemia earlier than day 7 of infection with a pathogenic trypanosome is therefore yet to be established.

(e) Erythrocytes life-span and Sialic acid in Trypanosomiasis:-

Few erythrokinetic studies have been undertaken in animals infected with pathogenic African trypanosomes.

Mamo and Holmes (1975) observed that the survival time of ^{51}Cr-labelled red cells was markedly decreased in *T. congolense* infected calves. It was shown that the ^{51}Cr-labelled

red cell half-life was 216 ± 47 days in control Zebu calves while in the infected calves this was reduced to 142 ± 33 days. The significant shortening of erythrocyte life span due to the accelerated loss of erythrocytes from circulation would appear to account for the anaemia observed (Mamo and Holmes, 1975).

It has also been observed that the mean time taken for ^{51}Cr-labelled red cell activity to fall by 50% was 3.7 ± 0.7 days in *T. congolense* infected calves as compared to 6.1 ± 0.8 days in the control calves (Mamo *et al.*, 1977). From this, it was shown that approximately 138ml of red cells were being lost from the circulation daily (Mamo *et al.*, 1977).

Valli *et al.*, (1978) also observed that the half-life of ^{51}Cr-labelled red blood cells dropped from an initial normal value of 10 days to 4.7 days at about 30 days after infection in *T. congolense* infected calves. After this time there was a gradual return towards normal red cell half-life at about 12 weeks. Thus the red cell life span was roughly halved during the acute phase of the infection (Valli *et al.*, 1978).

While there is a concensus that the red cell life span is reduced, the mechanism involved in the reduction of red cell life span has not been established.

Sialic acid, the acylated neuraminic acid (Blix, Gottschalk, and Klenk, 1957) occurs in appreciable quantities on the erythrocyte surface structures of several animal species (Eylar, Madoff, Brody, and Oncley, 1962; Seaman and Uhlenbruck, 1963). The bulk of the net negative surface charge of erythrocytes of most species of animals is due to ionized sialic acid (Eylar *et al.*, 1962). It has been shown that older erythrocytes migrated less in an electrical field due to decreased surface negative charge (Yaari, 1969) and decreased sialic acid content with increased agglutinability has been observed in older red cells (Greenwalt and Steane, 1973).

Reduction in sialic acid and surface charge has also been observed in older erythrocytes of humans, rats and rabbits (Durocher, Payne and Conrad, 1975). The latter workers used *Vibrio cholerae* neuraminidase to cleave off sialic acid from ^{51}Cr-labelled erythrocytes of rats and rabbits which were then injected into rats and rabbits. Radiochromium survival studies in these animals showed rapid clearance of these radio-labelled desialylated erythrocytes from circulation, with sequestration of such erythrocytes being mainly by the liver. Furthermore, these studies suggested that reduction in erythrocyte sialic acid content might be a mechanism of erythrocyte destruction and indeed might be important in erythrocyte senescence. Thus, loss

of cell surface sialic acid results in faster ageing and clearance of such desialylated erythrocytes from circulation by the reticulo-endothelial system where these erythrocytes undergo erythroclasis.

Removal of sialic acid from the surface of erythrocytes reveals new antigenic sites (Pirofsky, 1969), and it has been shown that anti-RBC antibodies are formed in human patients following removal of sialic acid from the surface or erythrocytes (Katz, Klajman, Yaretzky, Steiner, and Knyszynski, 1977). Thus the anti-RBC antibodies, which are incomplete and unbound, were found in the plasma of patients with immunological diseases such as autoimmune diseases, secondary immune deficiency, drug-induced immune diseases and cardiac traumatic haemolysis (Katz *et al.*, 1977). These incomplete anti-RBC antibodies may thus serve as opsonins and promote phagocytosis and destruction of erythrocytes by macrophages (Eisen, 1974).

In animal trypanosomiasis, however, there is very little information on sialic acid that might be of relevance to the pathogenesis of the anaemia.

Magaji (1975) has reported of elevated levels of serum sialic acid levels in cattle infected with *T. brucei* and *T. vivax*. It was however suggested that increased synthesis of this substance

by the organs invaded by these parasites might have accounted for the elevated serum sialic acid levels (Magaji, 1975). Banks (1979) has also shown that *Trypanosoma congolense* binds to bovine, sheep and goat erythrocytes *in vitro*. The binding was through the neuraminic acid receptors on the erythrocytes.

The charge on erythrocytes has been reported to decrease during trypanosome infections (Zuckerman, 1964 - cited by Mamo *et al.*, 1977).

It would appear therefore, that trypanosomes may directly or indirectly, through a factor, cause a cleavage of sialic acid from erythrocytes early in infection. This cleavage of sialic acid may in turn result in faster ageing and or faster clearance from the circulation of such desialylated erythrocytes, leading to anaemia that is characteristic of animal trypanosomiasis.

(f) Bone marrow response:-

Depression of erythropoiesis has long been observed in *T. brucei* infections of rabbits (Boycott and Price-Jones, 1913). It appeared the defect occurred in the earliest stage of the marrow growth and the conclusion was offered that the defective marrow response might not be related to inadequate stimulus, but to an inhibitory effect of the infection on the generation of red cells (Boycott and Price-Jones, 1913).

Fiennes (1954) observed that in *T. congolense* infected cattle, the red marrow of the long bones showed some degeneration and in post-critical period was replaced by yellow marrow and gelatinous material. However, the marrow of the sternum, ribs and the vertebrae appeared to be unaffected. It was therefore concluded that the primary cause of the anaemia was partly the result of the inhibition of the haemopoietic tissues of the bone marrow, whereby red cells lost were not replaced (Fiennes, 1954).

Naylor (1971) has also reported of degenerative changes in the bone marrow of *T. congolense* infected cattle. These changes included vacuolation of the cytoplasm and nuclei of the early stages of the granulocytic series, which also involved some of the mature cells. The erythrocytic series which also showed a tendency towards megaloblastic anaemia led to the conclusion that dyshaemopoiesis had a role to play in the induction of this anaemia (Naylor, 1971).

The contribution of depression of haematopoiesis in the development of anaemia of animal trypanosomiasis has also been reviewed (Losos and Ikede, 1972). The anaemia which accompanies trypanosome infections was described to result from inhibition of the haemopoiesis, and this conclusion was based on the absence of immature red blood cells in the circulating blood and the lack of evidence of stimulation of the marrow of the long bones (Losos and Ikede, 1972).

Losos *et al.*, (1973) had observed that the femoral bone marrow was reddish brown at the proximal epiphysis and this often extended down the shaft for one or two inches on the periphery of the cavity. Only rarely was there active marrow in the distal epiphysis of these cattle infected with *T. congolense*. There was serous atrophy of fat in the rest of the marrow which therefore led to the conclusion that there was erythropoiesis (Losos *et al.*, 1973). Similar observation of active erythropoiesis have been reported in *T. brucei* infected rabbits (Boreham, 1968) and rats (Jennings *et al.*, 1974; Murray *et al.*, 1974) and also in *T. evansi* infected rats (Assoku, 1975).

Jennings *et al.*, (1974) had shown that ^{59}Fe disappeared rapidly from plasma and the activity of the circulating red cells had indicated that the ^{59}Fe was rapidly being incorporated into the red cells. This observation would partly indicate that there was no loss in erythropoiesis.

Mackenzie and Cruickshank (1973) had also observed hyperplasia of the erythropoietic tissues of the bone marrow of sheep infected with *T. congolense*. This, together with the finding of reticulocytosis clearly indicated increased production of erythrocytes.

Saror (1975) also investigated bone marrow changes in cattle infected with *T. congolense* and *T. vivax* and observed that there was active erythropoiesis; a similar observation has recently been reported in goats infected with *T. vivax* (Saror, 1980).

The erythrokinetic studies of Mamo and Holmes (1975) also indicated there was active erythropoiesis during trypanosomiasis. The elevated plasma-iron turnover rate and the increased rate of utilisation of radio-iron in the red cells clearly demonstrated that erythropoiesis was increased in infected cattle, suggesting also that dyshaemopoiesis might be playing very little or no part in the aetiology of the anaemia (Mamo and Holmes, 1975).

The radio-labelling studies in rabbits infected with *T. congolense* by Holmes and Jennings (1976) have also shown increased erythropoiesis, which was essentially similar to that previously described in cattle by Mamo and Holmes (1975).

Van den Ingh *et al.*, (1976) also reported that the bone marrow of cattle infected with *T. vivax* was hyperplastic, indicating again that dyshaemopoiesis probably played no important part in the aetiology of this anaemia.

Maxie *et al.*, (1976; 1979) have reported on a comparative study of cattle infected with *T. congolense* and *T. vivax* and shown from bone marrow differential counts that the erythroid response was much more marked in *T. congolense* group than in the *T. vivax* group.

The anaemia in *T. congolense* infected cattle has further been shown to be responsive to sharply decreased myeloid: erythroid ratio and increased mean corpuscular volume (Valli *et al.*, 1978).

The ferrokinetic studies of Dargie, (1978); Dargie *et al.*, (1979ab) have confirmed that the rates of plasma iron turnover and red cell iron utilisation indicate erythropoiesis was faster in cattle infected with *T. congonlese* when compared to controls, therefore there was no overt evidence of dyshaemopoiesis. Nevertheless, the erythropoietic responses were surprisingly moderate for the degree of anaemia and the number of cells being removed. It was therefore suggested that the ability of the infected animals to increase red cell production was impaired, at least in relation to iron metabolism (Dargie, 1978; Dargie *et al.*, 1979ab).

Although there are numerous reports of active erythropoiesis during animal trypanosomiasis, recent experimental data seem to indicate some impairment in the ability of infected animal to increase production of red cells.

(g) Pharmacologically vasoactive factors in trypanosomiasis

Changes in pharmacologically active substances have been reported during animal trypanosomiasis. Although these substances may not cause anaemia as a result of direct haemolysis of the red blood cells, they might contribute to the pathogenesis of animal trypanosomiasis through their effects on the vascular system.

Tizard and Holmes (1977) reported that living or lysed *T. congolense* released a soluble vasoactive material of low molecular weight which was possibly peptide in nature. The material was not cytotoxic, but caused local inflammatory reaction when implanted intraperitoneally into rats. It was considered that this material might be contributing to the pathogenesis of *T. congolense* infection in animals.

Kinins were released in rabbits and cattle infected with *T. brucei*. The levels of the α_2 - globulin precursor, kininogen decreased at the same time as kinin was detected in the blood (Boreham, 1968; 1970 - cited by Boreham, 1978). It was observed that immune complex caused the release of kinins *in vitro* from a kininogen substrate. Injection of immune complex *in vivo* into rabbits caused a profound hypotension, which was inhibited by aprotinin. Trypanosomal antigen injected intravenously into rabbits with preformed antibody also resulted in hypotension caused by activation of the Kallikrein-kinin system (Boreham and Wright, 1976ab - cited by Boreham, 1978).

Van den Ingh *et al.*, (1976) also reported decreased blood serotonin levels, and also an initial rise with a subsequent fall in bradykinin levels in the blood of cattle infected with *T. vivax*. It was suggested that the increase in bradykinin levels was probably mediated by an immune reaction. Also in goats infected with *T. vivax*,

a gradual decrease in blood serotonin level during the acute phase, followed by a gradual return to almost normal values during the chronic phase had been observed (Veenendal, van Miert, Van den Ingh, Schotman, and Zwart, 1976). In this study, low bradykinin - like activity was detected despite the peaks of temperature, but this only increased very slightly during the chronic phase. It was concluded that bradykinin was not a major factor in the pathogenesis of chronic *T. vivax* infection and that the fluctuations of blood serotonin levels during temperature peaks, associated with peaks of parasitaemia and the presence of many platelet thrombi, suggested a correlation between *T. vivax* platelet aggregation and blood serotonin decrease.

How *T. vivax* activates bradykinin release is not clear, but since it was increased as early as 4 days post infection in the study by Zwart and Veenendal (1978), immune complex might not be responsible for the activation of bradykinin release, at least during the acute phase. These workers observed increased bradykinin levels, and attempted to correlate these with pathological and clinical changes attributed to bradykinin activity and/or trypanosomiasis, but the results were inconclusive. For example, no correlation between elevated bradykinin levels and increased capillary permeability was shown, although both conditions had been noted in studies of bradykinin in trypanosomiasis.

