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Anaemia in bovine African trypanosomiasis

A review

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Introduction

The development of anaemia is a well recognised and inevitable consequence of trypanosome infections in domestic animals in general and cattle in particular (Hornby, 1921; Murray, 1974; Morrison et al., 1981a). Maasai herds-men recognise that cattle in tsetse-infested areas can “run out of blood”. It has now been definitively established that the measurement of anaemia gives a reliable indication of the disease status (Murray, 1979) and productive performance (ILCA, 1986a, b) of trypanosome-infected cattle.

The severity of the anaemia which follows infection is affected by several factors. These include differences in virulence that exist among different species of trypanosome and among the large number of strains belonging to each species. At the same time, host factors such as age, nutritional status and breed are important (Murray et al., 1982).

The purpose of this paper is to consider the basic kinetics of trypanosome-induced anaemia, evaluate the possible mechanisms responsible, and discuss the factors which are important in influencing the severity of anaemia. Successful identification and understanding of the key mechanisms that limit the severity of trypanosome-induced anaemia might provide genetic markers for selection in conventional breeding programmes and might also permit the production by therapeutic or immunological means, or by molecular genetics, of animals that are more resistant to the effects of infection and hence more productive. That this is possible is shown by the fact that nature has already achieved these goals in the form of the large herds of wild Bovidae that roam the tsetse-infested forest and savanna lands of Africa and which are almost completely resistant or “tolerant” to the effects of trypanosome infection and do not develop anaemia.

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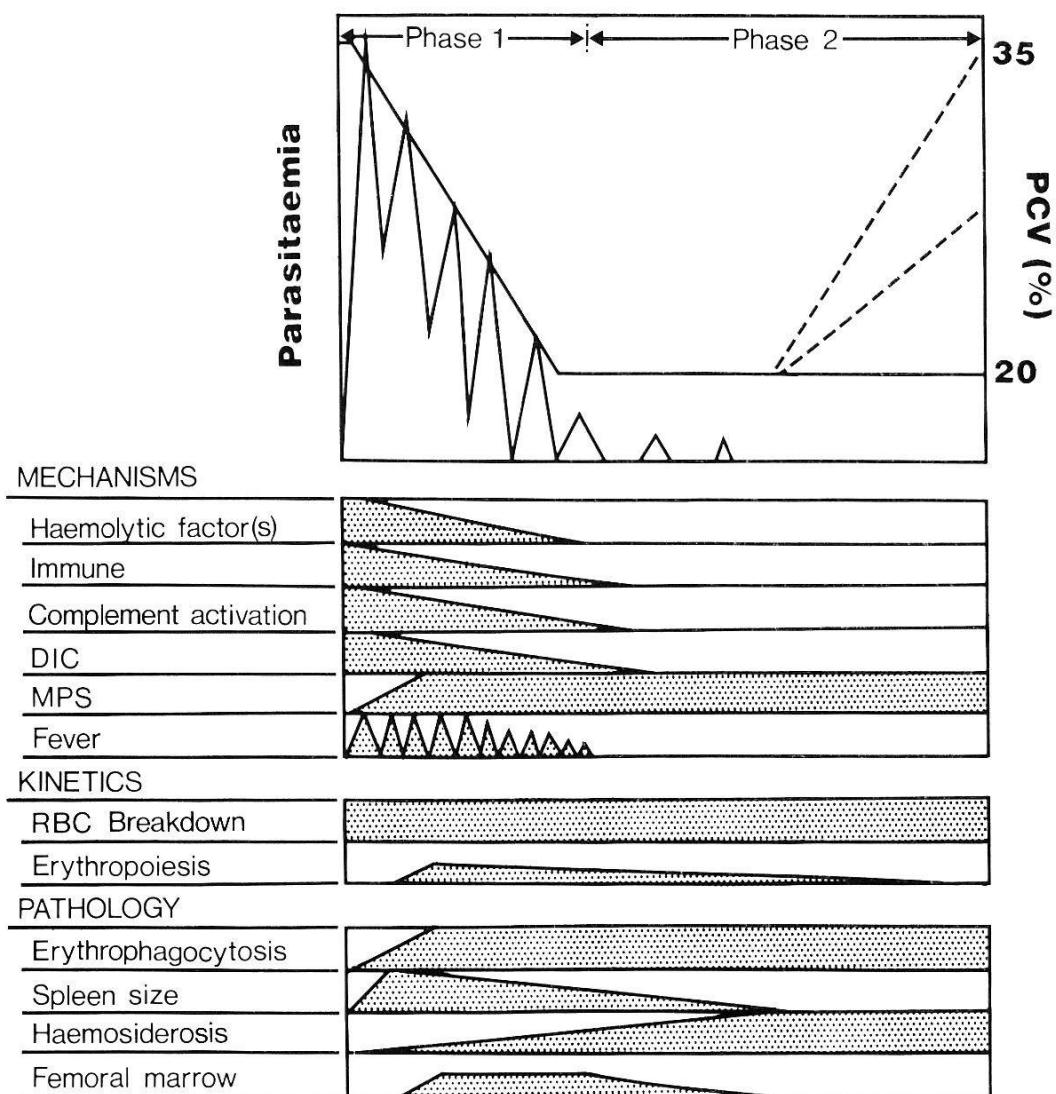


Fig. 1. Anaemia of bovine African trypanosomiasis in relation to parasitaemia, time, underlying mechanisms, erythrokinetics and post-mortem findings. Hatched areas represent an estimation of the importance of a reaction at any one time. DIC = disseminated intravascular coagulation; MPS = mononuclear phagocyte system.

This review will be devoted largely to presenting what is known in ruminants, particularly, cattle and wild Bovidae, as, in our opinion, several aspects of the pathogenesis of the disease in laboratory animals are sufficiently different as to make extrapolation misleading.

Kinetics of anaemia

Bird's eye view

To lay the basis for discussion, Fig. 1 has been included. This figure is based on the authors' own experience and innumerable publications quoted later in the paper as the discussion unfolds. It is a modified version of that produced by

Murray (1979) and attempts to present a "bird's eye" view of anaemia in relation to parasitaemia, erythrokinetics, possible mechanisms and post-mortem findings. While the schematic presentation of the anaemia and parasitaemia give an indication of the pattern of events over time, no scale is given for parasitaemia or time because of the considerable variation that can occur. Throughout this paper packed red cell volume percent (PCV) is used as the index of anaemia, as no evidence of haemodilution has been found in trypanosome-infected cattle (Dargie et al., 1979a).

Based on the presence or absence of trypanosomes, response to trypanocidal drug treatment, and pathological findings, the anaemia in trypanosome-infected cattle can be divided into two distinct but overlapping phases (Fig. 1).

The first is an "acute" phase characterised by progressive anaemia accompanied by parasitaemia. The initial fall in PCV values is associated with the first wave of parasitaemia in the blood. It is not clear from available data just when the first changes in red cell dynamics occur, i.e., whether they commence with appearance of the first parasites in the blood, or at the peak of parasitaemia or subsequent to the first peak of parasitaemia. These considerations are important when possible pathogenic mechanisms are evaluated. Most workers conclude that significant changes occur only after the first peak of parasitaemia (Fiennes, 1954). Furthermore, in animals infected with bloodstream forms of trypanosomes, peak parasitaemia can occur within a few days of inoculation (Murray et al., 1979e,f), whereas, in cattle subjected to a tsetse-transmitted trypanosome infection, parasites cannot be detected in the blood until the second or third week, considerable multiplication taking place in the skin (chancre) prior to dissemination to the bloodstream (Emery et al., 1980a). It is interesting to speculate as to whether the route of infection could influence the mode of induction of anaemia, especially with regard to the role of the immune response, when such differences exist in time to first parasitaemia.

Progressive decrease in PCV takes place over a period of 4 to 12 weeks (average 6 weeks) after infection by which time values of around 20% are reached. At this stage, PCV values may continue to fall until the animal dies (PCV = 15% or less).

The presence of the parasite is the basis of the progress of the anaemia during this phase. If animals are treated with a trypanocidal drug, there is a return to normal haematological values (Holmes and Jennings, 1976), which is especially rapid and dramatic the earlier animals are treated (innumerable personal observations).

Cattle that survive then progress into phase 2 of the disease syndrome that can end in death, spontaneous recovery or survival with persisting low grade anaemia. This phase of the disease process is characterised by low transient parasitaemia or the complete absence of detectable parasites in the blood, while PCV values stabilise at 20 to 25% for a variable period of time (Fig. 1). Some cattle, mainly of the trypanotolerant breeds, e.g., N'Dama, West African Short-

horn, make a complete recovery as judged by PCV values of 30% or more and clinical condition; this may occur between 2 and 4 months after infection. In more susceptible breeds such as the Zebu/Boran, the rate of recovery is slower or only partial (PCV = 25 to 30%). Other animals, despite the absence of detectable parasites, maintain persistently low PCV values (20 to 25%) and make no clinical improvement. This syndrome is rarely seen under experimental conditions, but was commonly encountered in a longitudinal epidemiological investigation of some 2000 N'Dama cattle carried out over 5 years in The Gambia, West Africa (Murray et al., 1979c, d). In this study, a significant number of cattle, previously found to be infected with trypanosomes, continued to exhibit a low grade anaemia for several months in the absence of parasites. Blood and tissues taken from such animals failed to infect mice and rats (Murray, 1979). While some animals suffering from the "chronic trypanosomiasis syndrome" die, many remain alive but in poor health characterised by stunting, wasting and infertility, despite continuing to eat. Animals that eat but do not produce are the scourge of the savanna lands of Africa.

In contrast to the early parasitaemic phase (phase 1) of the disease, the response to trypanocidal drug treatment during phase 2 is poor or is non-existent.

This "bird's eye" picture is one that is produced in cattle by most strains of *Trypanosoma congolense*, *T. vivax* and *T. brucei*. In general, the onset of the anaemia and the extent to which PCV values fall correlate closely with the appearance, height and duration of parasitaemia. Apart from an extravascular phase in the skin (Luckins and Gray, 1978; Akol and Murray, 1982) and the occasional presence in cerebrospinal fluid (Masake et al., 1984), *T. congolense* parasites are confined to the circulation. Once in the circulation they remain free or attach themselves by their anterior end to red cells or endothelial cells lining blood vessels (Bungener and Muller, 1976; Banks, 1979). Some strains of *T. congolense* produce fluctuating parasitaemia (never greater than 10^6 trypanosomes per ml) for 6 to 8 weeks followed by gradually diminishing levels and recovery of the majority of cattle. Other strains result in more sustained parasitaemia which may persist for 6 to 8 months. The majority of these cattle die at any time from 4 weeks to several months after infection but a few animals manage to survive to the stage when parasitaemia starts to diminish, and thereafter their haematological values slowly return to normal.

In contrast to *T. congolense*, both *T. vivax* (Van Den Ingh et al., 1976; Emery et al., 1980; Murray et al., 1980; Masake, 1980) and to an even greater extent *T. brucei* (reviewed by Morrison et al., 1983) have the capacity to invade tissues in domestic ruminants. Infection with certain isolates of *T. vivax* can produce an acute syndrome resulting in death within 2 to 3 weeks of infection. This syndrome is characterised by very high sustained levels of parasitaemia (around 10^7 trypanosomes per ml), with massive haemorrhage particularly into the alimentary tract (Hudson, 1944; Mwongela et al., 1981). Other strains of

T. vivax are not as pathogenic and produce an anaemia that is less severe and less progressive than that observed in most infections with *T. congolense*.

In cattle infected with *T. brucei*, the level of parasitaemia is usually low (less than 10^5 parasites per ml) and the degree of anaemia is less than encountered with the other trypanosome species (Murray et al., 1979e). However, such infections can lead to severe tissue damage, including myocarditis (Morrison et al., 1979) and meningoencephalitis (Morrison et al., 1983).

Finally, it must be pointed out when considering the significance of different strains and species of trypanosomes in the pathogenesis of anaemia that mixed infections of *T. congolense*, *T. vivax* and sometimes *T. brucei* do occur under natural field conditions.

Kinetics

Using radioactive isotope tracers in combination with microscopic and post-mortem findings, we have attempted to construct a kinetic model of the anaemia of bovine African trypanosomiasis prior to evaluating the mechanisms likely to be responsible.

The most composite studies carried out on red cell kinetics have been performed by Dargie et al. (1979a, b) who investigated cattle from 7 to 16 weeks after infection with bloodstream forms of *T. congolense* and *T. brucei*, respectively. By simultaneous use of ^{125}I -labelled albumin, ^{51}Cr -labelled red cells and ^{59}Fe -labelled transferrin it was possible to measure directly plasma and circulating red cell volumes, and the rates at which red cells were added to or withdrawn from the circulation by synthesis and breakdown. Other studies (Mamo and Holmes, 1975; Holmes and Jennings, 1976; Holmes, 1976; Valli et al., 1978; Preston et al., 1979) examined only one or only some of these parameters, but nevertheless made a significant contribution in that they were carried out over a range of time periods, allowing assessment of the kinetics of the anaemia from day 0 of infection until several months afterwards.

In cattle infected with *T. congolense*, increased red cell breakdown commences with the development of parasitaemia (Holmes, 1976; Preston et al., 1979) and would appear to be most rapid during the next 2 to 4 weeks (Preston et al., 1979). Two factors acting singly or in concert could theoretically account for these changes, namely, haemorrhage or haemolysis. Although the former occurs in cattle with the acute haemorrhagic syndrome caused by certain isolates of *T. vivax* (Hudson, 1944) and would be expected to be accompanied by hypoferraemia (discussed by Berry and Dargie, 1978), the majority of trypanosome-infected cattle have normal serum irons and high plasma iron pools (Dargie et al., 1979a, b). At the same time, at necropsy widespread haemorrhage is not a feature, and only occasional petechial and ecchymotic haemorrhages are found scattered throughout the carcass (Losos and Ikede, 1972; Murray et al., 1979d). Also, there is no evidence to indicate that intravascular haemolysis contributes to any significant extent to the anaemia of this phase of the infec-

tion. Jaundice is not a feature, serum bilirubin is not raised, and haemoglobin-aemia and haemoglobinuria do not occur.

It would appear that the major mode of red cell destruction in cattle during the initial parasitaemic phase of the disease is extravascular and is the result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (Murray et al., 1979d; Murray et al., 1980), as is also the case in small ruminants (MacKenzie and Cruickshank, 1973; MacKenzie et al., 1978), in man at least with *rhodesiense* (Woodruff, 1973; Woodruff et al., 1973) and in laboratory animals (Jennings et al., 1974; Murray et al., 1974a, b). In cattle at necropsy, splenomegaly can be marked with spleens weighing as much as 2 kg (400 to 500 g might be considered normal in adult cattle in Africa, Murray et al., 1979d). Splenomegaly is mainly the result of red cell sequestration and a massive macrophage population phagocytosing red cells. Erythrophagocytosis is not confined to the spleen and is widespread throughout the body in the lungs, haemal nodes, bone marrow and circulation; it is particularly marked in the liver which is usually swollen and pale at necropsy and histologically exhibits a striking increase in the number of Kupffer cells and in their activity (Murray et al., 1979d, 1980).

At the same time, judging by the rates of plasma iron turnover and red cell iron utilization, erythropoiesis is increased in infected cattle. This was observed as early as 2 weeks after infection (Holmes, 1976) and by 7 weeks there was nearly a three fold increase in haemopoiesis (Dargie et al., 1979a). There was, therefore, no evidence of overt dyshaemopoiesis. During the parasitaemic phase of the disease the anaemia is usually in our experience normocytic normochromic but we have observed macrocytic responses in certain individuals, a change reported by several other groups (Fiennes, 1954; Naylor, 1971; Mamo and Holmes, 1975; Valli et al., 1978; Maxie and Valli, 1979; Preston et al., 1979; Valli and Mills, 1980). A marked erythroid response has been reported in the sternal bone marrow of infected cattle with the myeloid: erythroid ratio decreasing by about three fold by the fifth week of infection (Maxie and Valli, 1979; Valli and Forsberg, 1979). While erythropoietic and to a lesser extent granulopoietic activity can be marked in the bone marrow of trypanosome-infected cattle, we have not found any equivalent extramedullary activity by histological examination, in contrast to the striking response in the spleen and liver of mice infected with *T. congolense* (Morrison et al., 1981a, 1982) or with *T. brucei* (Murray et al., 1974a).

However, Dargie et al. (1979a) were of the opinion that an erythropoietic response which was only three fold was surprisingly moderate in relation both to the degree of anaemia and to the number of cells being removed. At present, there are no data available on the maximum erythropoietic capacity of cattle, but if it approximates that of sheep, pigs and man, all of which can increase haemopoiesis by up to six times during parasitic or chemically-induced anaemia (Dargie, 1975; Bush et al., 1956; Wintrobe, 1967) then there is some in-

dication of impairment of the ability of cattle during the parasitaemic phase of the infection to increase red cell production.

It has also been proposed that during the early phases of infection haemo-dilution might contribute to the development of anaemia (Holmes, 1976; Maxie and Valli, 1979). However, Dargie et al. (1979a, b) and Dargie (1980) concluded that this was not the case. Independent measurement of plasma volumes (with ^{125}I -albumin) and circulating red cell volumes (with ^{51}Cr -labelled red cells) showed that while the plasma volumes of infected cattle were significantly higher and the circulating red cell volumes were significantly lower than those of controls, the total blood volumes were not altered by infection. It would appear that the expansion of plasma volume was a normal homeostatic response for maintaining blood volume and pressure in the face of the drop in red cell mass (Dargie, 1980). Previous studies had used ^{59}Fe results to help compute plasma volumes (Holmes, 1976). However, because of its rapid transfer from the plasma (Dargie et al., 1979a, b), ^{59}Fe does not equilibrate with the total plasma space and hence seriously overestimates the plasma volumes in anaemic animals. On the other hand, there was no difference in the transcapillary exchange rate of albumin between infected and control cattle (Dargie, 1980).