(h) Haematological changes in Bovine Trypanosomiasis

(i) Anaemia

Anaemia has long been recognized as the principal pathological feature of animal trypanosomias (Hornby, 1929; Hornby and Bailey, 1931; 1933). Since these preliminary observations were made, there have been numerous studies on the anaemia of animal trypanosomiasis. Fiennes (1954) investigated the haematological changes found in cattle with *T. vivax* infection. He confirmed that anaemia was primarily responsible for the disease process. He attributed the causes of the anaemia to haemolysis, haemodilution, removal and destruction of erythrocytes by the spleen and failure of the bone marrow to replenish red blood cells into circulation. He concluded that the anaemia was macrocytic in the acute stage while in the chronic stage it was microcytic.

Naylor (1971b) concluded from his studies that *T. congolense* infected cattle had a macrocytic normochromic anaemia in the acute stages of the disease but as the disease advanced into chronicity, the anaemia became of the normocytic normochromic type. Losos *et al.*, (1973) reported that zebu cattle with *T. congolense* infection developed a normocytic normochromic anaemia with a conspicuous absence of reticulocytes in circulation. Wellde *et al.*, (1974) also observed that the anaemia in cattle with *T. congolense* infection was normocytic normochromic. These workers, however, observed some circulating normoblasts in the

peripheral circulation of infected cattle. Saror (1975) observed anisocytosis, polychromatophilia and basophilic stippling in zebu cattle with *T. vivax* and *T. congolense* infections suggesting the possibility of macrocytosis. Further studies on the haematological changes in zebu cattle with *T. vivax* infection revealed that the anaemia was normocytic normochromic early in the disease with a tendency to being macrocytic normochromic as the disease progressed (Saror, 1979).

Further studies on the haematological changes in zebu cattle with *T. vivax* and *T. congolense* infection (Maxie *et al.*, 1976) also revealed that the anaemia in both infected groups was originally normocytic and became macrocytic later in the infection. Valli *et al.*, (1978) reported that the anaemia caused by *T. congolense* infection in calves was of moderate severity and was macrocytic normochromic in the acute phase changing to normocytic normochromic with chronicity. It is apparent that reports on the classification of trypanosomal anaemia in cattle show marked variation. It seems reasonable to conclude that the anaemia of bovine trypanesomiasis is basically normocytic normochromic and tends to become macrocytic normochromic in some cattle. This conclusion is in consonnance with other published reports indicating that the anaemia is regenerative in nature at least up to 12 weeks post infection (Murray, 1978). The magnitude of macrocytosis would therefore be influenced by the degree of regenerative response of the host. The regenerative

response could be affected by a number of factors including nutrition, age and the presence of other intercurrent infections.

(ii) Leukocyte response in animal trypanosomiasis

There have been relatively few studies in the changes in circulating leukocytes in animals with trypanosomiasis.

Cross, Redman and Bohl (1968) had described an association between *Trypanosoma theileri* infection and lymphocytosis, although *T. theileri* is considered to be non-pathogenic. Five of 25 cattle examined by blood culture methods were found infected with *T. theileri*. Four of the 5 infected cattle had at least 4 haemograms indicating lymphocytosis sufficiently high to be classified as positive for lymphosarcoma (Cross *et al.*, 1968). Attempts were made to reproduce the lymphocytosis. Blood culture was made from a cow which had a lymphocytosis of 30 months duration. Two splenectomized 6-month old calves were innoculated intravenously with culture medium containing trypanosomes. In each of these calves, lymphocytosis developed and reached a peak within 12 to 13 days following innoculation. The lymphocytosis persisted for 6 months and 1 month respectively. Trypanosomes were re-isolated from each calf (Cross *et al.*, 1968).

Hare, Soulsby and Abt, (1969) have also reported that trypanosomiasis due to *T. theileri* infection leads to lymphopoiesis and thus persistent

lymphocytosis.

Wilde and French (1945) observed that in Zebu cattle infected with *T. rhodesiense*, variations in white cell counts occurred which however, showed no significant correlation with the course of the disease. This finding was probably due to the fact that the cattle might not be the normal host for *T. rhodesiense*.

However, with the trypanosomes that are pathogenic to the domestic animals, the situation is different. Fiennes, Jones, and Laws (1946) observed that in adult cattle infected with *T. congolense* there was a gradual decrease in the leukocyte count, the lowest level being reached at the period of anaemic crisis. They further reported that the leukopaenia was really the result of a deficiency of neutrophils, the condition being a pure neutropenia. In calves however, the leukocyte counts increased, reaching the highest level at the point of anaemic crisis. This was also found to be due to a relative and absolute increase in lymphocyte counts. Other leukocytes however, remained within the normal ranges (Fiennes *et al.*, 1946). They suggested then that the absence of neutropenia in the calves, as compared with adults, was probably insignificant but the increase in lymphocytes in the calf probably indicated a defence reaction (Fiennes *et al.*, 1946).

Naylor (1971b) observed that in *T. congolense* infected cattle, blood levels of eosinophils, lymphocytes and neutrophils were all depressed early in the infection. Monocyte levels however showed little change, basophils tending to disappear while plasma cells tended to increase in number, the latter finding has not been confirmed by other investigators.

Losos *et al.*, (1973) also observed a rapid development of leukopenia within 1 week after infecting cattle with *T. congolense*, which was found to be due to a decrease in both the lymphocytes and neutrophils. The neutropenia was characterized by the presence of predominantly immature cells, indicating a degenerative left shift.

Wellde *et al.*, (1974) reported that leukopenia persisted for the duration of *T. congolense* infection in cattle, particularly low values being recorded during periods of high parasitaemias.

Maxie and Valli (1978a) and Maxie *et al.*, (1976, 1979) have also studied the comparative leukocyte change in cattle infected with *T. congolense* and *T. vivax*. The investigations revealed that although there was leukopenia, which was accounted for by concomitant lymphopenia and neutropenia in both infections, the leukopenia, however occurred earlier and was more transient in the *T. vivax* group than in the *T. congolense* group; significant eosinopenia also occurred in the *T. congolense* group.

Valli, Forsberg and Lumsden (1979) have also observed that in cattle infected with *T. congolense*, the total leukocyte count dropped to a minimum 7 days after infection and gradually returned to its normal value by 18 weeks. This finding was thought to be due to initial relative neutropenia and subsequent rise by an absolute lymphocytosis. Toxic changes were also observed in the neutrophils of the infected cattle.

Saror, Ilemobade and Nuru (1979) also observed that in cattle infected with *T. vivax* there was an early transient leukopenia followed closely by an increase to above pre-infection levels, the latter the result of significant lymphocytosis.

Available experimental data now seems to confirm marked leukocyte changes in cattle with trypanosomiasis, and these changes are characterized by an initial leukopenia, resulting from decreases in both circulating neutrophils and lymphocytes and a subsequent leukocytosis, the latter the result of increased lymphocytosis.

Leukophagocytosis has also been observed in the acute phase of *T. congolense* infection in sheep (Mackenzie and Cruickshank, 1973) and may be relevant to the reported leukopenia. It has also been suggested that leukopenia in acute *T. congolense* infection in cattle might be the result of loss of stem cells

caused by a circulating toxic factor, presumably released by trypanosomes, the nature of which is not yet known (Kaaya, Losos, Maxie, and Valli, 1978). The reported eosinopenia is probably referrable to the host's reaction to the stressing effects of trypanosomiasis.

Further studies are therefore necessary to explain the neutrophil, cosinophil and lymphocyte changes in animal trypanosomiasis.

(iii) Thrombocytopenia:-

Rhesus monkeys experimentally infected with *Trypanosoma rhodesiense* developed a pronounced thrombocytopenia (Sadun, Johnson, Nagle, and Duxbury, 1973). It has also been reported that rats injected intraperitoneally with human strain of *Trypanosoma rhodesiense* consistently developed trypanosomiasis and severe thrombocytopenia, without changes in haematocrit values (Davis, Robbins, Weller and Braude, 1974). Also rabbits infected with *Trypanosoma brucei* have developed pronounced thrombocytopenia (Jenkins, Forsberg, Brown and Parr, 1974).

Recent studies have also shown that thrombocytopenia regularly occurs in bovine trypanosomiasis. Maxie *et al.*, (1976; 1979) and Maxie and Valli, (1978) have actually quantitated the level of

thrombocytes in the peripheral circulation of cattle infected with either *T. vivax* or *T. congolense*. In either infection, there was an initial thrombocytopenia, the development of which was closely associated with the first peak of parasitaemia. Hypertrophy of the reticuloendothelial system has been suggested as the primary cause of the thrombocytopenia in human trypanosomiasis (Robins-Brown *et al.*, 1975 - cited by Maxie *et al.*, 1979), but Maxie and colleagues (Maxie *et al.*, 1979) have hypothesised that thrombocytopaenia was the result of an immunologically-mediated mechanism.

Wellde, Kovatch, Chumo and Wykoff (1978) also observed that cattle infected with *Trypanosoma congolense* developed a thrombocytopenia which was most severe during the early part of the infection when parasitaemia was highest. Thrombocyte levels returned to normal during the course of the infection. Indeed, in acutely ill, thrombocytopenic animals, treatment with trypanocidal drug resulted in rapid thrombocytosis with return to normal levels (Wellde *et al.*, 1978).

Normal bone marrow megakaryocytes have also been reported in cattle infected with *T. congolense* (Wellde *et al.*, 1974), but although Valli and Forsberg (1979) had observed megakaryocytes

to be large in the bone marrow of infected calves with no significant differences between the overall megakaryocytic diameters, significant differences existed between the nuclear diameters. It was concluded that this asynchrony of cytoplasmi maturation with megakaryocytic and nuclear volumes in the infec calves indicated dysthrombopoiesis (Valli and Forsberg, 1979)

Quite clearly, the exact cause of thrombocytopenia in bovine trypanosomiasis has yet to be determined.

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PART II

Trypanosoma vivax stock Y-58: *In vitro* PRODUCTION OF NEURAMINIDASE (= SIALIDASE EC 3.2.1.18) BY TRYPANOSOMA LYSATE

SUMMARY

Neuraminidase (= Sialidase EC 3.2.1.18) was detected in *Trypanosoma vivax* stock Y-58 lysate *in vitro*. The neuraminidase activity (pink colour) is reproduceable and has a linear relationship to the concentration of trypanosomes at lower concentrations. It is suggested that if trypanosomes do produce neuraminidase *in vivo*, the neuraminidase might be cleaving erythrocyte surface sialic acid in *Trypanosoma vivax* infections.

INTRODUCTION

The terminal sialic acid from the sugar residues and from glycoproteins is cleaved by the enzyme neuraminidase, and the detection of the neuraminidase rests on the assay of free sialic acid split from a substrate (Webster and Campbell, 1972). The presence of virus-coded neuraminidase antigens of the influenza virus is well documented (Gottschalk, 1958; Laver, 1963; Laver and Valentine, 1969; Webster, 1970; Webster and Campbell, 1972). This enzyme is also found in many bacterial filtrates; *Vibrio cholera* neuraminidase has been frequently used by many workers, including Gasic and Gasic, (1970) and Fabia,

Gattegno, Gluckman, and Cornillot (1978) during *in vitro* cleavage of erythrocyte surface sialic acid. Neuraminidase was found in large quantities in the cultures of a protozoon parasite, *Trichomonas foetus*, maintained for several years under *in vitro* conditions (Muller and Saathoff, 1972).

Immunosuppression is known to occur during infections with the pathogenic African trypanosomes (Goodwin *et al.*, 1972; Urquhart *et al.*, 1973; Murray *et al.*, 1974 ; Holmes *et al.*, 1974; Mackenzie *et al.*, 1975; Greenwood, 1974; 1976; Scott *et al.*, 1977; Nielsen *et al.*, 1978; Whitelaw *et al.*, 1979) and in influenza virus infections of human patients (Reed *et al.*, 1972 Kantzler *et al.*, 1974). It has been suggested that viral neuraminidase plays a role in the immunosuppression of influenza virus infections (Woodruff and Woodruff, 1976).

This study was undertaken to determine the production of neuraminidase by *Trypanosoma vivax*.