Less data are available on the kinetics of the anaemia which occurs in the more chronic phase of the disease. However, pathological findings (Murray et al., 1979d) and the work of Dargie et al. (1979a, b) and of Preston et al. (1979) who investigated cattle up to 16 weeks and 28 weeks after infection, respectively, provide key observations of what is going on during the period when PCV values have been persistently low for several weeks, despite scanty parasitaemia or the complete absence of detectable parasites in the blood. Thus, while splenomegaly is no longer a feature during this time, erythrophagocytosis by an obviously active mononuclear phagocytic system is striking and widespread in the spleen and throughout the body. An important new development is the appearance of haemosiderin deposits to the extent that in some animals they can be massive not only in the spleen, liver, lungs and bone marrow but throughout the body. At the same time, it can be difficult to find any evidence of erythropoietic activity in bone marrow which has a yellow gelatinous appearance.

Erythrokinetic studies confirmed the histological observations of increased red cell destruction (Dargie et al., 1979a, b) even as long as 28 weeks after infection (Preston et al., 1979). However, as alluded to previously, the erythropoietic responses were surprisingly moderate for the degree of anaemia and for the number of red cells removed (Dargie et al., 1979a). Iron metabolism data gave indications of dyshaemopoiesis developing (Dargie et al., 1979a). Although the amount of iron carried to the bone marrow was increased, the rate of ^{59}Fe clearance from the plasma was slower than expected from the degree of anaemia. Because erythropoiesis (Reissmann, 1964) and plasma ^{59}Fe clearance rates (Berry and Dargie, 1978) are markedly reduced in the presence of a low

protein intake, the relatively poor erythropoietic response may partly reflect the reduced appetite that can be shown by infected cattle (unpublished data). It is also evident that a proportion of the iron carried to the marrow was not incorporated into red cells. Of the total iron incorporated into red cells, a substantial proportion either was reutilised extremely slowly or became unavailable for further haemoglobin synthesis following erythrophagocytosis. This phenomenon has two possible explanations. Either the iron was excreted, which seems unlikely, or its release into the plasma and subsequent transport to the bone marrow was blocked by the reticuloendothelial system, thereby disturbing the normal exchange between plasma and storage pools. Whatever the cause, the change was obviously substantial because by the 7th week, the circulating iron pool of the infected Zebu, i.e., total red cell haemoglobin value $\times 3.4$, was 3000 mg compared to 6500 mg for the controls (Dargie et al., 1979a). As a consequence of their anaemia, the infected cattle lost about 55% of their circulating iron. This deficit, combined with the presence of massive haemosiderin deposits within the MPS, indicates defective iron utilization and raises the possibility that in severe or long-standing chronic infections, the marrow is effectively starved of iron and the anaemia complicated by dyshaemopoiesis. This theory is supported by a number of observations. First, the serum iron and transferrin concentrations and the plasma iron pool of the one animal that died in the study of Dargie et al. (1979a) were only about half normal: the plasma iron turnover rate was comparable to the average for the controls, and the PCV was deteriorating rapidly. Second, microcytosis (Fiennes, 1954), hypoferaemia (Tartour and Idris, 1973), and low plasma iron turnover rates (Preston and Wellde, 1976) attend the anaemia of long-term cases. Third, in animals necropsied longitudinal sections of femur showed the marrow to be yellow and gelatinous, indicating almost total unresponsiveness. These findings suggest that the anaemia is ultimately complicated by some degree of marrow dysfunction, the basis of which is probably reticuloendothelial iron blockage. Marrow dysfunction would explain the poor clinical response to trypanocidal drug therapy in animals with long-standing infections and would mean that the condition is morphologically, biochemically, and kinetically analogous to the anaemias associated with chronic human disorders (reviewed by Cartwright and Lee, 1975) and experimentally-induced inflammation in laboratory rodents (Hershko et al., 1974). The fact that many of these anaemias are resolved by testosterone or erythropoietin (Haurani and Green, 1967; Zucker et al., 1974) suggests that, in addition to their direct action on haemopoietic progenitor cells, such hormones are capable directly or indirectly, of releasing iron from the reticulo-endothelial system. This offers the interesting possibility that their administration may have therapeutic value in trypanosomiasis.

In conclusion, the anaemia of bovine African trypanosomiasis would appear to fall into two phases. During the first phase, the onset of anaemia and the extent to which PCV values fall would appear to correlate closely with the

appearance, intensity and duration of parasitaemia. The basis of the anaemia is the increased rate of destruction of red blood cells by an expanded and active mononuclear phagocytic system. This is accompanied by an increase in erythropoiesis, the magnitude of which is possibly limited. In the second phase of the disease, the anaemia may persist in the absence of parasites or in the presence of only a few. While there is evidence that the rate of red cell destruction by a mononuclear phagocytic system, which is still expanded and active, continues to be increased, there is also ferrokinetic and morphological evidence of dyshaemopoiesis as a result of iron trapping by the mononuclear phagocytic system.

Mechanisms of anaemia

As the infection progresses in trypanosome-infected cattle, the kinetics of the anaemia change and with them almost certainly, the underlying mechanisms (Fig. 1).

In phase 1, the trypanosome is key in initiating and maintaining the progress of the development of anaemia. Thus, the onset of anaemia is associated with the first wave of parasitaemia, while the rate of development and severity of anaemia usually reflects the intensity and duration of parasitaemia. In certain animals, the elimination of the parasite or self cure heralds recovery, while treatment with a trypanocidal drug results in the return of haemopoietic values to normal.

During phase 2, however, the role of the trypanosome in the maintenance of anaemia is less important. Thus, anaemia persists in the absence of detectable parasites in the blood, while the response to trypanocidal drug treatment is slow or not at all.

Phase 1

The development of anaemia becomes obvious after the first peak of parasitaemia when, as a result of the antibody responses, a major trypanolytic crisis, of which there are subsequently several, occurs. These crises lead to the formation of antigen-antibody complexes (Murray, 1974; Lambert and Houba, 1974) and probably to the release of a whole range of biologically-active factors known to be present in trypanosomes (Tizard et al., 1978b). There is also evidence that living trypanosomes may lead to red cell damage (Banks, 1979; Esievo, 1983) and therefore may play a key role in the induction of anaemia.

Trypanosome haemolysins

Landsteiner and Raubitschek (1907) found that degenerating trypanosomes, probably *T. brucei*, generated a lipid soluble factor that could lyse red cells. Subsequently, Fiennes (1954) demonstrated the intermittent presence in cattle infected with *T. congolense* of a plasma factor that was capable of lysing normal bovine red cells. Subsequently, we were able to show haemolytic activity

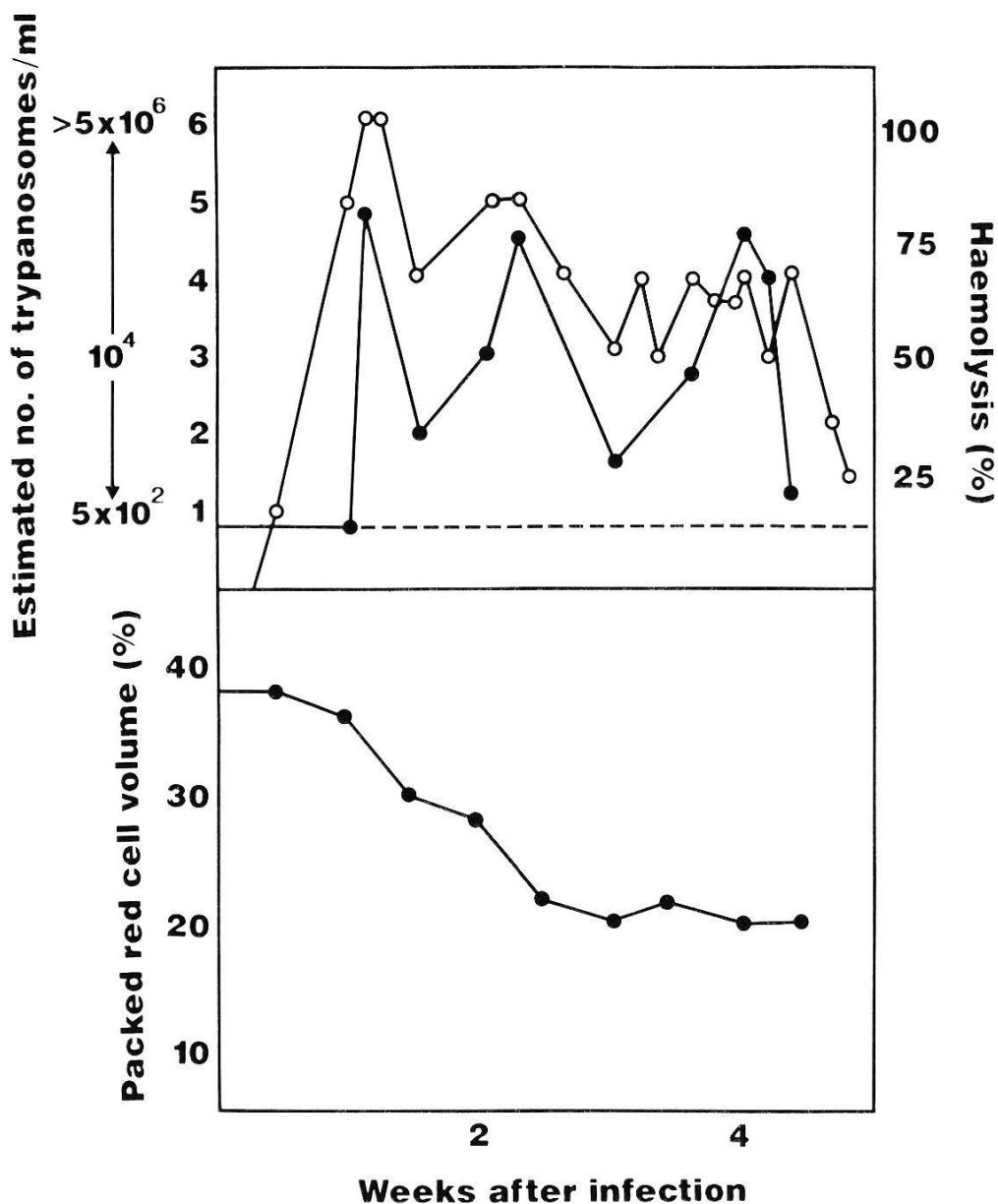


Fig. 2. Development of anaemia in relation to parasitaemia (○) and plasma haemolytic activity (●) in a Boran steer infected with *Trypanosoma vivax*.

in plasma of cattle infected with *T. vivax* (Fig. 2, unpublished data). The peaks of haemolytic activity followed waves of parasitaemia and accompanied the progressive anaemia. Our findings in *T. congolense* and *T. brucei* infected cattle were less consistent, although activity was found on a few occasions. Studies on mice infected with *T. congolense*, *T. vivax* and *T. brucei* (Murray et al., 1979b) and in rats infected with *T. brucei* (Murray, 1979) also indicated the presence of haemolysins in plasma; this activity appeared within 2 to 3 days of infection and correlated strongly with the development of parasitaemia and the onset of anaemia. Furthermore, anaemia occurred in rats subjected to total body irradiation and infected with *T. brucei* and it was possible to demonstrate haemolytic activity in the plasma of these animals (Fig. 3, unpublished data), indicating that immunological competence is not essential for the development of

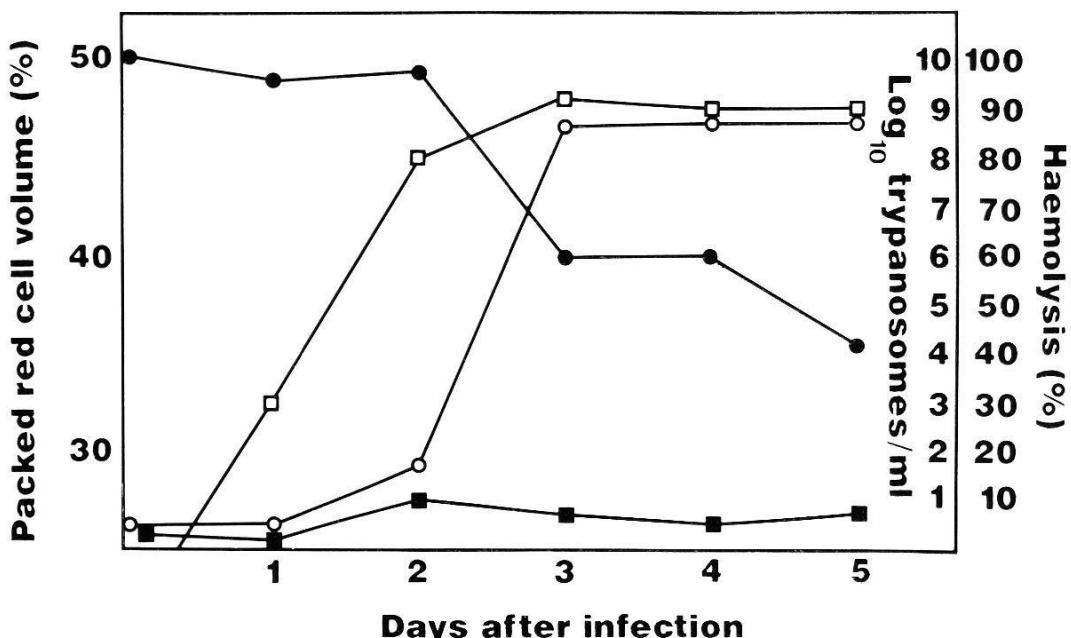


Fig. 3. Development of anaemia (●) in relation to parasitaemia (□) and plasma haemolytic activity (○) in irradiated rats infected with *Trypanosoma brucei*. Haemolytic activity in control rat plasma is also shown (■).

anaemia. The foregoing results were obtained from plasma samples frozen immediately after collection. Blood samples left on the bench for a few hours or collected as serum had significantly greater haemolytic activity, a finding suggesting that haemolytic activity is generated as trypanosomes die. Heating test samples at 56°C for 1 hour had no effect (Murray et al., 1979b).

Haemolytic activity has now been demonstrated in lysates of *T. brucei* (Huan et al., 1975), as well as in *T. congolense* and *T. vivax* (Murray et al., 1979b). However, the nature of the haemolytic factors particularly those responsible for the haemolytic activity in the plasma has yet to be extensively investigated. A range of enzymes that could play a role in red cell damage has now been identified in African trypanosomes. These include proteases (reviewed by Lonsdale-Eccles and Grab, 1986), phospholipases (reviewed by Mellors, 1985) and neuraminidases (Esievo, 1983), while Huan et al. (1975) identified a heat stable, trypsin sensitive substance with a molecular weight of 12 kDa and an isoelectric point ranging from pH 5.0 to 5.5 in the substrate of *T. brucei*.

Proteases

It is now known that trypanosomes contain a number of proteases (Rautenberg et al., 1982; Lonsdale-Eccles and Grab, 1986). It has been shown that lysosomes of *T. congolense* and *T. brucei* contain at least two classes of proteolytic activity, both thiol-dependent and thiol-independent enzymes (Lonsdale-Eccles and Mpimbaza, 1986; ILRAD Annual Report, 1985). A serine peptidase, which appears to be released into the culture medium when parasites are

damaged, has been partially purified from bloodstream forms of *T. brucei*. Another peptidase, apparently of parasite origin, has been identified in the plasma of cattle infected with *T. congolense* and mice infected with *T. brucei* (ILRAD Annual Report, 1986; Knowles et al., 1987). Such enzymes free in the circulation could contribute to red cell damage.

Phospholipases

On autolysis, *T. congolense* and *T. brucei* have been shown to generate an active phospholipase A (reviewed by Tizard et al., 1978b; Mellors, 1985). While phospholipases themselves may have a direct effect on red cell membranes, their main activity would appear to be through their action on endogenous phosphatidylcholine with the release of large quantities of free fatty acids (FFA) including palmitic, stearic and linoleic acids with lesser amounts of oleic and arachidonic acids (Mellors, 1985). As a result of their detergent qualities, FFA, particularly linoleic acid, are haemolytic (Tizard et al., 1978a). It is generally felt, however, that the haemolytic properties of these trypanosome-derived FFAs are not significant *in vivo*, because of the fact that they are rapidly bound to serum albumin and are not haemolytic when in the bound state (Starinsky and Shafrir, 1970). However, it should be emphasised that trypanosomes, particularly *T. congolense*, tend to congregate in dense clusters in capillary beds attaching to red cells or vascular endothelium; it is quite possible that FFA generated in such locations may reach sufficiently high concentrations to provoke damage. In the same way, the massive destruction that follows the termination of a parasitaemic wave could result in a transient but high enough levels of FFA to cause red cell damage.

Large quantities of phospholipase A, probably of trypanosome origin, have been found *in vivo* in tissue fluids of rabbits infected with *T. brucei*. This enzyme was also demonstrated in plasma but at a considerably lower level. It was of interest that within 2 weeks of infection a phospholipase plasma inhibitory factor, possibly antibody, was detected (Hambrey et al., 1980). Thus, apart from the possible localised harmful effects of FFA, trypanosomal phospholipases could have a direct pathological effect on red cell membranes.

Neuraminidase

There is evidence that sialic acids may play a role in the pathogenesis of the anaemia of bovine African trypanosomiasis. There are reports that *T. vivax* produces neuraminidase (Esievo, 1981, 1983), that *T. congolense* attaches to sialic acids on red cells (Banks, 1979), and that sialic acids are constituents of the carbohydrate moieties of the variable surface glycoproteins of *T. congolense* (Rautenberg et al., 1981).

It has been found that unlysed *T. vivax* exhibit neuraminidase activity *in vitro*. The degree of sialidase activity was linear with the number of trypanosomes and was inhibited by influenza serum, further evidence of neuramini-

dase activity in the trypanosome (Esievo, 1983). Based on these findings, it might be that the early anaemia which occurs in infected animals could be attributed to the activity of trypanosome sialidase which might cleave the surface sialic acid (Durocher et al., 1975), rendering erythrocytes more prone to phagocytosis, directly (Durocher et al., 1975), by immunoglobulin and complement opsonisation (Jancik et al., 1978) or by activation of the classical or alternate pathway of complement (Brown et al., 1983). Furthermore, cleavage of sialic acid from red cell membranes would expose new epitopes on the surface of affected cells (Pirofsky, 1969), an effect that could theoretically lead to antibody production against these exposed epitopes, and increased erythrophagocytosis.

It should also be noted that proteolytic enzymes, which, as already discussed, may be released into the circulation from damaged trypanosomes, can remove variable fractions of sialic acid from red cell membranes in the form of glycopeptides (Cook et al., 1960).

The possibility that a parasite neuraminidase might play a role in red cell damage in vivo was further supported by the finding that the onset of anaemia in *T. vivax*-infected cattle was preceded by a significant decrease in mean red cell surface sialic acid concentrations, starting 4 days after infection with the biggest drop occurring between 6 and 14 days, followed by a return to near normality by day 15 (Esievo et al., 1982). What was also significant was that these changes reflected the parasitaemia. The prepatent period was 2 to 3 days, peak parasitaemia was on day 8 and, by day 17, parasites were only just detectable. Moreover, free serum sialic acid showed an increase, though not significant, on day 8, an observation also made by Magaji (1975) in cattle infected with *T. vivax* and *T. brucei*.

Another aspect of the possible role of sialic acid in the pathogenesis of anaemia is the observation that *T. congolense* binds in vitro to bovine red cells through neuraminic acid receptors (Banks, 1979). Banks (1980) showed that damage to red cells occurred only when the organisms had attached to red cells, and antibody and complement had bound to the parasite. It was concluded that red cell damage was mediated through binding of anti-trypanosomal antibody and complement activation, with complement attaching to "bystander" red cells leading to increased erythrophagocytosis.

Trypanosome proteases, phospholipases and neuramidases could play a significant role in the pathogenesis of anaemia of bovine trypanosomiasis. Furthermore, these enzymes might lead to damage of other haemopoietic cells, of endothelial cells lining the circulation, and, depending on their invasive capacity, of body tissues. In this respect *Trypanosoma cruzi*, the causative organism of Chagas' disease, has been found to contain a neuraminidase and live parasites have been shown to have the capacity to remove sialic acid not only from red cells (Pereira, 1983) but also from the surface of endothelial and myocardial cells (Libby et al., 1986).

Immunological mechanisms

Even before the turn of the century, there was evidence that immunological mechanisms might be involved in the anaemia of African trypanosomiasis. In 1898, Kanthack et al. observed autoagglutination and increased sedimentation rates in cattle and also humans with trypanosomiasis. There are now several reports of possible immunological sensitization of red cells in trypanosome infections. Woodruff et al. (1973) demonstrated immunoglobulin and complement on the red cells of patients infected with *T. rhodesiense*, using indirect haemagglutination. This confirmed the findings of Zoutendyk and Gear (1951) and Barrett-Connor et al. (1973), also in humans, using direct haemagglutination. Immunoglobulins have been detected by immunofluorescence on red cells of mice infected with *T. brucei* (Amole et al., 1982).

Using the direct haemagglutination test, IgM and IgG, and C3 were demonstrated on red cells of cattle infected with *T. congolense* (Kobayashi et al., 1976) and with *T. vivax* (Facer et al., 1982). Assoku and Gardiner (in press) also showed IgM and IgG, and C3 on red cells of *T. vivax*-infected cattle, using immunodiffusion and immunofluorescence evaluated by microscopy or by cytofluorography. In the case of *T. congolense*, the development of parasitaemia, the decline in PCV and the detection of immunoglobulin on red cells occurred 7 to 10 days after infection (Kobayashi et al., 1976). The anti-globulin reaction was strongest between 3 and 9 weeks after infection and was positive sporadically until the termination of the experiment at 18 weeks, when the parasitaemia was very low and transient. The kinetics of events were similar in cattle infected with *T. vivax* (Facer et al., 1982; Assoku and Gardiner, in press). Thus, erythrocyte-bound IgG and IgM with or without C3 were demonstrated in infected cattle. In both studies, the animals became positive just after the first peak of parasitaemia and positive reactions were recorded during the 2 months of study. However, Facer et al. (1982) found that only 3 of the 6 animals infected became positive and, thereafter, they were inconsistently positive, whereas, in the experiments of Assoku and Gardiner (in press) all 10 cattle infected were consistently positive with peak activity occurring about one month after infection. Facer et al. (1982) noted that PCV values were always lower whenever a positive direct haemagglutination test was obtained.

With respect to the specificity of the immunoglobulin on the red cells, it was found in *T. congolense*-infected cattle that the IgM and IgG eluates contained antibody activity against *T. congolense* (Kobayashi et al., 1976), while in the studies of Facer et al., (1982) on *T. vivax*-infected cattle IgG and IgM in red cell eluates had antibody activity against *T. vivax* antigen.

It has been proposed that soluble trypanosome antigen, released from dying trypanosomes, is adsorbed on the red cells with subsequent opsonization of antibody and complement. Thus, Herbert and Inglis (1973) showed that syngeneic red cells exposed to plasma from mice infected with *T. brucei* could be used to immunise mice against homologous challenge with *T. brucei*, suggesting

that trypanosome variable surface glycoprotein can be adsorbed on the red cells. In the same way, Woo and Kobayashi (1975) found that sonicated *T. brucei* readily adsorbed on to normal rabbit red cells in vitro, which then lyse in the presence of complement and homologous anti-trypanosome antibody. Moreover, antibodies could be eluted from red cells of infected rabbits, and red cells from infected rabbits lysed in the presence of fresh complement.

In the same way, trypanosomal antigen was demonstrated by the indirect fluorescent antibody test on the surface of red cells of sheep infected with *T. congolense*. The reaction was not evident until after the initial peak of parasitaemia, and was thought to be the result of trypanolysis (MacKenzie et al., 1978). An alternative explanation for red cell sensitization is that trypanosome antigen-antibody complexes, themselves unrelated to erythrocytes, become passively attached to the membrane of red cells, which as discussed earlier, may or may not be damaged. In *T. congolense* infections in rabbits, Banks (1980) demonstrated in an in vitro test system that the binding of antitrypanosomal antibody and complement activation with ensuing red cell damage only occurred when the organisms had attached to red cells; when bystander trypanosome antigen-antibody complexes were added to the in vitro test system no red cell damage was detected.

In contrast, Assoku and Gardiner (in press) found that the IgM and IgG on the surface of red cells from *T. vivax*-infected cattle was specific for red cells. The enzyme-linked immunosorbent assay demonstrated antibodies to normal erythrocytes in the plasma of infected animals, just after the first peak of parasitaemia (around days 10 to 15). Plasma from cattle, taken after 32 days, precipitated radiolabelled proteins from autologous red cells, while it was found by cytofluorography that normal red cells adsorb IgM and IgG following incubation in plasma from infected animals. Immunoglobulins were demonstrated in the eluates of red cells from infected cattle by immunodiffusion. These were shown to restain normal erythrocytes in immunofluorescence tests, but they did not react with infecting trypanosomes.

Auto-antibodies to a variety of host cells or their products have been widely reported in man and laboratory animals infected with trypanosomes (Houba et al., 1969; MacKenzie and Boreham, 1974; Kobayakawa et al., 1979) and have been hypothesised to play a role in trypanosome-induced anaemia (Kobayakawa et al., 1979; Rickman and Cox, 1979).

It might be speculated in the results reported by Assoku and Gardiner (in press) that parasite-derived factors were the primary cause of red cell damage and that by exposing hidden epitopes on the red cell membrane had induced the production of autoantibodies which then played a key role in the maintenance and progression of the anaemia. In this respect, there is evidence that the initial drop in PCV associated with the first wave of parasitaemia does not require an intact immune system, in that there is no difference in PCV changes between normal *T. brucei*-infected rats and in infected rats immunologically compro-

mised by total body irradiation (Fig. 3). On the other hand, a certain degree of immunological competence may be important for the maintenance of anaemia, as suggested by the finding that corticosteroids can attenuate anaemia in *T. brucei*-infected mice (Balber, 1974; Shoyinka and Uzoukwu, 1986). However, it should also be pointed out that cortisone treatment can inhibit red cell sequestration in rats, particularly in the liver (Kaplan and Jandl, 1961) and may account for the ameliorating effect on haemolytic anaemia.

Complement

That complement might play a role in red cell damage and increased rate of destruction is supported by the demonstration of C3 on the surface of red cells of cattle infected with *T. congolense* (Kobayashi et al., 1976) or with *T. vivax* (Facer et al., 1982; Assoku and Gardiner, in press). Furthermore, cattle experimentally infected with *T. congolense* and *T. vivax* develop a marked hypocomplementemia (Kobayashi and Tizard, 1976; Rurangirwa et al., 1980; Tabel et al., 1980), as demonstrated by low levels of complement components C1, C1q, and C3 but not C8, and decreased haemolytic complement activities (Nielsen et al., 1978a). While it has been shown that there is a marked increase in catabolism of C1 and C3 during *T. congolense* infections in cattle (Nielsen et al., 1978b), it would also appear likely that considerable amounts of complement components are activated and consumed in vivo by immune complexes formed by antibodies to variable surface glycoproteins of the trypanosomes (Murray, 1974; Lambert and Houba, 1974).

There is evidence that trypanosomes can activate complement in the absence of antibody. Thus, in vitro studies indicated that soluble fractions of *T. brucei* and *T. congolense* can activate directly human complement, guinea pig complement and bovine complement (reviewed by Nielsen, 1985). Musoke and Barbet (1977) reported that purified surface glycoproteins of *T. brucei* and *T. congolense* activated the classical pathway, in the absence of antibody, while Ferrante and Allison (1983) found that *T. congolense* and *T. brucei* lacking a glycoprotein coat activated the alternative pathway of complement and questioned the previous result. Tabel (1982) provided evidence that homogenates, as well as particulate and soluble fractions, of *T. congolense* activated the alternative complement pathway in bovine serum and showed activation during the course of *T. congolense* infection in sheep (Malu and Tabel, 1986).

Another possible mode of complement activation might be brought about by the action of trypanosome enzymes, e.g., neuroaminidase or protease as discussed earlier, whereby removal of sialic acid from red cell surfaces could lead to the activation of the classical and alternative pathway of complement (Fearon, 1979; Brown et al., 1983).

Complement activation in the absence of antibody might play a role in the induction of anaemia prior to the development of antibody response or perhaps in the maintenance of anaemia in immunosuppressed trypanosome-infected hosts.

Elevated levels of immunoconglutinin have been reported in *T. brucei* infections in rats (Rickman et al., 1981), rabbits and cats (Ingram and Soltys, 1960), and in *T. rhodesiense* infections in man (Woodruff et al., 1973). Such a response reflects widespread fixation of complement possibly on red cells and, if so, could further facilitate increased red cell clearance from the circulation by causing agglutination. In contrast, Tizard et al. (1980) did not find elevated immunoconglutinin levels in *T. congolense*-infected cattle. The result possibly reflects the difference in species of trypanosome and the difference in host.

In conclusion, there is evidence, particularly from the work of Assoku and Gardiner (in press), to indicate that immunoglobulin and complement play a role in the anaemia that occurs during the parasitaemic phase of trypanosome infections in cattle by facilitating red cell destruction through phagocytosis via FC and complement receptors on the macrophage. With the reagents and techniques now available, there is a need to reassess the role of immunological mechanisms in the pathogenesis of the anaemia of bovine African trypanosomiasis in terms of quality, quantity and specificity.

Disseminated intravascular coagulation (DIC)

DIC can lead to a form of haemolytic anaemia, termed microangiopathic haemolytic anaemia, in which the red cells are damaged by widespread fibrin depositon in the microvasculature; the red cells then appear as distorted cells or schistocytes which are liable to lysis or phagocytosis. DIC and various degrees of consumption coagulopathy with severe thrombocytopenia have been described in man, laboratory animals and small ruminants infected with trypanosomes (reviewed by Davis, 1982; Jenkins and Facer, 1985).

Coagulation abnormalities also occur in trypanosome-infected cattle and could contribute to the haemolytic anaemia. The massive bleeding diathesis that occurs in some acute *T. vivax* infections in cattle (Hudson, 1944) is consistent with DIC, while in less acute trypanosome infections the petechial and ecchymotic haemorrhages which are regularly observed on mucous membranes and at necropsy (Morrison et al., 1981a) are also consistent with coagulation abnormalities. In *T. vivax*-infected cattle, prolonged bleeding and prothrombin times were found to be accompanied by elevated levels of fibrinogen and fibrin degradation products early in the infection, when parasitaemia was high (Wellde et al., 1983). Evidence of a mild to moderate consumption coagulopathy was also obtained in cattle infected with *T. congolense* (Wellde et al., 1978; Forsberg et al., 1979). Partial thromboplastin times were consistently prolonged, fibrin degradation products were detected, fibrinogen levels were reduced as was the half-life of fibrinogen, although the prothrombin time was not increased. At the same time, fibrin deposits of microthrombi have been reported in *T. vivax*-infected cattle (Isoun and Esuruso, 1972; Van Den Ingh et al., 1976). However, while these obervations may be true for *T. vivax*, we have not found convincing evidence of such lesions in *T. congolense*-infected cattle. A

critical evaluation of the microvasculature of trypanosome-infected cattle using the latest histochemical and electron microscopical techniques is required.

Further evidence of possible microangiopathy during the initial parasitaemic phase of *T. vivax* infections in cattle was the presence of irregularly-shaped red cells characteristic of microangiopathic haemolytic anaemia (Assoku and Gardiner, in press). The abnormal red cell population were composed of schistocytes (all fragments), "burr" cells (conspicuous spines), poikilocytes (varied shapes), anisocytes including macrocytes and a few microcytes (varied sizes), acanthocytes (crenated with fine surface projections) and helmet cells (helmet-shaped). On the other hand, in cattle infected with *T. congolense*, while spherocytes have been observed (Valli et al., 1978), no dramatic red cell abnormalities have been reported, apart from macrocytes in the initial phase of the disease (Valli et al., 1978) and microcytes in the later more chronic stage (Fiennes, 1954). It might be concluded and it is our impression that microangiopathy is most likely to play a significant role in those infections with *T. vivax* that are acute and characterised by high parasitaemia.

Thrombocytopaenia is a constant finding in both animal and human trypanosomiasis (reviewed by Davis, 1982) and is marked in trypanosome-infected cattle (Wellde et al., 1978; Forsberg et al., 1979; Preston et al., 1982; Wellde et al., 1983). It has long been speculated that the development of thrombocytopaenia associated with African trypanosomiasis is causally related to DIC (Barrett-Connor et al., 1973; Robins-Brown et al., 1975; Van Den Ingh et al., 1976; Davis, 1982). While the induction process that leads to platelet aggregation, defective function (Jenkins and Facer, 1985), reduced platelet half-life (Preston et al., 1982) and activation of the coagulation pathway is not known, there is evidence to suggest that thrombocytopaenia could play a pivotal role. Thus, thrombocytopaenia precedes the other coagulation abnormalities in trypanosome-infected cattle (Wellde et al., 1978; Forsberg et al., 1979) and occurs in rats by the second or third day after infection before any antibody reaction is likely to be involved (Davis et al., 1974; Davis, 1982). Since platelets release a phospholipid, platelet factor 3, that triggers the coagulation cascade, it seems likely that the coagulation disorder of trypanosomiasis begins before the production of autoantibodies or immune complexes, factors that might be subsequently involved. The role of the trypanosome in the induction of thrombocytopaenia is supported by several observations. First, the striking relationship between the onset, severity and persistence of the thrombocytopaenia and the onset, intensity and prevalence of parasitaemia (Wellde et al., 1978, 1983). Second, a quantitative study in cattle, as well as in sheep, goats and rats, showed that there was a dose response relationship between the height of parasitaemia and the degree of thrombocytopaenia, a relationship that was established regardless of strain or trypanosome species (Davis, 1982); reductions in mean platelet counts were directly related to the degree of parasitaemia, with each increase in parasitaemia of greater than $1 \log_{10}$ per ml peripheral blood leading

to a significant reduction in platelet count. Third, platelet counts in *T. congolense*-infected cattle returned rapidly to normal after treatment with the trypanocidal drug Berenil (Wellde et al., 1978).

As discussed earlier, trypanosomes per se possess factors capable of causing cell damage, namely neuraminidase (Esevio, 1983), proteases (Londsdale-Eccles and Grab, 1986) and phospholipases (Tizard et al., 1978b). At the same time, Davis et al. (1974) showed by phase and electron microscopy that whole trypanosomes and trypanosome-free sonicates of *T. rhodesiense* caused marked platelet aggregation in vitro, independent of adenosine diphosphate, complement, kinins, antibody, fatty acids and the presence of the spleen. In the face of the persistent thrombocytopenia that occurs in trypanosome-infected cattle, it is possible that after initial damage to platelets by trypanosome products thrombocytopenia is maintained by immunological reactions in the form of trypanosome generated antigen-antibody complexes (Slots et al., 1977) or by autoantibodies to platelets which have been demonstrated in the plasma of cattle infected with *T. vivax* (Assoku and Gardiner, in press).

Thus, there are considerable grounds to conclude that microangiopathic changes do occur, especially in cattle acutely infected with *T. vivax*, and that the changes, possibly initiated by thrombocytopenia, could lead to red cell damage with resultant phagocytosis.

Fever

Undulating fever usually associated with a trypanolytic crisis is a well recognised feature of bovine African trypanosomiasis (Fiennes, 1954). Studies with human and rabbit red cells have shown that even small elevations in temperatures can have a major effect on red cells. In vitro studies in rabbits have demonstrated that red cells exposed to temperatures only a few degrees above normal body temperature for a few hours have increased osmotic fragility, increased permeability and reduced plasticity with the result that in vivo the red cell survival time is shortened due to increased destruction (reviewed by Karle, 1974), findings that indicate a possible role for fever in the anaemia of bovine African trypanosomiasis.