MATERIALS AND METHODS

Trypanosoma vivax

A suspension of *Trypanosoma vivax* stock Y-58 (Leeflang *et al.*, 1976) freshly isolated from an experimentally infected Zebu calf was purified by eluting blood samples through Diethyl-Amino-Ethyl (DEAE) cellulose (Lanham and Godfrey, 1970): 25ml of blood was collected from the calf, by jugular venipuncture into a solution of heparin,

the final concentration of anticoagulant being approximately 10 I.U./ml of blood. The blood sample was immediately placed in ice, and was diluted 1:3 with cold buffer within 10 minutes of collection and stored in the refrigerator (4°C) for about 4h.

Buffer solution

Phosphate-buffered saline glucose (PSG) 4:6 ionic strength of 0.145 and pH 8.0 was prepared as described by Lanham and Godfrey (1970).

DEAE - cellulose column

250g of DEAE-Cellulose (DE-52) was suspended in 1 litre of PSG. The pH of the suspension was brought to pH 8.0 with 5% (v/v) Orthophosphoric acid ($H_3 PO_4$). The suspension was allowed to settle and the supernatant was decanted. The DE-52 was washed a further 4 times by decantation with 1 litre of the buffer solution, equilibrated and stored in the refrigerator (4°C) overnight. When required, the equilibrated DE-52 was removed from the refrigerator. A Whatman No.41 filter paper was placed in a slit-sieve disc and the slurry of equilibrated DEAE - cellulose was packed into the disc; another Whatman No. 41 filter paper was placed on the equilibrated DEAE - cellulose and the surface kept moist with PSG.

Isolation of Trypanosomes

The diluted blood was removed from the refrigerator and gently shaken. This was poured onto the DEAE - cellulose column, care being

taken to avoid spillage of blood into the collecting veseel. Eluates from the column was collected in tubes placed in ice. Trypanosomes were washed 3 times with PSG by centrifugation and decantation. The purified eluate containing approximately 6.5 x 10^6 trypanosomes per ml in PSG, pH 8.0, 10mg of Biocycline^R and equal volume of 2mM stabilizer was deep frozen until required.

Preparation of Stabilizer solution

This was prepared by the method of Kilgour *et al.*, (1975). 0.0308g. of dithiothreitol[a] (DTT) designated as A, and 0.0262g. of 5 - Amino-n-caproic acid[b] (ACA) designated as B were preweighed into separate bijou bottles. Another bijou bottle contained 0.0745g. of Ethylene diaminotetra acetate (EDTA) solution in approximately 1ml of water; this was designated as C. The pH of solution C was adjusted to pH 7.0 with N. Na OH. 1ml of C was added to A to dissolve, then added into B to give 200mM solution of DTT, ACA and EDTA.

Assay of Neuraminidase

Reagent: Fetuin[c] (substrate) Type III. 500mg. of freeze-dried fetuin was dissolved in 20ml of 0.2M sodium phosphate citrate buffer pH 6.7; the substrate solution was prepared fresh when required.

Biocycline^R *Oxytetracycline hydrochloride. The Upjohn Company, Kalamazoo, Michigan U.S.A.*

Dithiothreitol a *Sigma Chemical Co. Lot* 57C-0430

Amino-n-caproic acid b *Sigma Chemical Co. Lot* 37C-0317

Fetuin c *Sigma Chemical Co. Lot* 18C-9530

The frozen packed trypanosomes were allowed to thaw, followed by freezing and thawing 3 times to lyse the trypanosomes. Immediately prior to the assay, the sample was streaked on sheep blood agar and incubated at 37°C for 48h, to determine if there was any bacterial contamination.

Neuraminidase was assayed following the procedures described by Webster and Camphel (1972). 0.5ml of PSG was placed in a series of 5 screw-cap (Gibco) tubes. Tests were set in duplicate, and serial 2-fold dilutions of the trypanosome lysate were made, the first tube containing lysate of approximately 3.25×10^6 trypanosomes. Duplicate samples of each dilution (0.5ml) were incubated with an equal volume of fetuin at 37°C for 30 minutes. 2 other tubes containing 0.5ml each of trypanosome lysate (without fetuin) and fetuin (without trypanosome lysate), respectively, served as controls. The N-acetylneuraminic acid liberated from the fetuin, after 30 minutes incubation, was then assayed by the method of Aminoff (1961). In a preliminary investigation, solutions of N-acetylneuraminic acid[d] (NANA), containing 2.0, 2.5, 5.0, 10.0 and 12.5mg/dl were prepared and their respective absorbances at 549nm determined by the method of Aminoff (1961) to establish the necessary standard.

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[d] Standard NANA Sigma Chemical Co. Lot 76C-0188

RESULTS

The neuraminidase content of the trypanosome preparation is presented inAppendix and the plot of the neuraminidase activity against dilution of trypanosome lysate is shown in Fig. 1. There was a linear relationship between the neuraminidase activity and the trypanosome lysate dilutions. The relationship was no longer linear at higher trypanosome concentration (Fig. 1).

A linear relationship between concentrations of standard NANA and their respective absorbances was observed (Fig. 2).

The fetuin used as substrate during the assay of neuraminidase contained 5.6% total NANA with free sialic acid of 0.11%. Calculations based on these figures showed that the quantity of bound NANA in the sample size of 0.5ml was 12.486mg.

No bacterial growth was observed in the blood agar after incubation at 37°C for 48h.

DISCUSSION

It was observed during this study that *Trypanosome vivax* produces neuraminidase *in vitro*. Similar observations of *in vitro* production of neuraminidase have been reported in influenza viruses (Laver, 1963; Laver and Valentine, 1969; Webster, 1970; Webster and Campbell, 1972), in bacteria (Gasic and Gasic, 1970; Fabia, Gattegno, Gluckman and Cornillot, 1978) and in *Trichomonas foetus* (Muller and Saathoff, 1972).

It has further been shown that neuraminidase cleaves erythrocyte surface sialic acid *in vitro* (Gasic and Gasic, 1970; Greenwalt and Steane, 1973; Durocher, Payne and Conrad, 1975; Fabia *et al.*, 1978). Although *in vivo* studies were not carried out in these animals, it seems logical to speculate that trypanosomes may also produce neuraminidase *in vivo*. The *in vivo* produced neuraminidase may act by cleaving sialic acid off the surface of erythrocytes, thus contributing significantly to the development of anaemia. This might be accomplished by the reduction in life-span of such desialylated erythrocytes (Greenwalt and Steane, 1973) and their subsequent removal from circulation by the reticulo-endothelial system. This mechanism of erythrocyte destruction has already been demonstrated by Durocher *et al.*, (1975) in rats and rabbits using *Vibrio cholerae* neuraminidase.

Determination of the *in vivo* significance of the trypanosome neuraminidase is necessary in view of the loss of linearity observed at higher trypanosome concentrations. However, complete cleavage, that is, exhaustion of all available sialic acid on the substrate (fetuin) might explain the loss of linearity at higher trypanosome concentrations observed during this *in vitro* study. The linearity observed with the standard[d] solutions (Fig. 2) indicates that the range of sialic acid concentrations over which the method is applicable is between 0 and 12.5mg/dl. Although from calculation the sample size of 0.5ml contained 12.486mg. of bound sialic acid, the dilution effect

due to the addition of trypanosome lysate in buffer solution during the assay procedure might have further reduced the substrate concentration. This further suggests that the loss of linearity at higher trypanosome concentrations (Fig. 1) was as a result of exhaustion of substrate. Additional support for this conclusion comes from the fact that when 0.5 ml of fetuin was added into 2 of 4 screw-cap (Gibco) tubes, each of which contained equal volumes of approximately 3.25×10^6 trypanosome lysates, and assayed along with 2 tubes containing 1 ml each of fetuin, the neuraminidase activities (as sialic acid generated - mg/dl) in the first 2 tubes were 9.0 and 9.3 respectively. The activities, corrected for dilution, increased in the other 2 tubes to 12.8 and 13.5 respectively. This additional neuraminidase activity at higher substrate concentration, further indicates that the loss of linearity (Fig. 1) was a result of exhaustion of substrate.

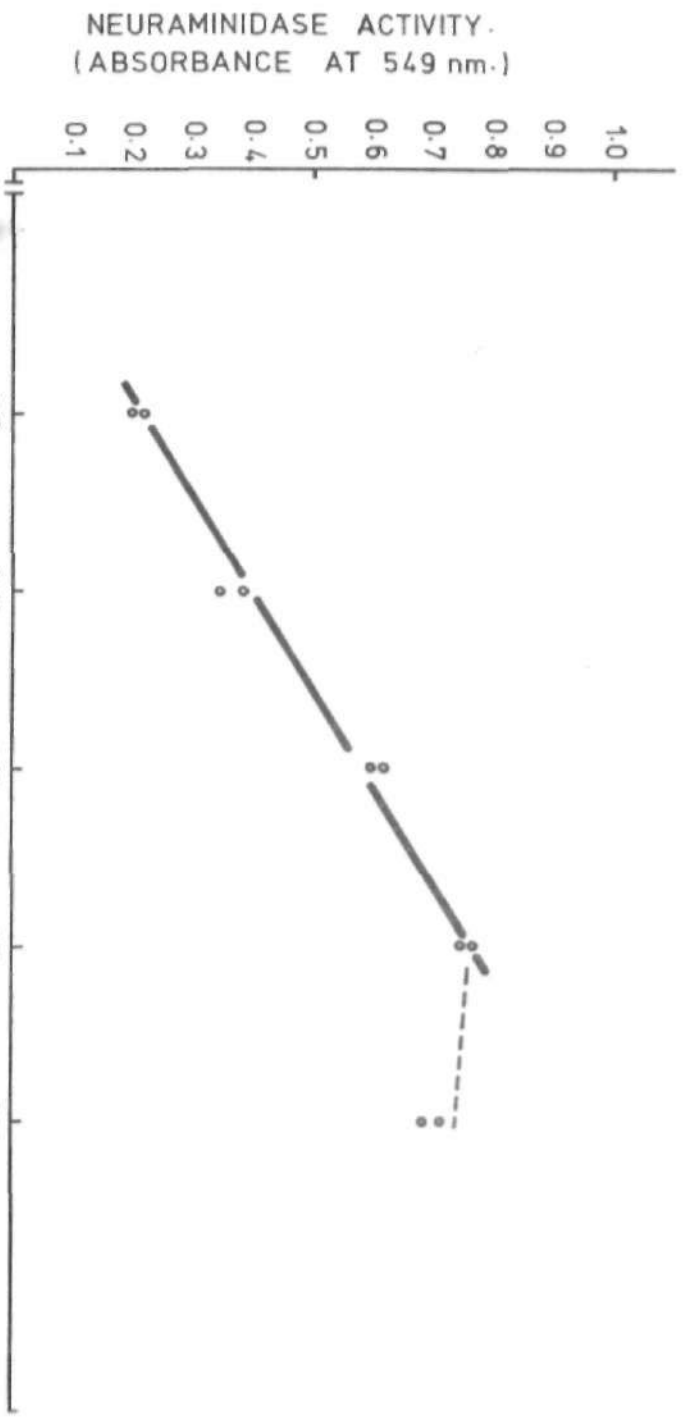


FIG. 1. Neuraminidase activity of Trypanosoma vivax (Y-58) lysate.