Mononuclear phagocytic system (MPS)

One of the most striking features of the pathology of bovine African trypanosomiasis is the expanded and active MPS that develops soon after infection and continues throughout the course of the disease (Murray, 1974; Murray et al., 1979d; 1980; Morrison et al., 1981a). Similar findings have been reported in sheep infected with *T. congolense* (MacKenzie and Cruickshank, 1973; MacKenzie et al., 1978). It is likely that this response of the MPS is caused by the massive intravascular presence of living, dying and dead trypanosomes, as well as from the resulting antigen-antibody complexes. It is known that the size and activity of the MPS is a direct function of its particulate work load (Jandl et al.,

1965). Furthermore, that splenomegaly and an expanded MPS per se are capable of causing anaemia has been shown by studies in which methyl cellulose (Palmer et al., 1953; Zuckerman et al., 1969), zymosan (Gorstein and Benacerraf, 1960), and *Corynebacterium parvum* (Nussenzweig, 1967) produced anaemia as a result of erythrophagocytosis. Furthermore, splenomegaly by extending travel through a lengthened vascular network and leading to more protracted contact with the numerous active macrophages lining the channels, must also contribute to red cell destruction (Jenkins and Facer, 1985), including increased removal by phagocytosis even of normal cells.

Conclusions

It would appear that phase one of the anaemia of bovine African trypanosomiasis depends on the presence of the trypanosome and is likely to have a multifactorial basis (Fig. 1), possibly involving trypanosome-generated enzymes, immunological mechanisms, complement activation through trypanosomes and or antigen-antibody reactions, microangiopathic damage, fever and an expanded and active MPS. Although each factor may function independently, it is much more likely that they interact, with trypanosome-derived factors possibly playing the key role in the inductive phase of red cell damage.

Phase 2

During the second phase of the disease process, the anaemia can persist despite the apparent absence of the parasite (Fig. 1). Under these circumstances, the high rate of red cell destruction continues and there is evidence of dyshaemopoiesis developing (Dargie et al., 1979a). As emphasised earlier, the response to trypanocidal drug treatment during this phase is poor.

Mononuclear phagocytic system

One possible explanation for the continuing red cell destruction by the MPS in the absence of trypanosomes comes from the work of Jandl et al. (1965) who found that after prolonged and repeated stimulation, the MPS in rats remains active long after the stimulant is withdrawn. It is possible that an analogous situation occurs in cattle and that the MPS stimulated by the massive trypanosome load and the resultant red cell phagocytosis, has become irreversibly expanded and active, even in the absence of trypanosomes.

Dyshaemopoiesis

While in the early phases of the disease in trypanosome-infected cattle erythropoiesis is increased, as the disease progresses there are indications that red cell synthesis is less than expected for the degree of anaemia, suggesting some impairment of bone marrow function (Dargie et al., 1979a). The effectiveness of the erythropoietic response can be determined by calculating radioactive iron incorporation into circulating red cells, i.e., red cell iron utilisa-

tion. Dargie et al. (1979a) found that this parameter was reduced in *T. congolense*-infected cattle studied between 7 and 15 weeks after infection, indicating diminished or ineffective erythropoiesis. As discussed earlier, the ferrokinetic results (Dargie et al., 1979a) combined with the histochemical finding of massive haemosiderosis (Murray et al., 1979d) suggested that defective iron metabolism caused by iron retention or trapping in the MPS could result in failure of erythropoiesis.

Lawson et al. (1980) evaluated bone marrow function in cattle infected with *T. congolense* using in vitro culture techniques. The mean number of erythroid (CFU-E)* as well as myeloid (GM-CFC) colonies were quantified from the sternal bone marrow. While there was a decrease in the number of myeloid colonies, there were no significant differences between infected and control calves in the number of erythroid colonies produced. However, a closer look at the data indicates especially in the calves with more longstanding infections (13 weeks) that there is a reduction in erythroid progenitor cells. Also, the degree of maturation as measured by haemoglobinization was decreased in the same animals, a result that probably reflects the lack of iron caused by the reticuloendothelial blockade.

Conclusions

These results would suggest that as the disease process progresses in trypanosome-infected cattle, the persistent anaemia is the result of continued increased red cell destruction by the expanded and active MPS and the development of dyshaemopoiesis, as a result of reticuloendothelial iron blockade and possibly a reduction in erythroid progenitor cells. It is interesting to speculate as to whether red cell autoantibodies or trypanosome mediated antigen-antibody reactions might have contributed to stem cell destruction. Sera from parasitaemic cattle infected with *T. congolense* or *T. vivax* failed to inhibit the development of bovine CFU-E colonies in vitro (Kaaya et al., 1979, 1980), although the same sera inhibited bovine GM-CFC colony formation in vitro.

While the search for erythroid colony inhibitors in the sera of trypanosome-infected cattle proved negative, surprisingly sonicated *T. congolense* and *T. brucei* actually stimulated bovine CFU-E colonies in vitro, sometimes resulting in a 20 fold increase in colony formation (Kaaya et al., 1980).

Leukopaenia

Accompanying the first waves of parasitaemia, a pancytopaenia develops in cattle infected with *T. congolense* and *T. vivax*. Thus, a 30 to 50% drop in total

* Committed granulocyte/macrophage colony forming cells capable of producing granulocyte/macrophage colonies in vitro are referred to as GM-CFC. Committed erythropoietic progenitor cells capable of producing erythroid colonies in vitro are referred to as Erythropoietin-dependent colony forming units or CFU-E.

leukocytes (Naylor, 1971; Vodrasky, 1969; Losos et al., 1973; Maxie et al., 1979; Kaaya et al., 1979; Valli and Mills, 1980; Saror et al., 1981; Ellis et al., 1987) accompanies the anaemia and thrombocytopenia. In all cases, the leukopenia was followed by a leukocytosis.

The leukopenia was the result largely of a decrease in lymphocytes and neutrophils (Naylor, 1971; Maxie et al., 1979; Saror et al., 1981; Ellis et al., 1987). In a study done on trypanotolerant N'Dama and trypanosusceptible Boran (Zebu) cattle infected with *T. congolense*, flow cytometric analysis using monoclonal antibodies (MoAbs) revealed that the leukopenia associated with the first wave of parasitaemia was due mainly to an absolute decrease in T cells expressing BoT2 and either BoT4 or BoT8, surface immunoglobulin M-positive B cells, nulls cells which did not express T cell, B cell or monocyte markers, and neutrophils. At the same time, there was a significant variation over time, but no overall increase or decrease, in the number of cells expressing class II major histocompatibility (MHC) molecules, or monocyte markers, or in the number of circulating eosinophils. A decrease in eosinophils has been reported by some workers in trypanosome-infected cattle (Naylor, 1971; Maxie et al., 1979), but again no significant changes in monocytes. In contrast, Saror et al. (1981) observed a transient increase in blood monocytes in N'Dama and Zebu cattle infected with *T. vivax* following the first peak of parasitaemia and then the values returned to normal. An increase in circulating monocytes might be expected based on the histological finding that monocytes and macrophages are numerous in the microcirculation of infected cattle (Murray et al., 1979d, 1980). Following the first wave of parasitaemia in N'Dama and Boran (which had to be treated with the trypanocidal drug Berenil to permit survival), there was a leukocytic response characterised by an increase in the total number of B cells, T cells and nulls and then a gradual return to preinfection levels, around 80 days after infection by which time parasites were rarely detectable in the blood (Ellis et al., 1987).

Prior to and throughout the course of infection, N'Dama had significantly higher numbers of B cells and null cells than Boran (Ellis et al., 1987), suggesting the capability of the trypanotolerant N'Dama to generate antibody responses that are qualitatively or quantitatively better than trypanosusceptible Boran. Superior antibody responses have been reported to *T. vivax* infections in N'Dama (Desowitz, 1959) and in Bauole cattle (Trypanotolerant West African Shorthorn) infected with *T. congolense* (Fig. 4; Akol et al., 1986; Pinder et al., 1988). Desowitz (1959) suggested that the basis of this superiority was a better innate capacity to mount a secondary immune response. Akol et al. (1986) and Pinder et al. (1988) found no difference in neutralising antibody responses between Bauole and Zebu to infecting *T. congolense* metacyclic parasites delivered by tsetse but showed that the Bauole mounted an earlier and much greater neutralising antibody response to the first peak bloodstream trypanosomes, possibly reflecting an inherent ability to produce a superior secondary response.

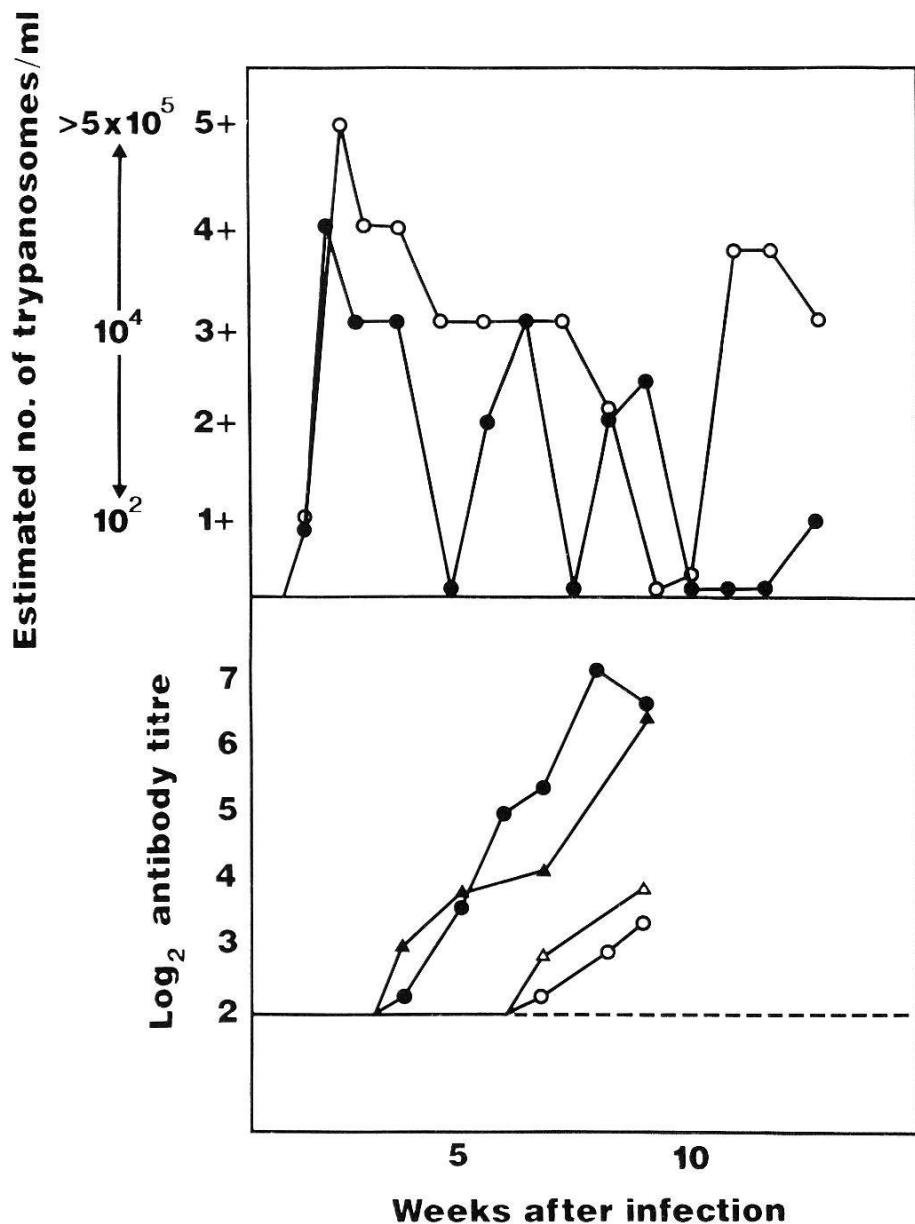


Fig 4. Parasitaemia in Baoule (●) and Zebu (○) cattle infected with *Trypanosoma congolense*. Also shown are neutralising antibody titres against bloodstream trypanosomes in the serum of two Baoule (● ▲) and two Zebu (○ △). The bloodstream trypanosomes were derived from the first peak parasitaemia of the respective animals. Limit of detection (---). Reproduced from Akol et al. (1986) with permission of Veterinary Immunology and Immunopathology.

In the same way, the higher number of B lymphocytes found in N'Dama compared with Boran (Ellis et al., 1987) might be due to the fact that animals used in this study had experienced a previous infection with an unrelated serodeme of *T. congolense*, possibly giving the N'Dama the opportunity to prime their superior immune system. In this respect, Saror et al. (1981) found no difference in total blood leukocytes in N'Dama and Zebu cattle with no previous exposure to trypanosomiasis.

The possible mechanisms that might be considered to be responsible for leukopaenia are similar to those discussed earlier for anaemia and thrombocytopenia. Thus, autoantibodies, pre-formed antigen-antibody complexes or ad-

sorbed trypanosome antigen have been suggested (MaxKenzie et al., 1978; Maxie et al., 1979; Jenkins and Facer, 1985) and could result in premature leukophagocytosis, an event that has been reported in *T. congolense* infected sheep (MacKenzie and Cruickshank, 1973) and in infected cattle (unpublished data). It has also been proposed that the increased marrow erythropoiesis which occurs, does so at the expense of the leukopoietic response (Valli et al., 1979).

An interesting observation has been the finding that sera from cattle infected with *T. congolense* or *T. vivax* inhibited GM-CFC colony formation with maximum inhibition being caused by sera collected during the first 2 to 3 weeks after infection, the period of maximum parasitaemia (Kaaya et al., 1979). The concentration of the leukopoietic inhibitor as assayed by inhibitory activity followed the same pattern as leukocyte counts in the circulation. Thus, it would appear that the leukopaenia resulted primarily from direct inhibition of stem cell differentiation rather than a stem cell deficit. The factor(s) was a heat stable protein with a molecular weight of between 100 kDa and 200 kDa (Kaaya et al., 1980). It was thought that it might be an immunoglobulin or a lymphokine. At the same time, trypanosome-derived factors were considered on the basis of correlation of activity with parasitaemia and rapid restoration of the leukocyte population after trypanocidal drug treatment. However, Kaaya et al. (1980) concluded that such factors were not involved as sonicated fractions of *T. congolense* (and *T. brucei*) did not have any effect on GM-CFC colony formation. Nevertheless, at this stage of our understanding trypanosome-related factors should not be excluded. Recalling the earlier discussion, it should be emphasised that the trypanosome-derived factors, which were found, at least in vitro, to damage red cells, were also toxic for peritoneal cells and buffy coat cells (Tizard and Holmes, 1976; Tizard et al., 1978b).

Factors affecting severity of anaemia

In preparing this "bird's eye" view of the anaemia of bovine African trypanosomiasis, and other disturbances of the haemopoietic system, it was obvious that the outcome of infection and the severity of anaemia were affected by variables in the parasite and in the host. Recognition of these variables and an understanding of the factors underlying them could lead to the development of novel methods for controlling bovine African trypanosomiasis.

The parasite

The factors involved in virulence depend on the characteristics of the species of the trypanosome, as well as the strain. The three trypanosomes responsible for the disease in cattle have quite different behavioural patterns, e.g., *T. congolense* is confined to the circulation where it often attaches to red cells or endothelial cells lining blood vessels; *T. vivax*, on the other hand, seems disinterested in red cells as it races about the circulation and can migrate into

extravascular locations; *T. brucei* is equally at home in the circulation or in the tissues. In addition, the capacity of different species to generate "toxins", or to activate complement or to undergo antigenic variation might affect their virulence.

A similar argument could be made for the variation in virulence among different strains of the same species. However, on the basis of the proposal that virulence might be related to variable antigen type (VAT) (McNeillage and Herbert, 1968; Van Meirvenne et al., 1975), Barry et al. (1979) examined a set of cloned populations of *T. brucei*, all of which bore the same VAT. The clones made close to the time of isolation were found to be moderately virulent for mice, whereas those prepared from the same stock following repeated passage were extremely virulent. It appeared that virulence and VAT were not necessarily related. Instead, it was concluded that virulence was limited by the capacity of the clone to differentiate morphologically, i.e., to become pleomorphic. This conclusion confirmed a well recognised situation in laboratory animals, namely, that trypanosome strains subjected to repeated passages become increasingly monomorphic and virulent. When the parasite differentiates it switches from the rapidly dividing slender form to the nondividing stumpy. Thus, the rapidly dividing parasites produce high parasitaemias and kill the host quickly, whereas, recent pleomorphic isolates produce lower parasitaemias and are less virulent. Furthermore, there is evidence that the stumpy forms of *T. brucei*, not the slender parasites, are responsible for induction of the immune response (Sendashonga and Black, 1982). Thus, virulence is related to the height of parasitaemia, a variable that is controlled by the growth capacity of the parasite and its ability to induce the immune response. Although, there is no morphological equivalent of the stumpy form in *T. congolense*, parasite growth control, as with *T. brucei*, appears to be related to the capacity to stimulate an antibody response (Roelants and Pinder, 1987).

Another possible aspect of trypanosome virulence was proposed by Sacks et al. (1980) who found that differences in the virulence of *T. brucei* were directly associated with the capacity of subcellular membrane fractions to induce immunosuppression.

There is much evidence to suggest that in *T. congolense*, *T. vivax* and *T. brucei* infections in cattle, the height of parasitaemia can determine the severity of anaemia (Murray et al., 1979f), of thrombocytopaenia (Davis, 1982) and of leukopaenia (Ellis et al., 1987). While, as will be discussed, the host can and does play an important role in controlling parasite growth, a major area for research must be on the parasite factors that regulate growth and induce the immune response.