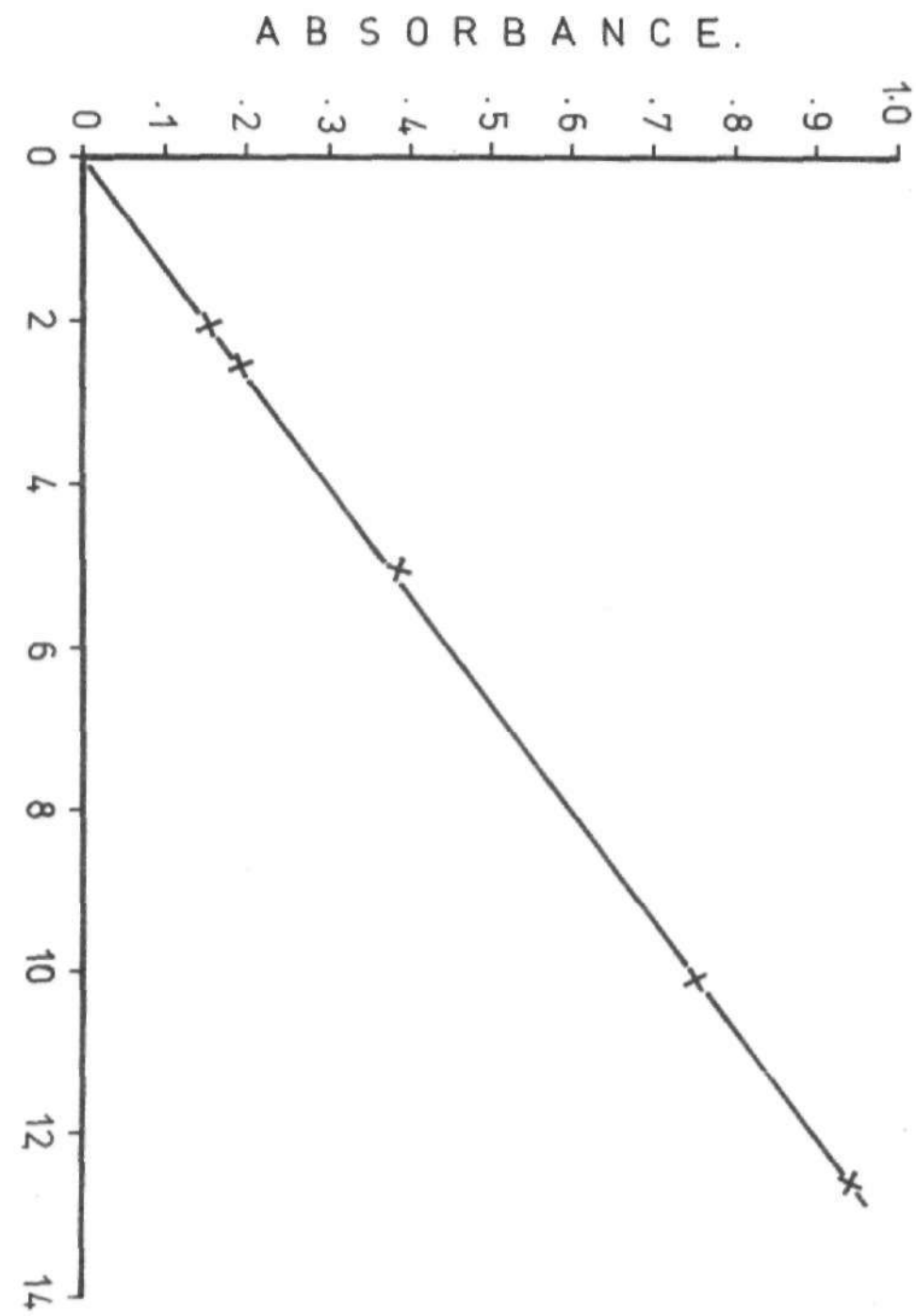


Fig. 2. Standard curve for neuraminidase assay.

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PART III

VARIATION IN ERYTHROCYTE SURFACE AND FREE SERUM SIALIC ACID CONCENTRATIONS DURING EXPERIMENTAL *Trypanosoma vivax* INFECTION IN CATTLE

SUMMARY

Erythrocyte surface and free serum sialic acid concentrations were determined during experimental *Trypanosoma vivax* infection in cattle. All infected calves developed mild trypanosomiasis, with significant decreases in mean PCV occurring on 15, 16, 20, 22 and 24 days post infection. The anaemia was preceded by significant decreases in mean erythrocyte surface sialic acid concentrations on days 7, 13 and 14, and yet another significant decrease on day 31 post infection. These decreases in erythrocyte surface sialic acid concentrations coincided with the parasitaemic waves. Free serum sialic acid concentration however showed an increase (albeit non-significant) on day 8, coinciding with both a decrease in erythrocyte surface sialic acid and the initial parasitaemic wave. It is postulated that the early anaemia observed in infected animals may be attributable to the activities of the circulating trypanosomes which produce neuraminidase which, in turn, cleaves off RBC surface sialic acid, thus rendering the RBC more prone to phagocytosis by the reticulo-endothelial system.

INTRODUCTION

Anaemia has long been established as one of the most significant clinico-pathological features of animal trypanosomiasis (Hornby, 1921; Fiennes, 1954) but its exact aetiology has been the subject of

much controversy (Tartour and Idris, 1973; Jennings *et al.*, 1974; Mamo and Holmes, 1975; Mamo *et al.*, 1977; Kobayashi *et al.*, 1976; Mackenzie and Cruickshank, 1973; Mackenzie *et al.*, 1978; Saror, 1975; Valli *et al.*, 1978; Dargie *et al.*, 1979).

Erythrophagocytosis (Fiennes, 1954; Naylor, 1971; Mackenzie and Cruickshank, 1973; Saror, 1975) and reduction in erythrocytes life span (Mamo and Holmes, 1975; Mamo *et al.*, 1977; Valli *et al.*, 1978) are prominent and important features in the aetiology of the anaemia during the acute phase of animal trypanosomiasis.

Although it is generally agreed that the onset of anaemia corresponds with the appearance of parasites in the blood and its severity with the level and duration of the initial peaks of parasitaemia (Naylor, 1971; Mackenzie and Cruickshank, 1973; Saror, 1975; Maxie *et al.*, 1976; 1979; Valli *et al.*, 1978; Dargie *et al.*, 1979) it has also been observed that the onset of anaemia is related to the decline of the first wave of parasitaemia and the rise in serum antibody (Balber, 1974; Assoku, 1975; Kobayashi *et al.*, 1976).

Sialic acids, the acylated neuraminic acid (Blix *et al.*, 1957) occur in appreciable quantities on the erythrocyte surface structures of several animal species (Eylar *et al.*, 1962; Seaman and Uhlenbruck, 1963). The bulk of the net negative surface charge of erythrocytes of most species of animals is due to ionized sialic acid

(Eylar *et al.*, 1962). It has been shown that older erythrocytes migrated less in an electrical field, due to decreased surface negative charge (Yaari, 1969) and decreased sialic acid content with increased agglutinability has been observed in older red cells (Greenwalt and Steane, 1973). Further evidence for reduced sialic acid and surface charge in older erythrocytes of humans, rats and rabbits has been reported (Durocher *et al.*, 1975). The latter workers cleaved sialic acid off ^{51}Cr-labelled erythrocytes with *Vibrio cholerae* neuraminidase, and injected the desialylated ^{51}Cr-labelled erythrocytes into rats and rabbits. They observed rapid clearance of desialylated erythrocytes from circulation, with sequestration by the liver. The reduction in sialic acid correlated with decreases in electrophoretic mobility of humans, rats and rabbits' erythrocytes (Durocher *et al.*, 1975). They therefore suggested that reduction in erythrocyte sialic acid is a mechanism of erythrocyte destruction and might be important in erythrocyte senescence.

Serum sialic acid was elevated in cattle infected with *Trypanosoma brucei* and *Trypanosoma vivax* and it was suggested that increased synthesis of the sialic acid by the organs invaded by these parasites might have accounted for the increased serum sialic acid (Magaji, 1975).

It has been shown that *Trypanosoma congolense* binds to bovine erythrocytes *in vitro* through the neuraminic acid receptors on the erythrocytes (Banks, 1979).

It was also observed that *Trypanosoma vivax* lysate hydrolysed sialic acid *in vitro* and the neuraminidase activity was proportional to the concentration of trypanosomes (Part II). The latter mechanism, if operative *in vivo*, might result in cleavage of sialic acid from erythrocytes, with subsequent clearance from circulation and possibly leading to clinical anaemia.

This investigation was designed to examine the pattern of behaviour of erythrocyte surface, and free serum sialic acid in cattle experimentally infected with trypanosomes.

MATERIALS AND METHODS

Experimental animals

Eleven Zebu calves ranging from 6 to 12 months in age were purchased from Jibiya, an area known to be free of tsetse-fly. On arrival at Zaria, the animals were dewormed with Thiabendazole[a] and vaccinated against anthrax, blackquarter, contagious bovine pleuropneumonia, and rinderpest. The animals were sprayed weekly with Pfizona[b] and fed a mixture of cotton seed, and wheat offal, with routine grazing daily. Water and salt licks were supplied *ad. lib.* The animals were not used for the experiment until 3 months had elapsed, during which baseline parameters were taken. Of these calves, 6 were

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Thiabendazole[a] - *Merck, Sharp and Dolme Ltd., U.K.*
Pfizona[b] - *(2-chloro-1-(2, 4-dichlorophenyl) vingl diethyl phosphate) i.e. chlorfevinphos. Pfizer (Nigeria) Limited.*

each infected by jugular venepuncture with 1.9×10^5 *Trypanosoma vivax* stock Y-58 (Leeflang, Buys and Blotkamp, 1976) while the other 5 served as uninfected controls.

Sample collection

Thirty-five ml of blood were collected from each calf by jugular venepuncture, daily for about 4 weeks, then every other day for the remaining 8 days when packed cell volume had levelled off. Of the blood samples collected, 5ml were placed into vacutainers[a] with disodium ethylene diaminotetra-acetate (EDTA) anticoagulant, 20ml placed into screw-cap bottles containing 3ml of acid citrate dextrose (ACD) anticoagulant solution A (Beutler, 1975) and the rest 10ml into vacutainers without anticoagulant for serum preparation. Serum was prepared within 1h by centrifugation of clotted blood, and frozen until required.

Haematological evaluation

Packed cell volume (PCV) was determined by the microhaematocrit method. Haemoglobin (Hb) concentration was determined as cyanmethaemoglobin using Coulter Haemoglobinometer[b]. Red blood cell (RBC) and total leukocyte (WBC) counts were determined by the Coulter Counter[c] (Model ZBI). Differential leukocyte counts were based on 100 leukocytes

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a. Terumo Corporation - *Tokyo, Japan*
b. Coulter Electronics - *Harpenden, Herts, England*
c. Coulter Electronics - *Hialeah, Florida, U.S.A.*

(Schalm, Jain and Carroll, 1975) and reticulocyte counts were performed using the new methylene blue stain (Schalm *et al.*, 1975).

Preparation of haemoglobin-free erythrocyte membranes (Ghosts)

Ghosts were prepared on the same day of blood collection, by the method of Dodge, Mitchell, and Hanahan, (1963). The 20ml blood in ACD were centrifuged at 1000g for 20 min., and the plasma and buffy coat removed by aspiration. The erythrocytes were washed 3 times with isotonic phosphate buffer, 310 ideal milli Osmol (im. Osm.) at pH 7.4. The erythrocytes were haemolysed immediately by pipetting 2ml aliquots of washed erythrocytes suspension into 28ml of hypotonic phosphate buffer, 20 im. osm. at pH 7.4, and centrifuged at 20,000g using refrigerated Centrifuge (Beckman Model J-21B centrifuge) for 40 min. Supernantant was decanted and ghosts were washed 3 times with the hypotonic buffer.

Assay of Erythrocyte surface sialic acid

0.5ml of washed erythrocyte ghosts suspension was incubated with 0.2ml of 0.1N sulphuric acid at 80°C for 1h to liberate surface bound sialic acid (Warren 1959; 1963) and the assay was continued without neutralization (Warren 1963) on the same day of blood collection, by the method of Aminoff (1961). The samples, blank and standard[d] were each treated with 0.25ml periodate reagent for

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d *Sigma Chemical Co. Lot 76C-0188*

30 min in a water-bath at 37°C; excess periodate was reduced with 0.2ml of sodium arsenite. After 2 min., 2ml of thiobarbituric acid were added; samples were covered and heated in a boiling water bath for exactly 7.5 min. The resultant coloured mixture of solutions was cooled in ice-water-bath and shaken with 5ml of acid-butanol, followed by short rapid centrifugation; the coloured solutions were read at 549nm in a spectrophotometer[e]. Readings were corrected for interferring compounds (Warren 1963), and dilution effect of sulphuric acid by multiplying with a factor 1.025.

Assay of serum sialic acid

Free sialic acid concentrations in serum were determined approximately every other day as outlined above but without prior mild acid hydrolysis.

Parasitaemia

A 1:200 dilution of each infected blood sample was made using *Trypanosoma* counting fluid, made by dissolving 3.2gm of potassium iodide, 1.0gm of sodium chloride, 5.0gm of disodium sulphate and 0.29 gm of iodine in 200ml of distilled water. The diluted blood was shaken for about 2 min. and placed dropwise into a haemocytometer. Trypanosomes were counted in the peripheral leukocyte chambers under the microscope (x 400) and the number found was multiplied by a factor 500 to determine the number of Trypanosomes per cubic mm.

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e *Spectronic 20. Bausch and Lomb*

Statistical analysis

Student's t-test (Snedecor and Cochran, 1974) was used to analyse the data obtained and values of P less than 0.05 were considered significant.

RESULTS

(i) Haematological Changes:

All the infected cows developed trypanosomiasis. The prepatent period following needle infection with *T. vivax* varied between 2 and 3 days. The disease that developed was mild in severity the affected animals becoming lethargic and beginning to lose weight soon after the onset of the anaemia. All the infected cows maintained good appetite during the course of the investigation.

Soon after establishment of patent infection, the parasitaemia continued to rise and reached a peak 7 days after infection. This was followed by a gradual decline over the next 8 days and by day 16 post infection, trypanosomes were too scanty to be counted although they were detectable with the aid of the microhaematocrit method. Circulating trypanosomes increased again between days 22 and 27 post infection, and thereafter, few trypanosomes could be detected (Fig. 3).

The development of the anaemia as determined by PCV tended to coincide with patency. The sharpest drop in PCV occurred at the

peak of parasitaemia and thereafter, declined very gradually, reaching its lowest point 15 days after infection. This was followed by a transient, but unsuccessful attempt at recovery such that by 36 days post infection, the PCV was still below pre-infection levels (Fig. 3). The PCV of uninfected controls calves as expected, remained quite stable throughout the course of the investigation.

The pattern of haemoglobin changes was similar to that of PCV (Table 1).

(ii) Changes in Erythrocyte Surface Sialic acid Concentration:

There was a gradual decline in mean erythrocyte surface sialic acid concentrations in the infected calves, beginning 4 days after infection. The greatest drop in RBC surface sialic acid concentration occurred between 6 and 13 days after infection. This was followed by a dramatic return to normality such that by 16 days post infection, the RBC surface sialic acid of the infected calves had returned to near pre-infection levels. Thereafter, the RBC surface sialic acid concentration of the infected calves remained lower than as found in the control animals, although these were found to be non-significant ($P > 0.05$). A transient but significant difference ($P < 0.05$) was again observed on day 31 post infection (Fig. 3).

(iii) Production of Serum Sialic acid:

The changes in free serum sialic acid in the infected and uninfected calves are presented in Fig. 4. In general, the

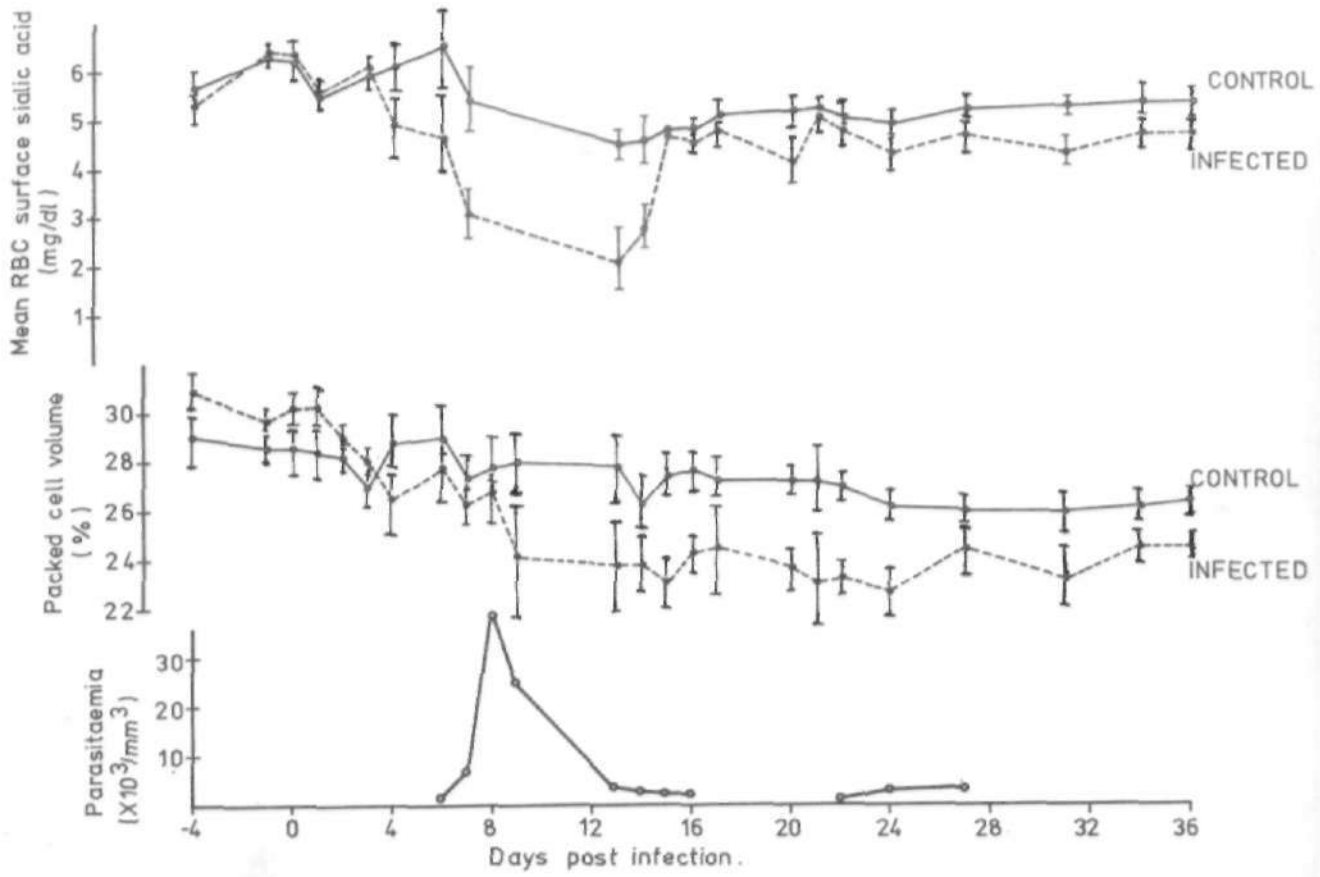


Fig. 2 Variation in erythrocyte surface sialic acid concentrations in relation to PCV and parasitaemia during experimental Trypanosoma Vivax infection in cattle. [⊢⊣ SE]

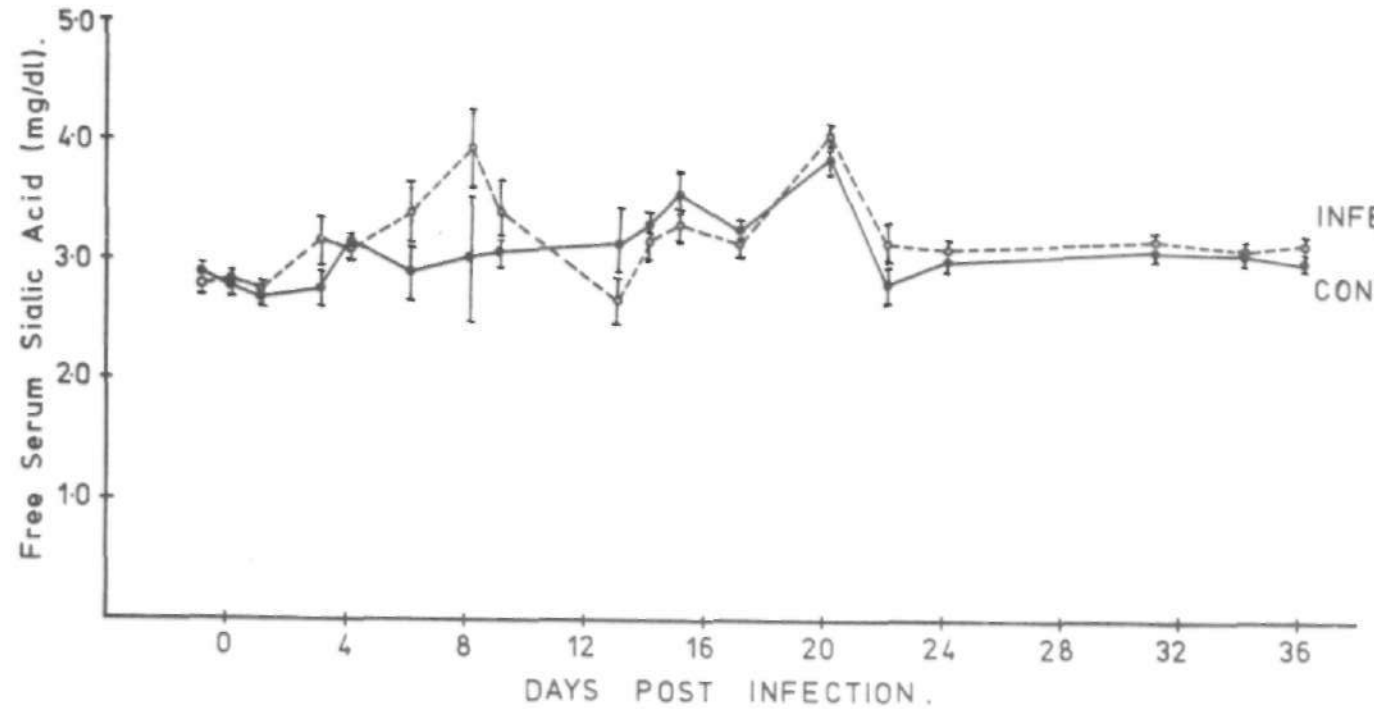


Fig. 4 Variation in free Serum Sialic Acid Concentrations during experimental Trypanosoma vivax infection in cattle. [M SE].

Table 1

Changes in Haemoglobin concentrations (gm %) during experimental *Trypanosoma vivax* infection in cattle.

DAYS P.I.	INFECTED	CONTROL	REMARKS
	MEAN SD	MEAN SD	
-4	10.5 ± 1.1	9.8 ± 0.9	
-1	10.1 ± 0.9	9.9 ± 0.8	
0	11.4 ± 1.2	9.9 ± 0.8	
1	10.6 ± 1.2	9.7 ± 1.2	
2	11.0 ± 1.5	11.3 ± 1.1	
3	9.8 ± 1.0	9.6 ± 1.0	
4	9.0 ± 1.2	9.9 ± 1.3	
6	10.1 ± 1.4	10.6 ± 1.2	
7	9.8 ± 1.5	10.4 ± 1.3	
8	9.7 ± 1.1	9.9 ± 1.3	
9	9.0 ± 1.2	10.3 ± 1.1	
13	10.2 ± 1.3	11.5 ± 1.3	
14	9.5 ± 0.7	10.6 ± 0.9	
15	8.9 ± 1.1	10.5 ± 1.1	
16	9.8 ± 0.9	11.1 ± 1.6	
17	9.3 ± 0.9	10.8 ± 1.1	
20	9.6 ± 1.3	10.7 ± 1.2	
21	8.5 ± 0.8	9.8 ± 0.5	($P < .05$)
22	8.6 ± 0.4	10.1 ± 1.1	($P < .05$)
24	8.8 ± 0.8	10.4 ± 0.9	($P < .05$)
27	9.1 ± 0.9	10.9 ± 0.8	
31	9.2 ± 1.4	10.1 ± 1.2	
34	9.7 ± 0.8	10.3 ± 0.9	
36	8.0 ± 0.9	9.0 ± 0.6	

changes were remarkably similar in both treatment groups. Free serum sialic acid concentratiosn in the infected calves did not show any significant differences between days 4 and 9 after infection. The values, however, soon declined to levels below those observed in non-infected control calves, but were still not significant. Thereafter, there was no discernible difference between free serum sialic acid levels in both infected and control calves.

DISCUSSION

The results indicate that the infected animals developed a mild infection. This may be attributed to a number of factors, including host resistance, virulence of the infecting trypanosomes, and possibly the level of nutrition. The decline in the RBC surface sialic acid tended to coincide with the appearance of the trypanosomes in the circulation, with the lowest values being recorded during the period of initial peak of parasitaemia. The decreased RBC surface sialic acid showed a recovery as the parasitaemia waned, although the PCV levels remained stable and persistent at recorded low levels. It therefore appeared that the loss of RBC surface sialic acid in Trypanosoma-infected cattle was related to the intensity of the parasitaemia and not necessarily the presence of circulating parasites. The slight decline in RBC surface sialic acid concentration in the infected calves observed 31 days after infection might be

related to the parasitaemic wave recorded earlier on day 27 post infection.