The host

Several host factors have now been shown to have a major effect on the severity of the anaemia of African trypanosomiasis. Of these the most significant are breed, nutritional status and age. Wild Bovidae must also be consid-

ered. In the studies reviewed in this document breeds of cattle evaluated have included Ayrshire, Friesian, Holstein, Hereford, and their crosses, as well as indigenous African breeds such as Zebu, Boran, West African Shorthorn and N'Dama. Their ages have varied from 1 week to 15 years, and they were maintained in villages, ranches or experimental stations where major differences in management and nutrition must have occurred.

Regulation of parasite growth

Because of the lower levels of parasitaemia that occur in cattle when compared with laboratory animals, standard quantitative techniques for enumerating parasites, such as the use of a haemocytometer, are not sufficiently sensitive and cannot be used (Paris et al., 1982). Thus, the development of the darkground/phase contrast buffy coat method (Murray et al., 1977) was an important advance in the study of parasite kinetics in cattle, particularly trypanotolerant breeds. This method is not only at least as sensitive as other techniques and permits trypanosome species identification but it also allows a semi-quantitative estimation of parasitaemia (Murray et al., 1979a; Paris et al., 1982).

It has long been recognised that certain breeds of cattle are able to survive in tsetse-infested areas and resist the effects of trypanosomiasis when other breeds rapidly succumb (Pierre, 1906; ILCA, 1979). This trait is termed trypanotolerance and is generally attributed to the indigenous taurine breeds of cattle in West and Central Africa, namely the N'Dama and West African Shorthorn.

Major comparative investigations on the question of trypanotolerance have been carried out on cattle in Nigeria (Roberts and Gray, 1973a, b), The Gambia (Murray et al., 1981a; reviewed by Murray et al., 1982), Senegal (Toure et al., 1978), Burkina Faso (reviewed by Roelants, 1986), Kenya (Njogu et al., 1985; Ismael et al., 1985; Paling et al., in press) and in the ILCA/ILRAD Trypanotolerance Network (1986a, b). The main breeds studied were the trypanotolerant N'Dama, and the trypanosusceptible Zebu (Boran).

In these investigations, cattle were infected by syringe inoculation with bloodstream trypanosomes, exposed to field challenge, laboratory challenge with wild caught tsetse or with tsetse experimentally infected. Irrespective of the mode of infection, the outcome of each study consistently confirmed the superior resistance of the N'Dama (and the West African Shorthorn) and showed that the basis of this trait lay in the capacity of these animals to develop less severe anaemia (Fig. 5). Furthermore, their resistance to anaemia appeared to be correlated with the ability to limit the intensity, prevalence and duration of parasitaemia to a significantly greater extent than the trypanosusceptible Zebu or European breeds (reviewed by Murray et al., 1982), rather than an innate capacity to mount and maintain a more efficient erythropoietic response (Dargie et al., 1979a). A similar capacity to control parasitaemia and resist anaemia has been shown by the Orma Boran cattle in Kenya (Ismael et al., 1985)

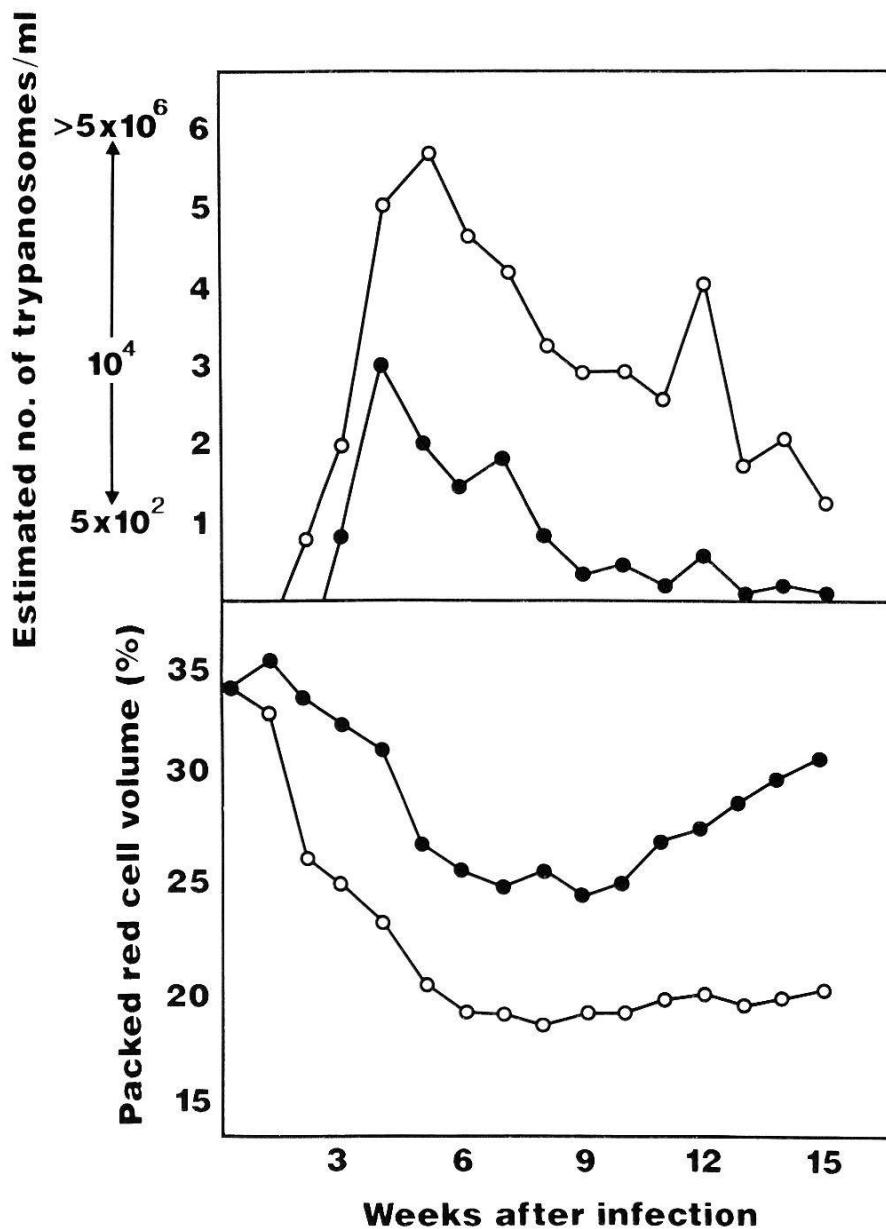


Fig. 5. Parasitaemia and anaemia in trypanotolerant (●) and trypanosusceptible (○) cattle infected with *Trypanosoma vivax*.

and to an even greater extent by several species of wild Bovidae (Murray et al., 1981b).

A study which indicated that the height of parasitaemia per se can influence the degree of anaemia was a dose response experiment in which groups of N'Dama and Zebu were inoculated subcutaneously with 10^3 , 10^5 or 10^8 blood-stream forms of *T. brucei* (Murray et al., 1979f). It was found that the number of parasites inoculated influenced not only the prepatent period but also the height of parasitaemia. Thus, the N'Dama and Zebu groups that received the largest doses had the shortest prepatent period and the highest parasitaemia and, correspondingly, developed the most severe anaemia (Fig. 6). In confirmation of their trypanotolerant nature, the N'Dama were better able to control parasitaemia and developed less severe anaemia than the Zebu which received the

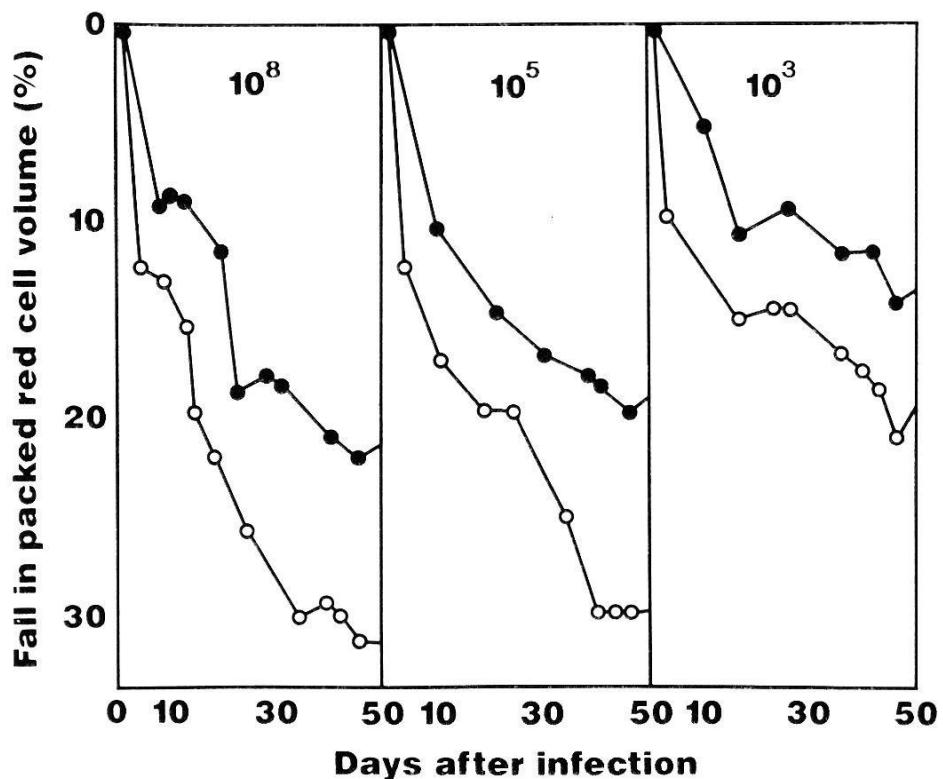


Fig. 6. Percentage fall in PCV from pre-infection values in N'Dama (●) and Zebu (○) cattle infected with 10^8 , 10^5 or 10^3 organisms of *Trypanosoma brucei*. Pre-infection PCV, N'Dama = $34 \pm 4\%$; Zebu = $35 \pm 4\%$ (mean \pm 1 standard deviation).

same dose; thus, it was necessary to challenge the N'Dama with $5 \log_{10}$ more organisms to produce similar levels of parasitaemia and similar severity of anaemia as in Zebu. This work, using *T. brucei* in cattle, is the only dose response study of which we are aware. It has important implications both in relation to control of parasitaemia and development of anaemia. However, it is our impression with *T. congolense* infections in cattle that while infective dose determines the prepatent period it does not affect the height of parasitaemia or the severity of anaemia. In the same way in mice, increasing doses of *T. congolense* (Morrison and Murray, 1985) or *T. brucei* (ILRAD, unpublished data) resulted in shorter pre-patent periods but there were no significant increases in the height of parasitaemia or the time to death after the first peak of parasitaemia.

It is generally believed that the superior capacity to control parasitaemia is associated with the immune response but in cattle, at any rate, there are only a few preliminary studies in N'Dama infected with *T. vivax* (Desowitz, 1959) and Bauole infected with *T. congolense* (Akol et al., 1986; Pinder et al., 1988) which indicated that this might be the case. Details of these results were discussed earlier in relation to leukopoiesis (Fig. 4).

Considerable understanding in the interrelationship between susceptibility, parasite growth and the immune response has been provided by the work of

Black et al. (reviewed in 1985) on the mouse model system. Mice vary in their susceptibility to *T. brucei* (Black et al., 1983), *T. congolense* (Morrison et al., 1978) and to *T. vivax* (Mahan et al., 1986). No critical studies have been done to compare the haemopoietic responses of these different strains of mice but it is our impression that they are minor, although the question requires investigation. Basically, it has been found that the more resistant strains of mice such as the C57B1 are better able to control parasitaemia and produce a superior antibody response. The differences in the immune response to infection could not be attributed to inherent differences in the immune response to the parasite, as no differences in antibody responses occurred between different strains of mice when immunized with non dividing irradiated *T. brucei* (Black et al., 1983), *T. congolense* (Morrison and Murray, 1985) or *T. vivax* (Mahan et al., 1986). In *T. brucei* infections, it was concluded that the height of parasitaemia is controlled by the availability of molecules which maintain the parasites as dividing forms. When the molecules reach a limiting concentration, the parasites cease dividing and start to degenerate. In vitro analysis suggests that *T. brucei* growth promoting activity is associated with serum components of molecular mass greater than 100 kDa (Black et al., 1985). The importance of parasite growth regulation in the control of parasitaemia is further emphasised by the findings which suggest that antibody responses against *T. brucei* are stimulated by fragments derived from senescent non dividing organisms but not by actively dividing parasites (Sendashonga and Black, 1982). As with *T. brucei*, the elimination of *T. vivax* and *T. congolense* from the blood is antibody mediated. There, however, would appear to be important differences in the role played by antibody in controlling *T. brucei*, *T. congolense* and *T. vivax* parasitaemia. Antibody responses to *T. brucei* (Sendashonga and Black, 1982; Black et al., 1983) and *T. congolense* (Mitchell and Pearson, 1986; Whitelaw et al., 1983) parasitaemia occur after most organisms have differentiated to non-dividing forms, the stumpy form in the case of *T. brucei*, and hence when parasite population expansion in the blood is no longer exponential. In contrast, antibody control of parasitaemia in C57B1/6 mice infected with *T. vivax* occurred during the exponential phase of parasite population increase in the blood (Mahan et al., 1986). Thus, the capacity to control *T. brucei* and *T. congolense* parasitaemia appears to lie in the ability to regulate parasite growth followed by the induction of the immune response, while in *T. vivax* infections it appears to be directly dependent on the ability to mount an immune response.

Another aspect of the inability of susceptible C3H/He mice to control parasitaemia following infection with *T. brucei* (Black et al., 1986) or with *T. vivax* (Mahan et al., 1986) resulted from an impaired capacity of parasite-induced antibody-containing cells to secrete immunoglobulin. Such cells regained the ability to secrete antibody within 24 h after trypanosome elimination by treatment with the trypanocidal drug Berenil (Black et al., 1986; Mahan et al., 1986), suggesting that the block in antibody secretion was maintained by living

parasites or short-lived components of degenerating parasites. Our own observations suggested that Ayrshire cattle might be the bovine equivalent of the C3H/He mouse with respect to susceptibility and response to infection with trypanosomes. Following infection with *T. congolense* or *T. vivax*, parasitaemia reaches a high level, antibody responses are low and transient and remission of parasitaemia is absent. It was a notable feature of both susceptible C3H/He mice and Ayrshire cattle that during infection the splenic architecture became rapidly disorganised (Murray and Black, 1985).

It was also possible to manipulate population growth rates in mice infected with *T. congolense* (Murray and Morrison, 1979), *T. brucei* (Murray and Morrison, 1979) and *T. vivax* (unpublished data). Mice treated with heat-killed *Propionibacterium acnes* (*Corynebacterium parvum*) prior to infection were able to control parasitaemia and survive for longer. The effect was most marked in strains of mice with the greatest innate resistance to trypanosome infection, e.g., the C57B1/6. Thus, sera from *P. acnes*-treated mice contained a group of hydrophilic molecules (100 kDa–1000 kDa) which competitively inhibited the capacity of foetal bovine serum (FBS) to support multiplication of *T. brucei* in vitro (S. J. Black, personal communication). Similar molecules were released by macrophages of *P. acnes*-treated mice, by macrophages fed *P. acnes* in vitro and, at a 4000 fold lower efficiency, by *P. acnes* incubated in vitro in the absence of macrophages. The competitive interaction between the growth-promoting factors in FBS and the growth-inhibiting factors produced by *P. acnes* might indicate a competitive interaction for a receptor site at the level of the trypanosome, a receptor usually reserved for the binding and uptake of serum nutrients which regulate multiplication.

The identification and characterisation of the factor(s) that promote and inhibit parasite growth, as well as the trypanosome-factors that inhibit immunoglobulin secretion in trypanosusceptible animals, could lead to new therapeutic or immunological approaches to prevent or control parasite growth. Prevention could result in a vaccine, better control might make it possible to turn the trypanosome status of a Zebu into that of a N'Dama, and of a N'Dama into that of a wild Bovid.

There is also evidence to indicate that the macrophage could play an important role in controlling parasitaemia. Thus, it was found that wildebeest (*Connochaetes taurinus*) infected with *T. brucei* were better able to control parasitaemia than their cattle counterparts and unlike cattle did not develop anaemia (Rurangirwa et al., 1986). Both wildebeest and cattle produced similar IgM, IgG₁ and IgG₂ antibody responses to the infecting trypanosome. However, it was found that serum from infected wildebeest had a higher capacity than cattle to induce adherence of trypanosomes to their own peripheral blood leukocytes (PBL) and that this difference could be attributed to the presence of IgM receptors in wildebeest PBL, which were not present on the PBL of the cattle studied. Such a mechanism could account for the lower parasitaemia

encountered in trypanotolerant animals. The cattle in this study were trypano-susceptible Zebu X Charolais and it is important that trypanotolerant N'Dama cattle be investigated in the same way.

Haemopoietic responsiveness

There is little doubt that the intensity of parasitaemia has a significant effect on the severity of the subsequent anaemia, an observation that has led to the general conclusion that the superior resistance of trypanotolerant animals lies in their ability to control parasitaemia rather than an inherent capacity to resist red cell destruction or mount a more efficient erythropoietic response (Dargie et al., 1979a).

However, differences in the severity of anaemia have been observed under circumstances where no difference in the intensity or prevalence of parasitaemia occurs.

Thus, a number of workers have confirmed that calves of less than one year are more resistant than adults to the effects of trypanosomiasis (Fiennes, 1970; Maxie and Valli, 1979; Wellde et al., 1981; Murray et al., 1982). While Wellde et al. (1981) concluded that parasitaemia was possibly less severe in calves infected with *T. congolense*, in an experiment carried out at the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi on Boran cattle aged 6 weeks and 18 months, we found that the calves developed anaemia that was significantly less severe than in adults, despite the fact that there were no differences in the intensity or prevalence of the parasitaemia. These results might indicate that younger animals have a superior erythropoietic response. In this respect, it is of interest that it is well recognised that calves are more resistant than adults to babesiosis (Callow et al., 1976; Levy et al., 1982).

Certain species of wild Bovidae can develop high levels of parasitaemia following trypanosome infection, e.g., waterbuck (*Kobus ellipsiprymnus*) infected with *T. brucei*. Despite this, no significant changes in red cell numbers occurred, in contrast to the marked anaemia in a trypanosusceptible cow with equivalent levels of parasitaemia (Fig. 7). These findings might suggest that the waterbuck red cells were more resistant to the destructive effects of the trypanosome.

In probably the most informative experimental study to date on the question of trypanotolerance, Paling et al. (in press) presented results on N'Dama and Boran cattle which indicated that the superior capacity of the N'Dama to control parasitaemia and resist anaemia are processes which are controlled genetically but are not directly linked to each other. The eight N'Dama used in the study were of Gambian origin and had been obtained by intrauterine implantation of embryos into Boran female cattle at ILRAD (Jordt et al., 1986). The N'Dama along with corresponding Boran controls had been challenged on four occasions with *Glossina morsitans centralis* infected with clones of *T. congolense* belonging to four different but equally virulent serodemes. To prevent

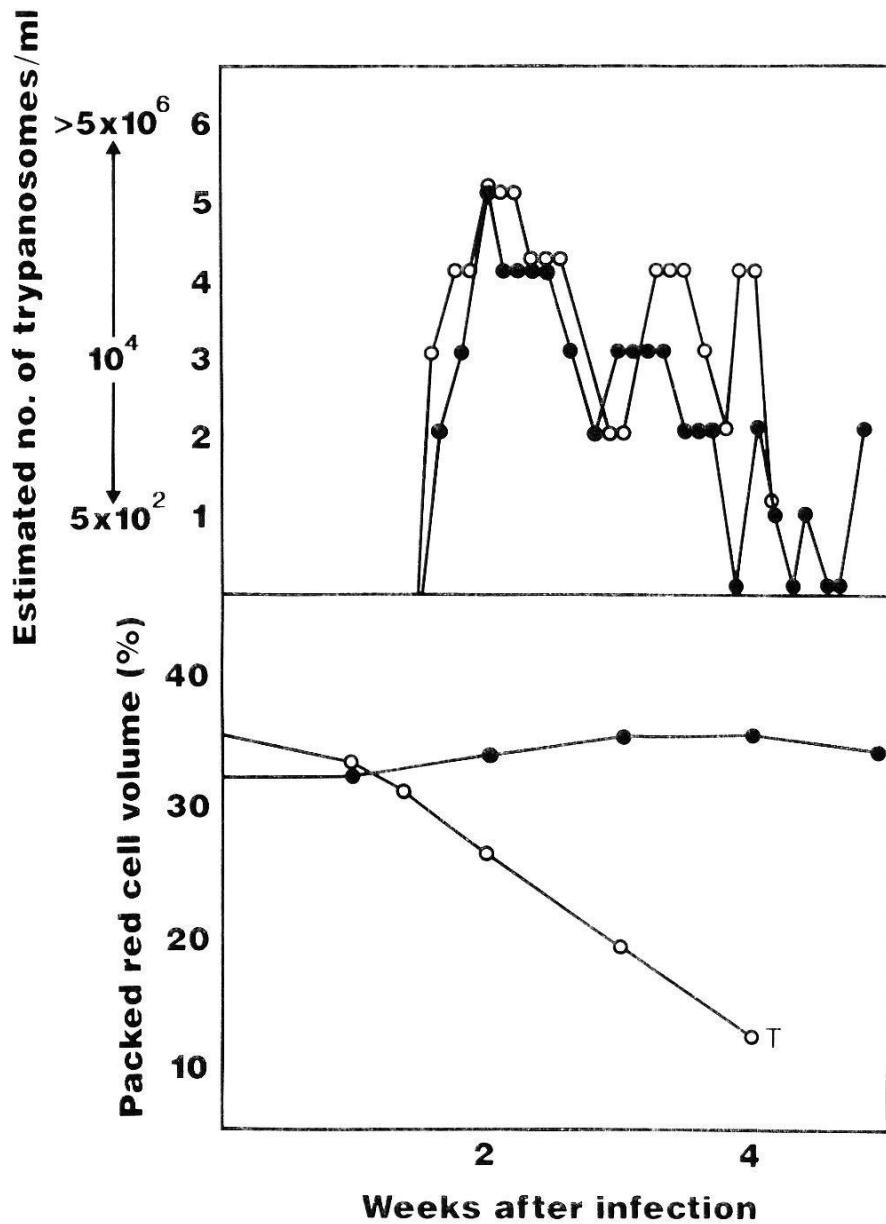


Fig. 7. Parasitaemia and anaemia in a waterbuck (●) and Ayrshire steer (○) infected with *Trypanosoma brucei* (T = treatment with trypanocidal drug).

death any infected animal with PCV value = 15% or less was treated with Berenil. In addition, all infections were terminated with Berenil on 164, 127, 149 and 133 days after each challenge.

No N'Dama required treatment but 77% of all infections in the Boran (32 animals) did. Moreover, in contrast to the severe weight losses experienced by trypanosome-infected Boran, compared with uninfected controls, trypanosome infection did not appear to affect body weight or liveweight gains in the N'Dama. Also in contrast to the Boran, infected female N'Dama continued to show normal oestrus cycle activity.

There were no significant differences between either the breed groups or among the infections of the four *T. congolense* clones in the prepatent period or in the number of days to the first peak in the parasitaemia. However, the

Table 1. Mean parasitaemia score¹ and packed cell volume percent of the N'Dama and Boran² infected with *Trypanosoma congolense* during four consecutive periods

<i>T. congolense</i> clone	Mean parasitaemia score		Mean PCV (%)	
	N'Dama	Boran	N'Dama	Boran
IL 1180.....	1.90	2.42	27.9	25.4
IL 2642.....	2.23	-	28.7	-
IL 1587.....	2.05	3.01	29.1	24.1
IL 2079.....	2.20	2.43	31.2	24.9
Overall	2.10	2.72	29.2	24.7

¹ Scoring method of Paris et al. (1982)

² Results are presented on the 3 Boran in group 2 which did not require treatment.

average number of parasites per ml blood at the first parasitaemia peak was slightly but significantly lower in N'Dama than in Boran. The overall mean parasitaemia score of the N'Dama during the four infection periods was significantly lower than the overall mean score of untreated Boran (Table 1). Within the N'Dama group individual animals demonstrated significantly higher or lower mean parasitaemia levels and this trend was consistent throughout all four infections (Table 2).

The kinetics of the anaemia in the group of N'Dama cattle during each of the four infection periods was very similar. First, there was a short phase during the development of parasitaemia from day 10 to 15 when the average daily PVC value in N'Dama and Boran dropped at the same rate. This was followed by a period from day 15 to 35, during which the average PCV in N'Dama decreased, but at a slower rate than that of the Boran. Might this suggest that N'Dama red cells are more resistant to destruction? From day 35 to 60, the average daily PCV value in N'Dama remained at the same level but most of the Boran required treatment. The lowest average PCV value reached by the N'Dama varied between 22.5% and 23% during the first two infections and between 25 and 27% during the second two infections. Next was a period of spontaneous recovery in the N'Dama, as assessed by a return to an average PCV value of 30%. In contrast, no spontaneous recovery occurred in the Boran. Most of the Boran required treatment and those that did not maintained a persistent average PCV value of 22%. The overall mean PCV value of the N'Dama was 29.2% during the four infection periods and this was significantly higher than the mean of 24.7% for the untreated Boran (Table 1).

While the mean PCV value of the N'Dama group increased from 27.9% during the first infection to 31.2% during the fourth infection, there was no corresponding decrease in trend in the parasite numbers measured during the four infections (Table 1). In the same way, some N'Dama demonstrated consis-

Table 2. Mean parasitaemia score and packed cell volume percent of N'Dama during four periods of infection with *Trypanosoma congolense*

Animal number	Parasitaemia score		PCV (%)	
	Mean	Ranking	Mean	Ranking
1	2.12	5	30.5	7
2	1.93	2	27.4	1
3	2.46	8	29.1	5
4	2.03	3	30.0	6
5	2.13	6	31.5	8
8	2.10	4	29.0	4
9	2.14	7	28.5	3
10	1.86	1	27.6	2

tently higher PCV values during all four infections while others showed better parasite control (Table 2). Thus, there was no correlation in the N'Dama between the level of parasitaemia, expressed as the mean parasitaemia score during the period of infection, and the level of anaemia expressed as the mean PCV.

The superior resistance of N'Dama compared with Zebu to trypanosome infection was once again confirmed by this study and the same general conclusion made, namely, that N'Dama exhibited a superior capacity to control parasitaemia and resist anaemia. However, when mean parasitaemia and PCV values of the N'Dama were computed over four infection periods no direct correlation could be established either at a group or individual level, indicating that both processes although controlled genetically are not directly linked to each other. PCV values of trypanotolerant livestock in tsetse-infested areas are known to be positively correlated with production traits such as reproductive performance and growth (ILCA/ILRAD, 1986a, b). Therefore, under controlled experimental conditions, PCV values induced during a primary trypanosome infection might serve as a selection criterion for trypanotolerance.

While control of parasite growth would still appear to be an important component of trypanotolerance other explanations must be sought for the capacity of certain groups of cattle and individuals within each group to resist anaemia. One interesting possibility has been reported by Esievo et al. (1986) who measured erythrocyte surface sialic acid concentrations in uninfected N'Dama and Zebu with no previous exposure to trypanosome. The sialic acid concentrations in the N'Dama were about seven fold greater than in the Zebu, although no differences in red cell or leukocyte numbers occurred. This might suggest that the red cell surfaces of the N'Dama are "stronger" than the Zebu and that, if the presence in trypanosomes of the sialic acid-cleaving enzymes, sialidase (Esievo, 1983) or proteases (Londsdale-Eccles and Grab, 1986) is

significant, sialic acid concentrations on red cell surfaces might play an important role in host resistance to trypanosome-induced anaemia.

In addition, in the study of Paling et al. (in press), there was evidence that the N'Dama had a greater inherent capacity to make better tertiary erythropoietic responses. Thus, the lowest average daily PCV values reached by the N'Dama group was 22.5, 23, 25 and 27% in consecutive infections, while the overall mean of all PCV values covering each of the four infection periods was 27.9, 28.7, 29.1 and 31.2% (Table 1). Both results show a significant upward progression. This improved responsiveness was not observed in the Boran subjected to four consecutive infections and was not related to improvement in control of parasitaemia or to a difference in virulence among the four clones of *T. congolense* used.

With respect to the improved response to consecutive infections, it is of interest that acquired resistance to trypanosomiasis has been reported in cattle on several occasions (reviewed by Murray et al., 1982) and has been assumed to be the result of acquisition of immunity. Thus, in Kilifi Plantations in Kenya, any cow (Sahiwal X Ayrshire) with a PCV value of less than 30% was treated with a trypanocidal drug; no systematic examination was made for parasites in the blood. Data analysis showed that, independent of age, the number of previous treatments influenced the number of subsequent treatments, i.e., the more previous treatments the less treatments that would be required (Murray et al., 1982). This was interpreted as circumstantial evidence of the acquisition of immunity to the trypanosome population in the locality. However, on the basis of the findings of Paling et al. (in press), it might be the result of tertiary responses of a primed haemopoietic system.

The way ahead

In seeking to understand the anaemia, and in general the pancytopaenia that occur in cattle infected with African trypanosomes, the availability of cattle which are highly resistant to infection and of cattle that are highly susceptible to infection offers an unparalleled opportunity to identify and understand the key mechanisms involved.

As reviewed, the kinetics and mechanisms responsible for the anaemia change during the course of the disease and it will be necessary to plan long term experiments to produce cattle in the chronic as well as in the acute phase of the disease. At the same time, several factors must be considered as likely to play important roles in the pathogenesis of the anaemia and in the outcome of infections. These include parasite virulence, parasite growth regulation, parasite-derived biologically-active substances, immune responses, macrophage function, red cell biochemistry and haemopoietic responsiveness.

Cattle that can resist anaemia remain productive (ILCA/ILRAD, 1986a, b). Therefore, the identification of markers that allow selection for this

trait, or the manipulation of the mechanism(s) responsible in order to induce resistance to anaemia will make a major contribution to improving livestock production in Africa. Furthermore, the availability of new chemotherapeutic approaches for the treatment of the phase of the disease that is unresponsive to the action of trypanocidal drugs could also improve the performance of cattle that currently are non productive.

Acknowledgments

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Akol G. W. O., Murray Max: Early events following challenge of cattle with tsetse infected with *Trypanosoma congolense*: development of the local skin reaction. *Vet. Rec.* **110**, 293–302 (1982).

Akol G. W. O., Audie E., Pinder M., Moloo S. K., Roelants G. E., Murray Max: Susceptibility and immune responses of Zebu and taurine cattle of West Africa to infection with *Trypanosoma congolense* transmitted by *Glossina mortisans centralis*. *Vet. Immunol. Immunopathol.* **11**, 361–373 (1986).

Amole B. O., Clarkson A. B., Shear H. L.: Pathogenesis of anaemia in *Trypanosoma brucei*-infected mice. *Infect. Immun.* **36**, 1060–1068 (1982).

Assoku R. K. G., Gardiner P. R.: Detection of antibodies to platelets and erythrocytes during haemorrhagic *Trypanosoma vivax* infection of Ayrshire cattle. *Vet. Parasit.* (in press).

Balber A. E.: *Trypanosoma brucei*: attenuation by corticosteroids of the anaemia of infected mice. *Exp. Parasit.* **35**, 209–218 (1974).

Banks K. L.: In vitro binding of *Trypanosoma congolense* to erythrocytes. *J. Protozool.* **26**, 103–108 (1979).

Banks K. L.: Injury induced by *Trypanosoma congolense* adhesion to cell membranes. *J. Parasit.* **66**, 34–37 (1980).

Barrett Connor E., Ugoretz R. J., Braude A.: Disseminated intravascular coagulation in trypanosomiasis. *Arch. intern. Med.* **131**, 574–577 (1973).

Barry J. D., LeRay D., Herbert W. J.: Infectivity and virulence of *Trypanosoma (Trypanozoon) brucei* for mice. IV. Dissociation of virulence and variable antigen type in relation to pleomorphism. *J. comp. Path.* **89**, 465–470 (1979).

Berry C. I., Dargie J. D.: The pathophysiology of ovine fascioliasis: the influence of dietary protein and iron on the erythrokinetics of sheep experimentally infected with *Fasciola hepatica*. *Vet. Path.* **4**, 327–339 (1978).

Black S. J., Sendashonga C. N., Lalor P. A., Whitelaw D. D., Jack R. M., Morrison W. I., Murray Max: Regulation of the growth and differentiation of *Trypanosoma (Trypanozoon) brucei brucei* in resistant (C57B1/6) and susceptible (C3H/He) mice. *Parasite Immunol.* **5**, 465–478 (1983).

Black S. J., Sendashonga C. N., O'Brien C. O., Borowy N. K., Naessens M., Webster P., Murray Max: Regulation of parasitaemia in mice infected with *Trypanosoma brucei*. *Curr. Top. Microbiol. Immunol.* **117**, 93–118 (1985).

Black S. J., Sendashonga C. N., Webster P., Shapiro S. Z.: Regulation of parasite-specific antibody responses in resistant (C57B1/6) and susceptible (C3H/He) mice infected with *Trypanosoma (trypanozoon) brucei brucei*. *Parasite Immunol.* **8**, 334–352 (1986).

Brown E. C., Joiner K. A., Frank M. M.: Interaction of desialated guinea pig erythrocytes with the classical and alternative pathways of guinea pig complement in vivo and in vitro. *J. clin. Invest.* **71**, 1710–1719 (1983).

Bungener W., Muller G.: Adhärenzphänomene bei *Trypanosoma congolense*. *Tropenmed. Parasit.* **27**, 370–371 (1976).

Bush J. A., Jensen W. N., Athens J. W., Ashenbrucker H., Cartwright G. E., Wintrobe M. M.: Studies on copper metabolism. XIX. The kinetics of iron metabolism and erythrocytes in copper deficient swine. *J. exp. Med.* 103, 701-712 (1956).

Callow L. L., Emerson F. R., Parker R. J., Knott S. G.: Infection rates and outbreaks of disease due to *Babesia argentina* in unvaccinated cattle on 5 beef properties in South-eastern Queensland. *Austral. Vet. J.* 52, 446-450 (1976).

Cartwright G. E., Lee G. R.: The anaemia of chronic disorders. *Brit. J. Haematol.* 21, 147-152 (1975).

Cook G. M. W., Heard D. H., Seaman G. V. F.: A sialomucopeptide liberated by trypsin from the human erythrocyte. *Nature (Lond.)* 188, 1011-1012 (1960).

Dargie J. D.: Application of radioisotopic techniques to the study of red cell and plasma protein metabolism in helminth disease of sheep. In: *Pathogenic processes in parasitic infections*, ed. by A. E. R. Taylor and R. Muller, Vol. 13, p. 1-26. Blackwell Scientific Publications, Oxford/London/Edinburgh/Melbourne 1975.

Dargie J. D.: Pathophysiology of trypanosomiasis in the bovine. In: *Isotope and radiation research on animal diseases and their vectors*. International Atomic Energy Agency, Vienna. IAEA-SM-240/28, p. 121-131 (1980).

Dargie J. D., Murray P. K., Murray Max, Grimshaw W. R. I., McIntyre W. I. M.: Bovine trypanosomiasis: the red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. *Parasitology* 78, 271-286 (1979a).

Dargie J. D., Murray P. K., Murray Max, McIntyre W. I. M.: The blood volumes and erythrokinetics of N'Dama and Zebu cattle experimentally infected with *Trypanosoma brucei*. *Res. Vet. Sci.* 26, 245-247 (1979b).