It has been shown earlier that trypanosomes produce neuraminidase *in vitro* (Part II; Pg. 64), and this enzyme has also been shown to cleave off erythrocyte surface sialic acid in a number of other species (Eylar *et al.*, 1962; Seaman and Uhlenbruck, 1963; Durocher *et al.*, 1975). The finding therefore that a reduced RBC sialic acid concentration occurred concurrently with an increased number of trypanosomes in the circulation, preceded by a significant development of anaemia, was an indirect evidence that the trypanosomes might be producing neuraminidase *in vivo* and that these signs might be functionally and causally related. Thus the observation also that the development of anaemia was most pronounced during this period was also presumptive evidence of a close relationship between circulating trypanosomes, the production of neuraminidase and accelerated erythrocyte destruction. It seems reasonable therefore to postulate that the early anaemia observed in infected animals may be attributable to the activities of the circulating trypanosomes, which produce neuraminidase, which in turn cleaves off RBC surface sialic acid from erythrocytes thus rendering them more prone to erythophagocytosis.

It has been observed that the severity of anaemia in cattle is related to the intensity and duration of the parasitaemia

(Dargie *et al.*, 1979). The finding of increased reduction in RBC surface sialic acid, concurrent with increasing parasitaemia and reduction in PCV lends credence to this observation.

The exact nature of the RBC surface damage is not known, but it might be physico-chemical during the early phase of the induction of the anaemia when it would not have been possible for anti-RBC antibodies to have been formed. Destruction of RBCs therefore is patently non-immunological.

There were no significant differences between free serum sialic acid concentrations in infected and control calves, nevertheless, slight but obvious increase found in the infected calves on day 8 may be noteworthy and significant since it coincided with the period of greatest RBC surface sialic acid loss and peak parasitaemia. One would have expected that the decline in RBC surface sialic acid would be associated with a concomitant increase in free serum sialic acid. However, although this was not the case and no ready explanation for this observation could be advanced, it is nevertheless reasonable to surmise that sialic acid removed from RBC surface is metabolised or detoxified just as soon as it is produced. Radiosotopic studies would be of immense assistance to determining the fate of the sialic acid cleaved from RBC surface in this way. It is pertinent also to observe here that the enzyme sialyltransferase, present in calf

thyroid gland (Spiro and Spiro, 1968) and in human serum and erythrocyte membrane (Kim, Perdomo, Bella, and Nordberg, 1971), has been suggested to regenerate sialic acid on the surface of cells (Kraemer, 1966). Although, sialyltransferase activity was not investigated in this study, the sharp increase in RBC surface sialic acid 15 days after infection might represent an accelerated replacement of sialic acid on RBC surface (Kraemer, 1966; Kim *et al.*, 1971) as a result of this enzyme. In addition, in combination with an influx of young red cells which normally have high sialic acid content (Yaari, 1969; Green-walt and Steane, 1973) and reduced neuraminidase production, rapid regeneration of erythrocyte sialic acid content could be explained.

The findings reported herein provide some insight into the mechanism of anaemia in bovine trypanosomiasis. It seems reasonable to postulate that as trypanosomes increase in circulation, they produce neuraminidase which cleaves off erythrocyte surface sialic acid thus causing physico-chemical damage to the erythrocyte surface and rendering them more prone to haemolysis by the reticulo-endothelial system. In addition, the cleavage of sialic acid off erythrocyte surfaces may be creating new antigenic sites on the surfaces of affected erythrocytes (Pirofsky, 1969). With time, antibodies could then be produced against these new antigenic sites and further render the RBC more prone to haemolysis.

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PART IV

GENERAL DISCUSSION

GENERAL DISCUSSION

Trypanosomiasis has long been recognized as a major contraint for livestock production in Nigeria. The economic losses resulting from stunting, debilitation, poor reproductive performance, and even death of affected animals have not been quantified, but must be staggering.

The course of the disease is generally considered to fall into 2 distinct phases: an initial acute phase, characterized by a rapidly developing anaemia, followed by a chronic phase during which there is low-grade anaemia, and muscle wasting.

Anaemia was observed in the infected calves during this investigation. It has long been established that anaemia is one of the main pathological features of animal trypanosomiasis (Fiennes, 1954).

Though the infected calves had good appetite throughout this investigation, the calves became lethargic and began to lose weight soon after the onset of anaemia. This finding is characteristic of animal trypanosomiasis.

Two parasitaemic waves were observed during this investigation: a much higher initial parasitaemia within the first 2 weeks of infection followed by a much lower parasitaemic wave within the fourth week of infection. Waves of parasitaemias during the course

of infection are also characteristic of animal trypanosomiasis. The anaemia observed during this investigation coincided with the initial parasitaemia and it is now generally agreed that the onset of the anaemia coincides with the appearance of parasites in the blood, its severity depending on the level and duration of the initial peak of parasitaemia (Naylor, 1971b; Assoku, 1975; Maxie *et al.*, 1976; 1979; Valli *et al.*, 1978; Dargie *et al.*, 1979ab).

Prevailing theories for the aetiology of early anaemia of animal trypanosomiasis include haemolysis, erythrophagocytosis, reduction of erythrocytes half-life, and haemodilution. Although haemodilution has long been suggested to contribute to the early anaemia of animal trypanosomiasis (Fiennes, 1954; Clarkson, 1968; Naylor, 1971b; Mamo and Holmes, 1975; Anosa and Isoun, 1976; Holmes and Jennings, 1976; Maxie and Valli, 1978; Valli *et al.*, 1978), the role of haemodilution in the pathogenesis of the early anaemia has recently been questioned, following some changes in methodology (Dargie, 1978; Dargie *et al.*, 1979ab), and it now appears that haemodilution does not contribute significantly to the development of early trypanosomal anaemia.

It has been previously suggested that dyshaemopoiesis is a factor in the development of trypanosomal anaemia, but recent experimental evidence (Saror, 1975; Valli *et al.*, 1978; Maxie

et al., 1979) indicate that the bone marrow of infected cattle is regenerative. The present results confirm the regenerative nature of the bone marrow, and strongly suggest that dyshaemopoiesis might not be a factor in the early phase of the anaemia. Recent studies on iron metabolism in cattle infected with *T. congolense* however suggest that in the chronic disease at least, the utilization of iron may be impaired, leading to dyshaemopoiesis (Dargie *et al.*, 1979ab).

It is again now generally agreed that the anaemia of animal trypanosomiasis is haemolytic in origin (Fiennes, 1954; Naylor, 1971b; Mamo and Holmes, 1975; Mamo *et al.*, 1977; Saror, 1975; Valli *et al.*, 1978) and that the bulk of the haemolysis occurs by way of erythrophagocytosis (Mackenzie and Cruickshank, 1973; Mackenzie *et al.*, 1978). Factors which render the RBC prone to haemolysis include the production of haemolysin by trypanosomes (Nguyen Huan *et al.*, 1975; Murray *et al.*, 1977; Tizard and Holmes, 1976; Tizard *et al.*, 1977), and immunologically-mediated extravascular haemolysis (Balber, 1972; Assoku, 1975; Kobayashi *et al.*, 1976; Kobayashi and Tizard, 1976; Nielsen *et al.*, 1978). Although haemolytic factors have been isolated from the sera of infected cattle, the role of haemolysin-mediated haemolysis is yet to be conclusively established. In the present study, neither haemoglobinaemia nor haemoglobinuria was observed. Indeed,

intravascular haemolysis has never been unequivocally shown to be a significant factor in bovine trypanosomal anaemia. The present studies were designed to investigate further those factors that could render the RBC prone to haemolysis and thus contribute to the early phase of the anaemia.

The results of this study have shown that *Trypanosoma vivax* lysates produced neuraminidase *in vitro*, and the neuraminidase activity was proportional to the concentration of trypanosomes, at least at the lower trypanosome concentrations. Similar *in vitro* production of neuraminidase has been observed with influenza viruses (Laver, 1963; Laver and Valentine, 1969; Webster, 1970; Webster and Campbell, 1972), bacteria (Gasic and Gasic, 1970; Fabia *et al.*, 1978) and *Trichomones foetus* (Muller and Saathoff, 1972) organism. However, the inability to directly determine serum neuraminidase levels suggests that radioisotopic studies are indicated to determine the *in vivo* production of neuraminidase during *Trypanosoma vivax* infection, and plans are underway for this investigation.

It has long been reported that neuraminidase cleaves off erythrocyte surface sialic acid in a number of species (Eylar *et al.*, 1962; Seaman and Uhlenbruck, 1963; Gasic and Gasic, 1970; Greenwalt and Steane, 1973; Durocher *et al.*, 1975; Fabia *et al.*,

1978). The present finding of reduced RBC surface sialic acid, occurring concurrently with increased circulating trypanosomes and the development of anaemia, is an indirect evidence that the trypanosomes might be producing neuraminidase *in vivo*. The observation also that the development of the anaemia was most pronounced during this particular periods is an important presumptive evidence of a causal relationship between these factors.

It has been reported that the erythrocyte of *T. congolense* infected cattle showed a significantly depressed half-life when compared with erythrocytes of control uninfected animals (Mamo and Holmes, 1975; Mamo *et al.*, 1977; Valli *et al.*, 1978). Again it has been observed that a reduction in surface sialic acid of old, aged RBC of humans and animals, and hence reduced negative surface charge with consequent reduction in electrophoretic migration (Eylar *et al.*, 1962; Seaman and Uhlenbruck, 1963; Yaari, 1969; Greenwalt and Steane, 1973; Durocher *et al.*, 1975) leads rapidly to death of such cells by sequestration. A similar artificial ageing process and accelerated destruction of RBC is suggested to be produced in cattle infected with *T. vivax*. Indeed, such a process has been demonstrated by the injection of *Vibrio cholerae* neuraminidase-treated red cells into rats and rabbits and the subsequent accelerated removal of the desialylated

RBCs (Durocher *et al.*, 1975).

It has been reported that serum sialic acid is elevated in cattle infected with *T. brucei* and *T. vivax* (Magaji, 1975). It has also been shown that *T. congolense* binds to bovine erythrocytes *in vitro* the binding being through the neuraminic (sialic) acid receptors on the erythrocytes (Banks, 1979). Again, preliminary studies with another set of cattle, showed that the erythrocytes from *T. vivax* infected cattle migrated less in polyacrylamide gel than those from uninfected controls, indicating probably reduced surface negative charge and reduced surface sialic acid (Unpublished). It seems reasonable therefore to postulate that the early anaemia observed in the infected animals might be attributable to the activities of the circulating trypanosomes which produce neuraminidase, which in turn cleaves off RBC surface sialic acid from erythrocytes thus rendering them more prone to destruction by the reticuloendothelial system. The exact nature of the RBC surface damage is not known, but it would appear to be physico-chemical damage at this early phase of the anaemia. It is also being suggested that the significant reduction in the RBC surface sialic acid in the infected animals, during the development of the anaemia, might be contributing, at least in part, to the reduced erythrocyte half-life observed in bovine trypanosomiasis by Mamo and Holmes, (1975) Mamo *et al.*, (1977) and Valli *et al.*, (1978).

A transient but non-significant increase in free serum sialic acid was observed in association with the decline in RBC surface sialic acid. This might indicate a rapid clearance of sialic acid from the plasma as soon as it was cleaved from the RBC. Radioisotopic studies might be required to elucidate more the fate of this cleaved sialic acid. However, the enzyme sialyltransferase, present in calf thyroid gland (Spiro and Spiro, 1968) and in human serum and erythrocyte membrane (Kim *et al.*, 1971), has been suggested to regenerate sialic acid on the surface of cells (Kraemer, 1966). Though sialyltransferase activity was not investigated, its probable presence might account for the non-significant increase in free serum sialic acid. Also, the rapid return of RBC surface sialic acid to normal levels 15 days after infection might partly be attributed to the increased replacement of sialic acid on RBC surface by sialyltransferase (Kraemer, 1966; Kim *et al.*, 1971). The influx of young red cells of high sialic acid content (Yaari, 1969; Greenwalt and Steane, 1973), combined with the reduced neuraminidase production as a result of significant reduction in parasitaemia, might also be important contributory factors.

The development of the anaemia early in the infection, during high parasitaemia, may be attributable to the possible physico-chemical damage to the erythrocytes following cleavage of sialic acid,

thus rendering them more prone to extravascular haemolysis by the reticulo-endothelial system. Cleavage of sialic acid from erythrocyte surface is known to create new antigenic sites on the surfaces of the affected erythrocytes (Pirofsky, 1969). Therefore, as the infection progressed, it was possible that auto-antibodies could then be mounted against these new antigenic sites, rendering these RBC to further reticulo-endothelial destruction.

Erythrophagocytosis has been reported to contribute substantially to the development of the anaemia of animal trypanosomiasis (Mackenzie and Cruickshank, 1973). Mackenzie *et al.*, (1978) for instance showed that the coating of red cells with trypanosomal antigen was responsible for the initiation of the phagocytic activity. Other hypotheses to account for the erythrophagocytosis have been reviewed (Jennings, 1976). It is also known that desialylation of erythrocytes creates new antigenic sites (Pirofsky, 1969) to which anti-RBC antibodies could be formed, leading to such human immunological disease like autoimmune haemolytic anaemias, secondary immune deficiency, drug-induced immune diseases and cardiac traumatic haemolysis (Katz *et al.*, 1977). It is probable that anti-RBC antibodies could have been produced following cleavage of sialic acid off the surface of circulating RBC of the infected calves, leaving new antigenic sites. This,

with analogy to others (Pirofsky, 1969; Eisen, 1974; Katz *et al.*, 1977) might have rendered such RBC susceptible to rapid erythrophagocytosis.

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APPENDIX I

PRINCIPLE OF ANALYSIS

N - Acetyl Neuraminic Acid

Principle

The periodate oxidation of the neuraminic acid back bone of sialic acids results in the formation of beta-formylpyruvic acid from carbon atoms 1 to 4. The N - acetyl or N - glycol group of sialic acids apparently does not interfere with periodate oxidation. Beta-formylpyruvic acid is then coupled with 2-thiobarbituric acid to form a red chromophore with a maximum absorption at 549nm (Warren, 1959; 1963).

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APPENDIX II

TABLES

Table 2 Neuraminidase assay of *Trypanosoma vivax* (Y-58) lysate

TRYPANOSOME LYSATE APPROX. NO. OF TRYPANOSOMES	OPTICAL DENSITY (ABSORBANCE) READINGS		NEURAMINIDASE ACTIVITY (as Sialic acid (mg/dl)	
3.25×10^6	0.69;	0.72	9.2;	9.6
1.625×10^6	0.77;	0.75	10.3;	10.0
0.8125×10^6	0.60;	0.62	8.0;	8.3
0.40625×10^6	0.38;	0.34	5.1;	4.5
0.203125×10^6	0.22;	0.20	2.9;	2.7
TRYPS. CONTROL (without Fetuin)	0.00;	0.00	0.0	0.0
FETUIN CONTROL (without tryps.)	0.00;	0.00	0.0	0.0

Table 3 Standard curve determination for Neuraminidase assay

Solutions of standard N-acetyl neuraminic acid (mg/dl)	2.0	2.5	5.0	10.0	12.5
Absorbance at 549nm	0.15	0.19	0.38	0.75	0.94

Table 4

Variation in erythrocyte surface sialic acid concentrations (mg/dl) during experimental *Trypanosoma vivax* infection in cattle

DAYS P.I.	INFECTED	CONTROL	REMARKS
	MEAN ± SD	MEAN ± SD	
-4	5.28 ± 0.4	5.64 ± 1.2	
-1	6.38 ± 1.2	6.33 ± 1.3	
0	6.35 ± 1.2	6.30 ± 1.3	
1	5.57 ± 2.4	5.46 ± 1.3	
2	-	-	
3	6.16 ± 1.9	5.93 ± 2.2	
4	4.94 ± 2.4	6.08 ± 0.7	
6	4.61 ± 1.8	6.55 ± 1.9	
7	3.12 ± 1.5	5.40 ± 1.5	($P < .05$)
8	-	-	
9	-	-	
13	2.13 ± 1.8	4.47 ± 0.7	($P < .02$)
14	2.78 ± 1.3	4.56 ± 1.2	($P < .05$)
15	4.71 ± 1.6	4.87 ± 1.7	
16	4.51 ± 1.9	4.86 ± 1.0	
17	4.78 ± 1.6	5.04 ± 2.0	
20	4.19 ± 1.4	5.19 ± 1.3	
21	5.04 ± 0.8	5.20 ± 0.8	
22	4.74 ± 1.3	4.99 ± 0.8	
24	4.33 ± 0.8	4.91 ± 0.4	
27	4.64 ± 0.8	5.20 ± 0.3	
31	4.32 ± 0.8	5.21 ± 0.4	($P < .05$)
34	4.68 ± 0.7	5.31 ± 0.4	
36	4.63 ± 0.7	5.29 ± 0.5	

Table 5

Changes in free serum sialic acid concentrations (mg/dl) during experimental *Trypanosoma vivax* infection in cattle

DAYS P.I.	INFECTED	CONTROL	REMARKS
	MEAN ± SD	MEAN ± SD	
-1	2.80 ± 0.4	2.90 ± 0.5	
0	2.82 ± 0.4	2.80 ± 0.4	
1	2.77 ± 0.3	2.69 ± 0.1	
3	3.17 ± 0.8	2.75 ± 0.4	
4	3.10 ± 0.3	3.14 ± 0.6	
6	3.39 ± 1.4	2.90 ± 0.8	
8	3.93 ± 0.6	3.02 ± 0.7	
9	3.40 ± 1.2	3.07 ± 1.2	
13	2.65 ± 0.8	3.12 ± 0.7	
14	3.14 ± 0.5	3.30 ± 1.2	
15	3.30 ± 0.7	3.56 ± 1.0	
17	3.12 ± 1.0	3.24 ± 0.3	
20	4.03 ± 0.9	3.86 ± 1.2	
22	3.14 ± 0.7	2.80 ± 0.5	
24	3.10 ± 0.4	3.00 ± 0.5	
31	3.17 ± 0.8	3.10 ± 0.4	
34	3.10 ± 0.3	3.10 ± 0.4	
36	3.14 ± 0.5	3.00 ± 0.5	

Table 6

Packed cell volume (%) changes during experimental *Trypanosoma vivax* infection in cattle.

DAYS P.I.	INFECTED	CONTROL	REMARKS
	MEAN ± SD	MEAN ± SD	
-4	30.8 ± 3.2	29.0 ± 2.2	
-1	29.7 ± 2.7	28.6 ± 3.0	
0	30.2 ± 2.7	28.6 ± 3.0	
1	30.3 ± 3.4	28.4 ± 3.0	
2	29.0 ± 3.0	28.2 ± 2.4	
3	28.0 ± 2.6	27.0 ± 1.6	
4	26.5 ± 3.8	28.8 ± 3.8	
6	27.8 ± 3.9	29.0 ± 3.1	
7	26.3 ± 3.9	27.4 ± 3.0	
8	26.8 ± 2.9	27.8 ± 3.7	
9	24.2 ± 3.4	28.0 ± 2.5	
13	23.8 ± 3.8	27.8 ± 2.5	
14	23.8 ± 2.9	26.2 ± 1.6	
15	23.1 ± 3.2	27.4 ± 2.3	($P < .05$)
16	24.3 ± 1.2	27.6 ± 2.4	($P < .02$)
17	24.5 ± 2.1	27.2 ± 2.3	
20	23.7 ± 2.9	27.2 ± 0.8	($P < .05$)
21	23.1 ± 2.6	27.2 ± 0.8	
22	23.2 ± 1.7	27.0 ± 2.3	($P < .01$)
24	22.7 ± 2.3	26.2 ± 1.5	($P < .02$)
27	24.5 ± 2.7	26.0 ± 1.2	
31	23.2 ± 2.6	26.0 ± 1.6	
34	24.5 ± 3.0	26.2 ± 1.3	
36	24.5 ± 3.3	26.4 ± 1.1	

Table 7

Mean parasitaemia during experimental *Trypanosoma vivax* infection in cattle.

DAYS P.I.	PARASITAEMIA ($\pm$ S.D.) X $10^3/mm^3$	REMARKS
-4	-	
-1	-	
0	-	
1	-	
2	-	Identified with Hct.
3	-	"
4	1.3 ± 0.4	
6	6.2 ± 3.4	
7	38.4 ± 24.2	
8	24.9 ± 15.9	
9	3.5 ± 2.7	
13	2.5 ± 0.5	
14	2.25 ± 0.4	
15	2.0 ± 0.0	
16	-	Identified with Hct.
17	-	"
20	-	"
21	1.0 ± 0.0	
22	3.0 ± 0.7	
24	3.5 ± 0.0	
27	-	Identified with Hct.
31	-	"
36	-	"

APPENDIX III

DATA: INDIVIDUAL ANIMALS

DATA: INDIVIDUAL ANIMALS

	PARAMETERS:	UNITS.
1.	PCV	%
2.	Hb	gm/dl
3.	RBC	x 10^6/ul.
4.	MCV	cubic microns.
5.	MCH	micro micrograms
6.	MCHC	%
7.	WBC	/ul
8.	Bands	/ul
9.	Neutrophils	/ul
10.	Lymphocytes	/ul
11.	Eosinophils	/ul
12.	Monocytes	/ul
13.	Basophils	/ul
14.	Total RBC membrane sialic acid	mg/dl
15.	Free serum sialic acid	mg/dl
16.	Total protein	gm/dl

02 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	34	11.8	8.2	5.41	-
-1	31	10.6	8.0	7.03	2.70
0	32	12.3	8.5	7.00	2.70
1	33	11.5	8.4	4.00	2.52
2	30	11.9	8.1	-	-
3	31	10.8	8.0	6.80	4.15
4	32	10.5	7.2	4.24	3.15
6	31	11.1	8.0	3.55	2.00
7	31	11.1	8.2	5.19	-
8	28	10.0	7.6	-	3.89
9	26	10.8	7.5	-	3.18
13	24	10.5	7.5	0.21	4.09
14	24	9.3	7.4	3.76	3.94
15	23	8.9	7.3	2.48	3.71
16	24	9.8	7.5	2.27	-
17	25	9.4	7.6	3.61	2.44
20	25	9.8	7.5	3.10	3.25
21	24	8.5	7.1	6.51	-
22	22	8.6	7.0	3.57	2.83
24	21	8.3	7.0	4.32	3.15
27	25	9.0	7.0	4.62	-
31	23	9.8	7.0	4.30	3.29
34	24	9.9	7.0	4.70	3.25
36	24	8.0	7.2	4.64	2.83

03 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	31	10.1	8.5	5.41	-
-1	30	9.9	8.3	6.10	2.25
0	30	10.8	8.5	6.05	2.25
1	32	11.4	8.5	4.00	2.70
2	30	12.4	8.4	-	-
3	29	10.2	8.0	3.32	2.34
4	27	9.0	8.0	6.83	3.00
6	31	11.5	8.4	6.97	5.23
7	29	11.2	8.4	4.09	-
8	28	10.4	8.4	-	3.35
9	27	9.8	8.3	-	4.32
13	28	11.5	8.6	3.01	2.20
14	26	10.4	8.3	3.96	3.11
15	26	9.9	8.4	4.99	3.58
16	26	10.8	8.4	4.05	-
17	26	10.3	8.5	3.94	4.72
20	22	10.4	8.0	5.95	4.53
21	25	8.9	8.2	5.28	-
22	25	9.3	7.2	4.67	3.69
24	26	9.9	8.5	5.28	3.00
27	26	9.8	8.4	5.60	-
31	28	11.7	8.5	5.22	4.15
34	29	10.8	8.3	5.60	4.20
36	30	9.2	8.0	5.48	3.70