Davis C. E.: Thrombocytopaenia: a uniform complication of African trypanosomiasis. *Acta trop. (Basel)* 39, 123-133 (1982).

Davis C. E.: Robbins R. S., Weller R. D., Braude A. I.: Thrombocytopaenia in experimental trypanosomiasis. *J. clin. Invest.* 53, 1359-1367 (1974).

Desowitz R. S.: Studies on immunity and host parasite relationships. I. The immunological response of resistant and susceptible breeds of cattle to trypanosomal challenge. *Ann. trop. Med. Parasit.* 53, 293-313 (1959).

Durocher J. R., Payne R. C., Conrad M. E.: Role of sialic acid in erythrocyte survival. *Blood* 45, 11-20 (1975).

Ellis J. A., Scott J. R., MacHugh N. D., Gettinby G., Davis W. C.: Peripheral blood leucocyte subpopulation dynamics during *Trypanosoma congolense* infection in Boran and N'Dama cattle: an analysis using monoclonal antibodies and flow cytometry. *Parasite Immunol.* 9, 363-378 (1987).

Emery D. L., Akol G. W. O., Murray Max, Morrison W. I., Moloo S. K.: Chancre - early events in the pathogenesis of African trypanosomiasis in domestic livestock. In: *Host-invader interplay*, ed. by H. Van Den Bossche, p. 345-356. Elsevier/North Holland Biomedical Press, Amsterdam 1980a.

Emery D. L., Barry J. D., Moloo S. K.: The appearance of *Trypanosoma (Duttonella) vivax* in lymph following challenge of goats with infected *Glossina morsitans morsitans*. *Acta trop. (Basel)* 37, 375-379 (1980b).

Esievo K. A. N.: In vitro production of neuraminidase (sialidase) by *Trypanosoma vivax*. International Scientific Council for Trypanosomiasis Research and Control. 16th Meeting. Yaounde, Cameroon, 1979. OAU/STRC, Publication No. 111, p. 205-210 (1981).

Esievo K. A. N.: *Trypanosoma vivax*, Stock V953: inhibitory effect of type A influenza virus anti-HAV8 serum on in vitro neuraminidase (sialidase) activity. *J. Parasit.* 69, 491-495 (1983).

Esievo K. A. N., Saror D. I., Ilemobade A. A., Hallaway M. H.: Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *Trypanosoma vivax* infection in cattle. *Res. Vet. Sci.* 32, 1-5 (1982).

Esievo K. A. N., Saror D. I., Kolo M. N., Eduvie L. O.: Erythrocyte surface sialic acid in N'Dama and Zebu cattle. *J. comp. Path.* 96, 95-99 (1986).

Facer C. A., Crosskey J. M., Clarkson M. J., Jenkins G. C.: Immune haemolytic anaemia in bovine trypanosomiasis. *J. comp. Path.* 92, 393-401 (1982).

Fearon D. T.: Activation of the alternative complement pathway. *Crit. Rev. Immunol.* 1, 1-27 (1979).

Ferrante A., Allison A. C.: Alternative pathway activation of complement by African trypanosomes lacking a glycoprotein coat. *Parasite Immunol.* 5, 491-498 (1983).

Fiennes R. N. T.-W.: Haematological studies in trypanosomiasis of cattle. *Vet. Rec.* 66, 423-434 (1954).

Fiennes R. N. T.-W.: Pathogenesis and pathology of animal trypanosomiases. In: *The African trypanosomiases*, ed. by H. W. Mulligan, p. 729-750. George Allen and Unwin/Ministry of Overseas Development, London 1970.

Forsberg C. M., Valli V. E. O., Gentry P. W., Donworth R. M.: The pathogenesis of *Trypanosoma congolense* infection in calves. IV: The kinetics of blood coagulation. *Vet. Path.* 16, 229-242 (1979).

Gorstein F., Benaceraff B.: Hyperactivity of the reticuloendothelial system and experimental anaemias in mice. *Amer. J. Path.* 37, 569-582 (1960).

Hambrey P. N., Tizard I. R., Mellors A.: Accumulation of phospholipase A₁ in tissue fluid of rabbits infected with *Trypanosoma brucei*. *Tropenmed. Parasit.* 31, 439-443 (1980).

Haurani F. I., Green D.: Primary defective iron neutralisation. Responses to testosterone therapy. *Amer. J. Med.* 42, 151-158 (1967).

Herbert W. J., Inglis M. D.: Immunisation of mice against *T. brucei* infection by the administration of released antigen absorbed to erythrocytes. *Trans. roy. Soc. trop. Med. Hyg.* 67, 268 (1973).

Hershko C., Cook J. D., Finch F. A.: Storage iron kinetics. The effect of inflammation on iron exchange in the rat. *Brit. J. Haematol.* 28, 67-75 (1974).

Holmes P. H.: The use of radioisotopes tracer techniques in the study of the pathogenesis of trypanosomiasis. In: *International Atomic Energy Agency, Nuclear Techniques in Animal Production and Health*, p. 463-474. IAEA, Vienna 1976.

Holmes P. H., Jennings F. W.: The effect of treatment on the anaemia of African trypanosomiasis. In: *Pathophysiology of parasitic infection*, ed. by E. J. L. Soulsby, p. 199-210. Academic Press, New York 1976.

Hornby H. E.: Trypanosomes and trypanosomiasis of cattle. *J. comp. Path.* 34, 211-240 (1921).

Houba V., Brown K. N., Allison A. C.: Heterophile antibodies, M-anti-globulins and immunoglobulins in experimental trypanosomiasis. *Clin. exp. Immunol.* 4, 113-123 (1969).

Huan C. N., Webb L., Lambert P. H., Miescher P. A.: Pathogenesis of the anaemia of African trypanosomiasis. Characterisation and purification of a haemolytic factor. *Schweiz. med. Wschr.* 105, 1582-1583 (1975).

Hudson J. R.: Acute and subacute trypanosomiasis in cattle caused by *T. vivax*. *J. comp. Path.* 54, 108-119 (1944).

ILCA: Trypanotolerant Livestock in West and Central Africa. Monograph No. 2. International Livestock Centre for Africa, Addis Ababa, Ethiopia 1979.

ILCA/ILRAD: The ILCA/ILRAD Trypanotolerance Network. Situation report, December 1985. Proceedings of a Network Meeting held at ILCA, Nairobi, June 1986. International Livestock Centre for Africa, Addis Ababa, Ethiopia 1986a.

ILCA/ILRAD: The African Trypanotolerant Livestock Network. Indications from results. 1983-1985. December, 1986. International Livestock Centre for Africa, Addis Ababa, Ethiopia 1986b.

ILRAD: Annual Report of the International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya 1985.

ILRAD: Annual Report of the International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya 1986.

Ingram D. G., Soltys M. A.: Immunity in trypanosomiasis. IV. Immuno-conglutinin in animals infected with *Trypanosoma brucei*. *Parasitology* 50, 231-239 (1960).

Ismael A. A., Njogu A. R., Gettinby G., Murray Max: Susceptibility of Orma and Galana Boran cattle to infection with bloodstream forms of *Trypanosoma congolense* and *T. vivax*. International Scientific Council for Trypanosomiasis Research and Control. 18th Meeting. Harare, Zimbabwe, 1985. OAU/STRC, Publication No. 113, p. 176-181 (1985).

Isoun T. T., Esuruoso G. O.: Pathology of natural infection of *Trypanosoma vivax* in cattle. Nig. Vet. J. 1, 42–45 (1972).

Jancik J. M., Schauer R., Andres K. H., von Düring M.: Sequestration of neuraminidase-treated erythrocytes. Studies on its topographic, morphologic and immunologic aspects. Cell and Tissue Res. 186, 209–226 (1978).

Jandl J. H., Files N. M., Barnett S. B., MacDonald R. A.: Proliferative response of the spleen and liver to haemolysis. J. exp. Med. 122, 299–325 (1965).

Jenkins G. C., Facer C. A.: Haematology of African trypanosomiasis. In: The immunology and pathogenesis of trypanosomiasis, ed. by I. R. Tizard, p. 13–44. CRC Press Inc., Florida, USA 1985.

Jennings F. W., Murray P. K., Murray Max, Urquhart G. M.: Anaemia in trypanosomiasis: studies in rats and mice infected with *Trypanosoma brucei*. Res. Vet. Sci. 16, 70–76 (1974).

Jordt T., Mahon G. D., Touray B. N., Ngulo W. K., Morrison W. I., Rawle J., Murray Max: Successful transfer of frozen N'Dama embryos from The Gambia to Kenya. Trop. Animal Hlth and Prod. 18, 65–75 (1986).

Kaaya G. P., Valli V. E. O., Maxie M. G., Losos G. J.: Inhibition of bovine bone marrow granulocyte/macrophage colony formation in vitro by serum collected from cattle infected with *Trypanosoma vivax* or *Trypanosoma congolense*. Tropenmed. Parasit. 30, 230–235 (1979).

Kaaya G. P., Tizard I. R., Maxie M. G., Valli V. E. O.: Inhibition of leukopoiesis by sera from *Trypanosoma congolense* infected calves: partial characterisation of the inhibitory factor. Tropenmed. Parasit. 31, 232–238 (1980).

Kanthack A. A., Durham H. E., Blandford W. F. H.: On nagana, or tsetse fly disease. Proc. roy. Soc. 64, 100–118 (1898).

Kaplan M. E., Jandl J. H.: Inhibition of red cell sequestration by cortisone. J. exp. Med. 114, 921–937 (1961).

Karle H.: The pathogenesis of the anaemia of chronic disorders and the role of fever in erythrokinetics. Scand. J. Haematol. 13, 81–86 (1974).

Knowles G., Black S. J., Whitelaw D. D.: Peptidase in the plasma of mice infected with *Trypanosoma brucei brucei*. Parasitology 95, 291–300 (1987).

Kobayakawa T., Louis J., Izui S., Lambert P. H.: Autoimmune response to DNA, red blood cells and thymocyte antigens in association with polyclonal antibody synthesis during experimental African trypanosomiasis. J. Immunol. 122, 296–301 (1979).

Kobayashi A., Tizard I. R.: The response to *Trypanosoma congolense* infection in calves: determination of immunoglobulins IgG₁, IgG₂, IgM and C₃ levels and the complement fixing antibody titers during the course of infection. Tropenmed. Parasit. 27, 411–417 (1976).

Kobayashi A., Tizard I. R., Woo P. T. K.: Studies on the anaemia in experimental African trypanosomiasis. II. The pathogenesis of the anaemia in calves infected with *Trypanosoma congolense*. Amer. J. trop. Med. Hyg. 25, 401–406 (1976).

Lambert P. H., Houba V.: Immune complexes in parasitic diseases. In: Progress in immunology II, ed. by L. Brent and J. Holborow, p. 57–67. North Holland Publishing Company, Amsterdam 1974.

Landsteiner K., Raubitschek H.: Beobachtungen über Hämolyse und Hämagglutination. Zentralbl. Bacteriol. Parasitenk. Infektionskr. Hyg. Abt. 1 Orig. 45, 660–667 (1907).

Lawson F. M., Valli V. E., Mills J., Forsberg C. M.: The quantification of *Trypanosoma congolense* in calves. IV. In vitro culture of myeloid and erythroid marrow cells. Tropenmed. Parasit. 31, 425–534 (1980).

Levy M. G., Clabaugh G., Ristic M.: Age resistance in bovine babesiosis: role of blood factors in resistance in *Babesia bovis*. Infect. Immun. 37, 1127–1131 (1982).

Libby P., Alroy J., Pereira M. E. A.: A neuraminidase from *Trypanosoma cruzi* removes sialic acid from the surface of mammalian myocardial cells and endothelial cells. J. clin. Invest. 77, 127–135 (1986).

Londsdale-Eccles J. D., Grab D. J.: Proteases in African trypanosomes. In: Cysteine proteinases and their inhibitors, ed. by V. Turk, p. 189–197. Walter de Gruyter, Berlin 1986.

Londsdale-Eccles J. D., Mpimbaza G. W. N.: Thiol-dependent proteases of African trypanosomes: analysis by electrophoresis in sodium dodecyl sulphate/polyacrylamide gels co-polymerized with fibrinogen. Europ. J. Biochem. 155, 469–473 (1986).

Losos G. J., Ikede B. O.: Review of the pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. rhodesiense* and *T. gambiense*. *Vet. Path.* 9, 1–71 (1972).

Losos G. J., Paris J., Wilson A. J., Dar F. K.: Pathology of the disease in cattle caused by *Trypanosoma congolense*. *Bull. epizootiolog. Dis. Afr.* 21, 239–248 (1973).

Luckins A. G., Gray A. R.: An extravascular site of development of *T. congolense*. *Nature (Lond.)* 272, 613–614 (1978).

MacKenzie A. R., Boreham P. F. L.: Autoimmunity in trypanosome infections. Tissue autoantibodies in *Trypanosoma (Trypanozoon) brucei* infections in rabbits. *Immunology* 26, 1225–1238 (1974).

MacKenzie P. K. I., Cruickshank J. G.: Phagocytosis of erythrocytes and leukocytes in sheep infected with *Trypanosoma congolense* (Broden, 1904). *Res. Vet. Sci.* 15, 256–262 (1973).

MacKenzie P. K. I., Boyt W. P., Nesham V. M., Pirie E.: The aetiology and significance of the phagocytosis of erythrocytes and leukocytes in sheep infected with *Trypanosoma congolense* (Broden, 1904). *Res. Vet. Sci.* 24, 4–7 (1978).

McNeillage G. J. C., Herbert W. J.: Infectivity and virulence of *Trypanosoma (Trypanozoon) brucei* for mice. II. Comparison of closely related trypanosome antigenic types. *J. comp. Path.* 78, 345–349 (1968).

Magaji: *J. Nig. Vet. med. Ass.* 4, 29–36 (1975).

Mahan S. M., Hendershot L., Black S. J.: Control of trypanodestructive antibody responses and parasitaemia in mice infected with *Trypanosoma (Duttonella) vivax*. *Infect. Immun.* 54, 213–221 (1986).

Malu M. N., Tabel H.: The alternative pathway of complement in sheep during the course of infection with *Trypanosoma congolense* and after Berenil treatment. *Parasite Immunol.* 8, 217–229 (1986).

Mamo E., Holmes P. K.: The erythrokinetics of Zebu cattle chronically infected with *Trypanosoma congolense*. *Res. Vet. Sci.* 18, 105–106 (1975).

Masake R. A.: The pathogenesis of infection with *Trypanosoma vivax* in goats and cattle. *Vet. Rec.* 107, 551–557 (1980).

Masake R. A., Nantulya V. M., Akol G. W. O., Musoke A. J.: Cerebral trypanosomiasis in cattle with mixed *Trypanosoma congolense* and *T. brucei brucei* infections. *Acta trop. (Basel)* 41, 237–246 (1984).

Maxie M. G., Valli V. E. O.: Pancytopenia in bovine trypanosomiasis. In: *Pathogenicity of trypanosomes*, ed. by G. Losos and Amy Chouinard. IDRA No.132e, p. 135–136 (1979).

Maxie M. G., Losos G. J., Tabel H.: Experimental bovine trypanosomiasis (*Trypanosoma vivax* and *T. congolense*). I. Symptomatology and clinical pathology. *Tropenmed. Parasit.* 30, 274–282 (1979).

Mellors A.: Phospholipases in trypanosomes. In: *The immunology and pathogenesis of trypanosomiasis*, ed. by I. R. Tizard, p. 67–74. CRC Press Inc., Florida, USA 1985.

Mitchell L. A., Pearson T. W.: Antibody responses in resistant and susceptible inbred mice infected with *Trypanosoma congolense*. *Immunology* 57, 297–303 (1986).

Morrison W. I., Murray Max: The role of humoral immune responses in determining susceptibility of A/J and C57BL/6 mice to infection with *Trypanosoma congolense*. *Parasite Immunol.* 7, 63–80 (1985).

Morrison W. I., Roelants G. E., Mayor-Withey K. S., Murray Max: Susceptibility of inbred strains of mice to *Trypanosoma congolense*: correlation with changes in spleen lymphocyte populations. *Clin. exp. Immunol.* 32, 25–40 (1978).

Morrison W. I., Murray Max, Sayer P. D.: The pathogenesis of tissue lesions in *Trypanosoma brucei* infections. In: *Pathogenicity of trypanosomes*, ed. by G. Losos and Amy Chouinard. IDRC No. 132e, p. 171–177 (1979).

Morrison W. I., Murray Max, McIntyre W. I. M.: Bovine trypanosomiasis. In: *Diseases of cattle in the tropics. Economic and zoonotic relevance*, ed. by Miodrag Ristic and Ian McIntyre. *Current Topics in Veterinary Medicine and Animal Science* Vol. 6, p. 469–497. Martinus Nijhoff Publishers, The Hague/Boston/London 1981a.

Morrison W. I., Murray Max, Bovell D. L.: The response of the murine lymphoid system to a chronic infection with *Trypanosoma congolense*. I. The spleen. *Laborat. Invest.* 45, 457–557 (1981b).

Morrison W. I., Murray Max, Hinson C. A.: The response of the murine lymphoid system to a chronic infection with *Trypanosoma congolense*. II. Lymph nodes, thymus and liver. *J. Path.* 103, 273–288 (1982).

Morrison W. I., Murray Max, Whitelaw D. D., Sayer P. D.: Pathology of infection with *Trypanosoma brucei*: disease syndromes in dogs and cattle resulting from severe tissue damage. In: *From parasitic infection to parasitic disease. Contributions to Microbiology and Immunology* 7, ed. by P. L. Gigase and E. A. E. Van Mark, p. 103–119. S. Karger, Basel 1983.

Murray Christine M., Murray Max, Murray P. K., Morrison W. I., Pyne C., McIntyre W. I. M.: Diagnosis of African trypanosomiasis in cattle. Improved parasitological and serological techniques. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia, 1977. OAU/STRC, Publication No. 110. p. 247–254 (1979a).