04 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	33	10.7	7.0	5.41	-
-1	33	11.1	7.3	7.04	3.20
0	32	11.7	7.0	7.05	3.30
1	33	11.6	7.2	5.80	
2	32	11.8	7.3	-	-
3	30	10.3	7.0	5.97	3.51
4	29	9.8	6.6	4.99	3.15
6	30	10.6	7.1	3.01	2.00
7	28	10.6	7.1	3.00	-
8	28	9.7	7.0	-	3.68
9	27	8.9	6.9	-	2.00
13	28	11.4	7.0	4.92	2.94
14	28	10.1	7.4	3.57	2.59
15	26	9.2	7.2	5.47	3.25
16	25	10.0	7.2	2.41	-
17	26	9.8	7.3	4.37	4.03
20	25	9.5	7.0	2.59	3.96
21	26	9.4	7.2	4.87	-
22	25	8.7	7.4	5.21	2.52
24	25	9.3	7.0	4.87	3.15
27	28	9.8	7.0	5.17	-
31	21	9.0	6.5	4.90	2.34
34	23	8.7	6.3	5.20	2.35
36	23	7.2	6.1	5.10	2.50

07 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	30	10.0	8.1	4.76	-
-1	30	10.3	8.5	8.00	3.10
0	30	11.4	8.5	7.75	3.10
1	30	10.2	8.2	10.07	2.70
2	27	10.2	8.0	-	-
3	26	9.0	8.0	9.26	2.68
4	24	8.5	8.0	3.21	3.47
6	26	9.7	7.1	2.60	2.42
7	25	9.1	8.1	2.74	-
8	28	9.8	8.5	-	3.38
9	24	8.4	8.4	-	2.31
13	22	9.4	8.0	0.12	2.00
14	22	9.3	8.4	2.49	2.60
15	21	8.3	8.1	6.97	2.00
16	23	8.7	8.0	5.14	-
17	23	8.2	8.1	7.90	2.00
20	22	9.1	8.0	5.66	3.70
21	20	8.1	8.0	4.05	-
22	21	8.3	8.0	2.96	4.22
24	22	9.2	8.0	4.67	3.47
27	23	8.7	8.0	4.99	-
31	24	9.1	7.8	4.67	3.52
34	26	10.2	7.5	4.97	3.50
36	25	8.4	8.5	5.00	4.20

09 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	25	8.7	7.1	5.90	-
-1	25	8.5	7.4	5.10	3.00
0	25	9.3	7.0	5.10	3.02
1	24	8.4	7.1	5.57	2.50
2	24	8.3	7.0	-	-
3	24	8.2	6.6	5.80	-
4	21	7.1	6.3	8.48	2.52
6	21	7.7	6.8	5.95	4.13
7	20	7.3	6.5	3.05	-
8	21	7.6	6.5	-	4.20
9	18	7.4	6.0	-	5.15
13	18	8.0	6.7	2.60	2.34
14	20	8.3	6.5	0.55	3.20
15	18	7.1	6.5	3.35	3.53
16	23	8.9	6.8	6.65	-
17	21	8.4	7.0	5.01	3.03
20	20	7.5	6.0	3.83	3.25
21	20	7.2	6.5	4.94	-
22	22	8.1	7.0	6.51	2.73
24	20	7.6	6.5	3.27	2.52
27	20	7.6	6.5	3.58	-
31	21	7.6	6.7	3.24	2.68
34	20	8.8	6.7	3.70	2.70
36	20	6.8	6.4	3.55	2.75

10 (INFECTED)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	32	11.4	8.1	4.76	-
-1	29	10.0	7.5	5.00	2.55
0	32	12.6	7.5	4.99	2.55
1	30	10.3	7.4	4.00	3.10
2	31	11.5	7.8	-	-
3	28	10.1	7.0	5.80	-
4	26	9.0	6.5	1.91	3.29
6	28	10.2	7.4	5.60	4.56
7	25	9.7	7.3	0.65	-
8	28	10.5	6.8	-	5.08
9	23	8.6	6.8	-	3.43
13	23	10.6	6.5	1.93	2.30
14	23	9.3	6.4	2.33	3.58
15	25	10.1	6.4	4.99	3.74
16	25	10.6	6.6	6.51	-
17	26	9.9	6.5	3.82	2.49
20	28	11.3	6.5	3.98	5.46
21	24	8.7	6.6	4.60	-
22	24	8.8	6.5	5.49	2.83
24	22	8.7	6.4	3.57	3.29
27	25	9.6	6.2	3.87	-
31	22	8.2	6.0	3.56	3.17
34	25	9.5	6.7	3.92	3.20
36	25	8.5	6.0	4.00	2.85

01 (CONTROL)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	30	10.2	8.0	6.40	-
-1	30	10.4	8.0	5.19	2.75
0	30	10.4	7.8	5.18	2.60
1	27	9.5	7.4	5.97	2.83
2	31	12.0	8.0	-	-
3	27	9.9	7.0	2.99	2.50
4	27	9.3	7.4	5.74	3.17
6	27	9.7	7.0	3.42	3.93
7	26	10.0	7.4	5.67	-
8	27	9.8	7.5	-	3.15
9	26	10.2	7.4	-	2.19
13	26	10.8	7.6	4.99	2.28
14	25	10.8	7.3	4.10	3.20
15	26	10.8	7.5	4.99	2.84
16	26	10.5	7.5	4.80	-
17	25	10.0	7.4	4.80	2.78
20	27	10.5	7.5	4.64	5.48
21	25	9.9	7.6	5.28	5.48
22	26	10.6	8.0	4.90	2.61
24	27	10.8	7.5	5.21	3.05
27	27	10.7	7.5	5.33	-
31	26	10.6	7.8	5.50	3.15
34	26	10.5	7.4	5.60	3.05
36	26	8.7	7.1	5.70	3.05

05 (CONTROL)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	32	10.9	7.3	4.92	-
-1	32	10.9	7.5	6.10	2.15
0	32	10.8	7.5	6.00	2.20
1	33	11.5	7.5	4.00	2.70
2	30	12.5	7.5	-	-
3	29	10.4	7.1	6.63	2.70
4	35	12.0	7.5	6.83	2.52
6	34	12.6	7.0	7.29	2.69
7	32	12.3	7.3	3.74	-
8	34	12.1	7.4	-	3.05
9	31	11.1	7.1	-	3.22
13	32	13.7	7.6	3.66	2.71
14	29	11.7	7.4	3.96	2.00
15	31	11.8	7.3	3.42	2.66
16	31	13.0	7.0	4.05	-
17	30	12.5	7.5	3.24	3.55
20	28	12.5	7.0	3.61	2.97
21	26	9.9	7.0	4.39	-
22	30	10.9	7.5	4.30	2.61
24	26	10.5	7.0	4.39	3.00
27	27	10.6	7.5	4.70	-
31	28	11.5	7.8	4.56	3.10
34	28	11.1	7.5	4.62	3.05
36	28	10.1	7.5	4.85	3.00

06 (CONTROL)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	28	9.3	8.0	4.76	-
-1	24	9.1	7.6	6.10	3.10
0	24	9.2	7.8	6.12	3.00
1	29	10.0	8.0	6.15	2.70
2	27	11.5	7.8	-	-
3	26	9.1	7.6	4.65	3.00
4	28	9.7	7.7	5.33	2.70
6	29	10.8	7.8	6.56	2.63
7	27	10.9	7.8	3.90	-
8	27	9.6	7.7	-	4.07
9	29	10.7	7.9	-	5.05
13	27	11.3	7.5	3.66	2.94
14	26	10.2	7.8	3.96	4.81
15	28	10.7	8.0	3.42	5.04
16	29	11.8	7.6	4.05	-
17	27	10.3	8.0	3.70	3.30
20	27	10.6	7.5	6.83	3.43
21	24	9.0	7.9	5.21	-
22	25	9.0	7.5	4.30	2.45
24	28	11.1	8.5	4.46	4.00
27	26	9.5	7.5	4.96	-
31	27	10.6	8.0	5.00	3.15
34	25	9.4	7.4	5.10	4.05
36	27	8.8	8.0	5.00	4.05

08 (CONTROL)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	26	8.6	7.4	7.38	-
-1	28	9.3	7.6	8.56	3.15
0	26	9.8	7.2	8.55	2.70
1	25	8.3	7.4	7.03	2.70
2	25	9.7	7.5	-	-
3	25	8.1	7.0	6.63	3.25
4	25	8.5	7.0	6.83	4.17
6	26	9.5	8.3	8.25	2.10
7	24	8.7	7.2	6.96	-
8	24	8.4	6.9	-	2.00
9	25	8.5	7.0	-	2.46
13	26	10.3	7.0	5.13	3.47
14	25	9.3	6.8	6.70	2.36
15	25	8.8	7.0	7.52	4.28
16	25	8.8	6.8	6.58	-
17	25	9.8	7.5	8.46	3.50
20	26	9.1	7.0	6.25	2.80
21	25	9.9	7.0	6.51	-
22	25	9.0	7.0	6.37	2.56
24	24	8.8	7.5	5.28	2.00
27	24	9.0	7.1	5.50	-
31	24	8.4	7.3	5.60	3.47
34	25	9.2	7.0	5.72	2.10
36	25	8.8	7.0	6.00	2.05

12 (CONTROL)

DAYS P.I.	PCV	Hb.	T.P.	Total RBC-memb. Sialic Acid	Free Serum Sialic Acid
-4	29	10.0	9.0	4.76	-
-1	29	10.0	8.8	5.70	2.95
0	29	9.5	8.8	5.69	2.80
1	28	9.4	8.6	4.16	2.50
2	28	10.9	8.6	-	-
3	28	10.3	8.5	8.77	2.30
4	29	10.0	8.3	5.69	3.15
6	29	10.6	8.6	7.25	2.20
7	28	10.0	8.5	6.75	-
8	27	9.7	8.7	-	2.83
9	29	10.9	8.9	-	2.43
13	28	11.6	8.3	4.92	4.19
14	26	10.8	8.3	4.10	4.13
15	27	10.3	8.3	4.99	2.98
16	27	11.3	8.4	4.80	-
17	29	11.3	8.5	5.01	3.06
20	28	10.7	8.5	4.64	4.62
21	26	10.4	8.5	4.60	-
22	29	11.2	8.5	5.10	3.75
24	26	10.7	6.5	5.21	2.73
27	26	10.4	8.5	5.30	-
31	25	9.3	8.5	5.40	2.54
34	27	11.2	6.0	5.50	2.70
36	26	8.8	8.5	4.90	2.70

STUDIES ON AN ASPECT OF THE ANAEMIA OF BOVINE TRYPANOSOMIASIS *(Trypanosoma vivax)*

by

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D.V.M., Ahmadu Bello University, 1974
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AN ABSTRACT OF A THESIS

Submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Veterinary Pathology and Microbiology

AHMADU BELLO UNIVERSITY, ZARIA

SEPTEMBER, 1980

ABSTRACT

The production of neuraminidase (= sialidase EC 3.2.1.18) by *Trypanosoma vivax* was investigated. *Trypanosoma vivax* (stock Y58) lysates were found to hydrolyse sialic acid from a substrate *in vitro*. The neuraminidase activity was proportional to the concent ration of trypanosomes, at lower concentrations of trypanosomes. Calculations based on the available bound sialic acid on the substrate indicated that loss of linearity between neuraminidase activity and higher concentrations of trypanosomes might have been due to exhaustion of the substrate.

Effect of *Trypanosoma vivax* infection on bovine erythrocyte surface sialic acid concentrations was therefore investigated. All infected calves developed mild anaemia with significant decreases in mean packed cell volume occuring on days 15, 16, 20, 22 and 24 post infection. The anaemia was preceeded by significant decreases in mean erythrocyte surface sialic acid concentrations on days 7, 13, 14 and then 31 post infection in the infected calves. Free sialic acid concentrations in the serum were also assayed during the study. An initial but non-significant increase in free serum sialic acid concentrations was observed. The initial increase coincided approximately with both reduced erythrocyte surface sialic acid and increased parasitaemia.

It was postulated that the early anaemia observed in the infected animals may be attributable to the activities of the circulating

trypanosomes which produce neuraminidase which in turn cleaves off RBC surface sialic acid thus rendering the RBC more prone to destruction. The significance of reduced erythrocyte surface sialic acid in reduction of erythrocyte half-life and erythrophagocytosis are discussed in relation to the development of the anaemia of African animal trypanosomiasis. Possible areas for further research are highlighted.