Murray Max: The pathology of African trypanosomiasis. In: *Progress in immunology* II, ed. by L. Brent and J. Holborow, p. 181–192. North Holland Publishing Company, Amsterdam 1974.

Murray Max: Anaemia of bovine African trypanosomiasis: an overview. In: *Pathogenicity of trypanosomes*, ed. by G. Losos and Amy Chouinard. IDRC No. 132e, p. 121–127 (1979).

Murray Max, Morrison W. I.: Non-specific induction of increased resistance in mice to *Trypanosoma congolense* and *T. brucei* by immunostimulants. *Parasitology* 79, 349–366 (1979).

Murray Max, Black S. L.: African trypanosomiasis in cattle: working with nature's solution. *Vet. Parasit.* 18, 167–182 (1985).

Murrax Max, Murray P. K., Jennings F. W., Fisher E. W., Urquhart G. M.: The pathology of *Trypanosoma brucei* in the rat. *Res. Vet. Sci.* 16, 77–84 (1974a).

Murray Max, Murray P. K., McIntyre W. I. M.: An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. roy. Soc. trop. Med. Hyg.* 71, 325–326 (1977).

Murray Max, Huan Chi Nguyen, Lambert P. H., Gerber Heidi: The anaemia of African trypanosomiasis. Demonstration of a haemolytic factor. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia. 1977. OAU/STRC, Publication No. 110, p. 460–469 (1979b).

Murray Max, McIntyre W. I. M., Murray P. K., Urquhart G. M., Jennings F. W., Greig W. A., Clifford D. J., N'Dow W. S. M., Touray B. N., Sanyang B. T., Bray R. S.: Cattle diseases and trypanosomiasis in The Gambia. I. Clinical studies. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia. 1977. OAU/STRC, Publication No. 110, p. 83–91 (1979c).

Murray Max, McIntyre W. I. M., Murray P. K., Urquhart G. M., Jennings F. W., Greig W. A., Clifford D. J., N'Dow W. S. M., Touray B. N., Sanyang B. T., Bray R. S.: Cattle diseases and trypanosomiasis in The Gambia. II. Pathological studies. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia, 1977. OAU/STRC, Publication No. 110. p. 92–98 (1979d).

Murray Max, Morrison W. I., Emery D. L., Akol G. W. O., Masake Rachel A., Moloo S. K.: Pathogenesis of trypanosome infections in cattle. In: *Isotope and radiation research on animal diseases and their vectors*. International Atomic Energy Agency, Vienna. IAEA-SM-240/19, p. 15–32 (1980).

Murray Max, Clifford D. J., Gettinby G., Snow W. F., McIntyre W. I. M.: A study on the susceptibility to African trypanosomiasis of N'Dama and Zebu cattle in an area of *Glossina morsitans submorsitans* challenge. *Vet. Rec.* 109, 503–510 (1981a).

Murray Max, Grootenhuis J. G., Akol G. W. O., Emery D. L., Shapiro S. Z., Moloo S. K., Dar Faiqa, Bovell D. L., Paris J.: Potential application of research on African trypanosomiasis in wildlife and preliminary studies on animals exposed to tsetse infected with *Trypanosoma congolense*. In: *Wildlife diseases research and economic development*, ed. by L. Garstad, B. Nestel and M. Graham. IDRC-179e, p. 40–45 (1981b).

Murray Max, Morrison W. I., Whitelaw D. D.: Host susceptibility to African trypanosomiasis: trypanotolerance. *Advanc. Parasit.* 21, ed. by J. R. Baker and R. Muller, p. 1–68. Academic Press, London 1982.

Murray P. K., Jennings F. W., Murray Max, Urquhart G. M.: The nature of immunosuppression in *Trypanosoma brucei* infections in mice. I. The role of the macrophage. *Immunology* 27, 815-824 (1974b).

Murray P. K., Murray Max, Wallace Margaret, Morrison W. I., McIntyre W. I. M.: Trypanosomiasis in N'Dama and Zebu cattle. I. An experimental investigation of susceptibility to *Trypanosoma brucei*, *T. congolense* and mixed infections. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia, 1977. OAU/STRC, Publication No. 110. p. 470-481 (1979e).

Murray P. K., Murray Max, Wallace Margaret, Morrison W. I., McIntyre W. I. M.: Trypanosomiasis in N'Dama and Zebu cattle. II. The influence of weight infection on the severity of the disease. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting. Banjul, The Gambia, 1977. OAU/STRC, Publication No. 110, p. 482-487 (1979f).

Musoke A. J., Barbet A. F.: Activation of complement by variant-specific surface antigen of *Trypanosoma brucei*. *Nature (Lond.)* 270, 438-440 (1977).

Mwongela G. N., Kovatch R. M., Fazil M. A.: Acute *Trypanosoma vivax* infection in dairy cattle in Coast Province, Kenya. *Trop. Animal Hlth and Prod.* 13, 63-69 (1981).

Naylor D. C.: The haematology and histopathology of *Trypanosoma congolense* in cattle. *Trop. Animal Hlth and Prod.* 3, 159-168 (1971).

Nielsen K. H.: Complement in trypanosomiasis. In: The immunology and pathogenesis of trypanosomiasis, ed. by I. R. Tizard, p. 133-144. CRC Press Inc., Florida, USA 1985.

Nielsen K. J., Sheppard J., Holmes H., Tizard I. R.: Experimental bovine trypanosomiasis. Changes in serum immunoglobulins, complement and complement components of infected animals. *Immunology* 35, 817-826 (1978a).

Nielsen K. H., Sheppard J., Holmes W., Tizard I. R.: Experimental bovine trypanosomiasis. Changes in the catabolism of serum immunoglobulins and complement components in infected cattle. *Immunology* 35, 811-816 (1978b).

Njogu A. R., Dolan R. B., Wilson A. J., Sayer P. D.: Trypanotolerance in East African Orma Boran cattle. *Vet. Rec.* 117, 632-636 (1985).

Nussenzweig R. S.: Increased non-specific resistance to malaria produced by administration of killed *Corynebacterium parvum*. *Exp. Parasit.* 21, 224-231 (1967).

Paling R. W., Moloo S. K., Scott J. R.: The relationship between parasitaemia and anaemia in N'Dama and Zebu cattle following four sequential challenges with *Glossina morsitans centralis* infected with *Trypanosoma congolense*. International Scientific Council for Trypanosomiasis Research and Control. 19th Meeting. Lome, Togo, 1987. OAU/STRC, Publication No. 114 (in press).

Palmer J. G., Eichwald E. J., Cartwright M. D., Wintrobe M. M.: The experimental production of splenomegaly, anaemia and leucopaenia in albino rats. *Blood* 8, 72-80 (1953).

Paris J., Murray Max, McDimba F.: An evaluation of the sensitivity of current parasitological techniques for the diagnosis of bovine African trypanosomiasis. *Acta trop. (Basel)* 39, 307-316 (1982).

Pereira M. E. A.: A developmentally regulated neuraminidase activity in *Trypanosoma cruzi*. *Science* 219, 1444-1446 (1983).

Pierre C.: L'élevage dans l'Afrique Occidentale Française. Gouvernement Général de l'Afrique Occidentale Française, Paris 1906.

Pinder M., Bauer J., Van Melick A., Fumoux F.: Immune responses of trypanoresistant and trypanosusceptible cattle after cyclic infection with *Trypanosoma congolense*. *Veterinary Immunol. Immunopathol.* 18, 245-257 (1988).

Pirofsky B.: Auto-immunization and the auto immune hemolytic anemias, p. 418-430. Williams and Wilkins, Baltimore 1969.

Preston J. M., Welde B. T.: Studies on African trypanosomiasis. Final Report to Department of the Army, Walter Reed Army Institute of Research, DAMD 17-76-9412 (1976).

Preston J. M., Kovatch R. M., Welde B. T.: *Trypanosoma congolense*: thrombocyte survival in infected steers. *Exp. Parasit.* 54, 129-133 (1979).

Preston J. M., Wellde B. T., Kovatch R. M.: *Trypanosoma congolense*: calf erythrocyte survival. *Exp. Parasit.* 48, 118–125 (1982).

Rautenberg P., Reinwald E., Risse H.-J.: Sialic acids are responsible for charge heterogeneity of the variant surface glycoprotein of *Trypanosoma congolense*. *Molec. biochem. Parasit.* 4, 129–138 (1981).

Rautenberg P., Schadler R., Reinwald E., Risse H.-J.: Study on a proteolytic enzyme from *Trypanosoma congolense*. Purification and some biochemical properties. *Molec. cell. Biochem.* 47, 151–159 (1982).

Reissman K. R.: Protein metabolism and erythropoiesis. I. The anaemia of protein deprivation. *Blood* 23, 137–145 (1964).

Rickman W. J., Cox H. W.: Association of autoantibodies with anaemia, splenomegaly and glomerulonephritis in experimental African trypanosomiasis. *J. Parasit.* 65, 65–73 (1979).

Rickman W. J., Cox H. W., Thoongsuwan S.: Interactions of immunoconglutinin of immune complexes in cold autoagglutination associated with African trypanosomiasis. *J. Parasit.* 67, 159–163 (1981).

Roberts C. J., Gray A. R.: Studies on trypanosome-resistant cattle. I. The breeding and growth performance of N'Dama, Muturu and Zebu cattle maintained under the same conditions of husbandry. *Trop. Animal Hlth and Prod.* 5, 211–219 (1973a).

Roberts C. J., Gray A. R.: Studies on trypanosome-resistant cattle. II. The effect of trypanosomiasis on N'Dama, Muturu and Zebu cattle. *Trop. Animal Hlth and Prod.* 5, 220–233 (1973b).

Robins-Browne R. M., Schneider J., Metz J.: Thrombocytopenia in trypanosomiasis. *Amer. J. trop. Med. Hyg.* 24, 226–231 (1975).

Roelants G. E.: Natural resistance to African trypanosomiasis. *Parasite Immunol.* 8, 1–10 (1986).

Roelants G. E., Pinder M.: The virulence of *Trypanosoma congolense* can be determined by the antibody response of inbred strains of mice. *Parasite Immunol.* 9, 379–388 (1987).

Rurangirwa F. R., Tabel H., Losos G., Tizard I. R.: Haemolytic complement and serum C3 levels in Zebu cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax* and the effect of trypanocidal treatment. *Infect. Immun.* 27, 832–836 (1980).

Rurangirwa F. R., Musoke A. J., Nantulya V. M., Nkonge C., Njuguna L., Mushi E. Z., Karstad L., Grootenhuis J. G.: Immune effector mechanisms involved in the control of parasitaemia in *Trypanosome brucei*-infected wildebeest (*Connochaetes taurinus*). *Immunology* 58, 231–237 (1986).

Sacks D. K., Selkirk M., Ogilvie B. M., Askonas B. A.: Intrinsic immunosuppressive activity of different trypanosome strains varied with parasite virulence. *Nature (Lond.)* 283, 476–478 (1980).

Saror D. I., Ilemobade A. A., Nuru S.: The haematology of N'Dama and Zebu cattle experimentally infected with *Trypanosoma vivax*. International Scientific Council for Trypanosomiasis Research and Control, 16th Meeting, Yaounde, Cameroon, 1979, p. 287–294. Organization for African Unity, OAU/STRC No. 111. Scientific Publications Division of Eleza Services, Nairobi 1981.

Sendashonga C. N., Black S. J.: Humoral responses against *Trypanosoma brucei* variable surface antigen are induced by degenerating parasites. *Parasite Immunol.* 4, 245–257 (1982).

Shoyinka S. V. O., Uzoukwu M.: The comparative effects of cortisol and voltaren on anaemia and temperature changes in experimental *Trypanosoma brucei* infections in rats. *J. comp. Path.* 96, 277–284 (1986).

Slots J. M. M., Van Miert A. S. J. P. A. M., Akkerman J. W. N., De Gee A. L. W.: *Trypanosoma brucei* and *Trypanosoma vivax*: antigen-antibody complexes as a cause of platelet serotonin release in vitro and in vivo. *Exp. Parasit.* 43, 211–219 (1977).

Starinsky R., Shafrir E.: Displacement of albumin-bound bilirubin by free fatty acids. Implications for neonatal hypobilirubinaemia. *Clin. chim. Acta* 29, 311–318 (1970).

Tabel H.: Activation of the alternative pathway of bovine complement by *Trypanosoma congolense*. *Parasite Immunol.* 4, 329–335 (1982).

Tabel H., Losos G. J., Maxie M. G.: Experimental bovine trypanosomiasis (*Trypanosoma vivax* and *T. congolense*). II. Serum levels of total protein, albumin, hemolytic complement, and complement component C3. *Tropenmed. Parasit.* 31, 99–104 (1980).

Tartour G., Idris O. F.: Iron metabolism in *Trypanosoma congolense* infection in Zebu cattle: serum iron and serum iron binding capacity. *Res. Vet. Sci.* 15, 24–32 (1973).

Tizard I. R., Holmes W. L.: The generation of toxic activity from *Trypanosoma congolense*. *Experientia* (Basel) 32, 1533–1534 (1976).

Tizard I. R., Holmes W. L., Nielsen K. H.: Mechanisms of the anemia in trypanosomiasis: studies on the role of the hemolytic fatty acids derived from *Trypanosoma congolense*. *Tropenmed. Parasit.* 29, 108–114 (1978a).

Tizard I. R., Nielsen K. H., Seed J. R., Hall J. E.: Biologically active products from African trypanosomes. *Microbiol. Rev.* 42, 661–681 (1978b).

Tizard I. R., Mittal K. R., Nielsen K. H.: Depressed immunoconglutinin responses in calves experimentally infected with *Trypanosoma congolense*. *Res. Vet. Sci.* 28, 203–206 (1980).

Toure S. M., Gueye A., Seye M., Ba M. A., Mane A.: A comparison between the pathology of a natural trypanosome infection in Zebu and N'Dama cattle. *Rev. Elev. Méd. Vét. Pays trop.* 31, 293–313 (1978).

Valli V. O. E., Forsberg C. M.: The pathogenesis of *Trypanosoma congolense* infection in calves. V. Quantitative histological changes. *Vet. Path.* 16, 334–368 (1979).

Valli V. O. E., Mills J. N.: The quantitation of *Trypanosoma congolense* in calves. I. Hematological changes. *Tropenmed. Parasit.* 31, 215–231 (1980).

Valli V. O. E., Forsberg C. M., McSherry B. J.: The pathogenesis of *Trypanosoma congolense* infection in calves. II. Anaemia and erythroid response. *Vet. Path.* 15, 732–745 (1978).

Van Den Ingh T. S. G. A. M., Zwart D., Van Miert A. S. J. P. A. M., Schotman A. J. H.: Clinico-pathological and pathomorphological observations in *Trypanosoma vivax* infection in cattle. *Vet. Parasit.* 2, 237–250 (1976).

Van Meirvenne N., Janssens P. G., Magnus E., Lumsden W. H. R., Herbert W. J.: Antigenic variation in syringe passaged populations of *Trypanosoma (Trypanozoon) brucei*. II. Comparative studies on two antigenic type collections. *Ann. Soc. belge Méd. trop.* 55, 25–30 (1975).

Vodrasky H. P.: Clinical signs, daily rate of infection, physical changes in the blood and pathomorphological changes in cattle artificially infected by *Trypanosoma vivax*. *Rev. Elev. Méd. vét. Pays trop.* 24, 257–263 (1969).

Welldé B. T., Kovatch R. M., Chumo D. A., Wykoff D. E.: *Trypanosoma congolense*: thrombocytopaenia in experimentally infected cattle. *Exp. Parasit.* 45, 26–33 (1978).

Welldé B. T., Hockmeyer W. T., Kovatch R. M., Bhogal M. S., Diggs C. L.: *Trypanosoma congolense*: natural and acquired resistance in the bovine. *Exp. Parasit.* 52, 219–232 (1981).

Welldé B. T., Chumo D. A., Adoyo M., Kovatch R. M., Mwongela G. N., Opiyo E. A.: Haemorrhagic syndrome in cattle associated with *Trypanosoma vivax* infection. *Trop. Animal Hlth and Prod.* 15, 95–102 (1983).

Whitelaw D. D., Macaskill J. A., Holmes P. H., Jennings F. W., Urquhart G. M.: Immune mechanisms in C57Bl/6 mice genetically resistant to *Trypanosoma congolense* infection. I. Effects of immune modulation. *Parasite Immunol.* 5, 85–94 (1983).

Wintrobe M. M.: Clinical haematology. 6th edition, p. 63–67. Henry Kimpton, London 1967.

Woo P. T. K., Kobayashi A.: Studies in the anaemia in experimental African trypanosomiasis. I. A preliminary communication on the mechanisms of the anaemia. *Ann. Soc. belge Méd. trop.* 55, 37–45 (1975).

Woodruff A. W.: Mechanisms involved in anaemia associated with infection and splenomegaly in the tropics. *Trans. roy. Soc. trop. Med. Hyg.* 67, 313–325 (1973).

Woodruff A. W., Ziegler J. L., Hathaway A., Gwata T.: Anaemia in African trypanosomiasis and “big spleen disease” in Uganda. *Trans. roy. Soc. trop. Med. Hyg.* 67, 329–337 (1973).

Zoutendyk A., Gear J.: Autoantibodies in the pathogenesis of disease. A preliminary study of autosensitisation of red cells in various diseases. *South Afr. med. J.* 25, 665–668 (1951).

Zucker S., Friedman S., Lysik R. M.: Bone marrow erythropoiesis in the anaemia of infection, inflammation and malignancy. *J. clin. Invest.* 53, 1132–1138 (1974).

Zuckerman A., Abzug S., Burg R.: Anaemia in rats with equivalent splenomegalies produced by methyl cellulose and *Plasmodium berghei*. *Milit. Med. (Special Issue; September)* p. 1084–1099 (1969).