Haematological and biochemical changes in human and animal trypanosomiasis. Part I

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On assiste, depuis environ les années 1970, à un regain d'intérêt pour la pathologie des trypanosomoses et de nombreuses études ont été rédigées sur les diverses manifestations, dont l'une des plus importantes est l'anémie, de ces maladies tant chez l'homme et le bétail que lors d'infections expérimentales chez les animaux de laboratoire. L'article de M. ANOSA rassemble la plupart des données parues jusqu'en 1986 concernant les répercussions hématologiques et biochimiques chez l'homme et chez l'animal de ces affections. L'auteur, en s'appuyant sur plus de 230 références, s'attache à décrire les modifications qu'entraîne la présence des parasites sur les hématies, la moelle osseuse, les leucocytes, le sérum, le liquide cérébro-spinal et leurs conséquences sur l'anémie, la coagulation sanguine et les réactions cellulaires et tissulaires des mammifères infectés. Il effectue en outre une synthèse des divers événements pathologiques décrits dans la littérature et tente d'en expliquer l'étiologie. Il nous a donc paru utile de publier, en deux parties, en raison de sa longueur, cet important travail qui constitue, à notre avis, une bonne revue de la pathogénie des trypanosomoses. En raison du grand nombre de références citées, la bibliographie sera publiée à part et adressée aux lecteurs qui en feront la demande.

Dans cette première partie, après une courte introduction, l'auteur reconnaît, dans la pathogénie des trypanosomoses, bien qu'elles soient très variables car fonction de la virulence et de la localisation des diverses espèces de parasites et de la sensibilité de leurs hôtes mammifères, trois phases successives au cours de l'infection : une phase aiguë, une phase chronique et une phase de rétablissement, chacune caractérisée par une parasitémie plus ou moins élevée et des symptômes hématologiques et biochimiques plus ou moins accusés. Sont ensuite revues successivement les altérations érythrocytaires (apparition et évolution de l'anémie ; pathologie érythrocytaire ; hémolyse d'ordre immunitaire et autres mécanismes hémolytiques ; érythrophagocytose et ses répercussions organiques ; modifications des volumes des érythrocytes, du plasma et du sang total ; métabolisme du fer), ainsi que les altérations de la moelle osseuse avec ses conséquences sur les quantités de réticulocytes, d'hématies et d'hémoglobine, et sur l'hémopoïèse extramédullaire. Enfin les causes de l'anémie : hémolyse, inhibition de l'érythropoïèse, hémodilution, hémorragie sont examinées.

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Les trypanosomoses se caractérisent, en général, par de l'anémie, de la leucopénie, de la thrombocytopénie, ainsi que par des troubles du métabolisme d'où résultent de l'hypoglycémie, une élévation du taux d'azote uréique du sang, de l'hypoalbuminémie et de l'hypogammaglobulinémie, conséquence d'une augmentation du taux des IgM. Bien qu'il y ait des variations, fonctions de l'hôte (homme, animaux domestiques et animaux d'expérience) et des espèces de trypanosomes (*T. brucei, T. gambiense, T. evansi, T. vivax, T. congolense*), la gravité des altérations d'ordre hématologique et biochimique, associées aux diverses combinaisons hôte-parasite, est déterminée par le niveau de parasitémie qui s'établit pendant la première phase de l'infection. On peut en effet distinguer trois phases successives au cours de ces infections :

— une crise aiguë caractérisée par une parasitémie élevée, une destruction très rapide des érythrocytes, de la thrombocytopénie, de la leucopénie et des perturbations biochimiques marquées.

— une crise chronique que l'on constate chez les animaux ayant survécu à la première phase. Elle se caractérise par une parasitémie plus faible, avec cependant persistance des altérations hématologiques, atténuation de quelques troubles du métabolisme comme l'hypoglycémie, mais persistance d'autres troubles comme les modifications des protéines plasmatiques.

— la troisième phase, ou rétablissement, survient chez les animaux qui ont surmonté les deux phases précédentes. Elle est caractérisée par une diminution nette de la parasitémie, voire même une stérilisation parasitaire, accompagnée d'un retour graduel à la normale des altérations qui s'étaient manifestées précédemment.

Le franchissement, pour un hôte infecté, de ces trois phases, dépend de son état nutritionnel, de la sévérité des lésions qui se développent pendant les crises aiguës et chroniques, et de l'existence d'infections secondaires. Les troubles d'ordre hématologique et biochimique induits par les trypanosomes résultent de leurs effets directs ou indirects, par l'intermédiaire de leurs produits de dégradation, sur les cellules de l'hôte (hématies, globules blancs, plaquettes) et ses tissus (foie, reins, moelle osseuse, organes lymphoïdes), d'où destruction cellulaire et mauvais fonctionnement organique, soustraction et addition de produits biochimiques chez l'hôte, liées au métabolisme du parasite. *Mots clés* : Homme - Animal - Trypanosomose - Modification hématologique - Biochimie.

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INTRODUCTION

The more important trypanosome species affecting man, domestic and experimental animals have been subdivided into two groups, the haematinic group (*Trypanosoma congolense, T. vivax*) which remains in the plasma, and the tissue-invading group (*T. brucei, T. evansi, T. gambiense, T. rhodesiense*, and *T. equiperdum*) which is found extravascularly and intravascularly (130). Because of their presence in the blood, they produce numerous changes in its cellular and biochemical constituents. Although considerable research has hitherto been carried out on these blood changes, there has been an attempt to compartmentalize the results obtained either in relation to the parasite species, or to the host species.

The aim of this paper is to summarize and compare the changes in the cellular and biochemical elements of the blood, as well as in the cerebrospinal fluid, which are produced by the important species of trypanosomes in man and other animals with a view to highlighting differences and similarities observed with various host and parasite species. An attempt will be made also to examine what mechanisms produce these changes. The comparative aspects of *T. vivax* and *T. brucei* infections in ruminants and mice have recently been reported (8), while the nature of diseases produced by *T. vivax* in ruminants, horses and rodents have also been summarized (7).

PATHOGENESIS OF TRYPANOSOMIASIS : A UNIFYING HYPOTHESIS

The haematological and other changes in trypanosome infections are determined by several factors including the virulence of the parasite, the susceptibility of the host, the period of the infection during which samples are taken, among others. Because of different combinations of these host, parasite and other factors, the pattern of a trypanosome infection is quite variable. Thus, peracute, acute, sub-acute and chronic infections have been described.

Nevertheless, an overview of *T. congolense* infections in cattle (46, 48, 118, 124, 125, 131, 164, 210, 212), goats (61) and mice (227), *T. vivax* infections of cattle, sheep and goats (10, 12, 185, 215), *T. brucei* infection of rodents (2, 6, 16, 114), and *T. rhodesiense* infection of monkeys (26, 182) suggests that there are three recognizeable phases of trypanosomiasis (Fig. 1) :

— Phase I : acute crisis, begins with the first appearance of trypanosomes in blood after the incubation period. The parasitaemia is high, although fluctuating, with most days showing positive parasitaemia. In the peracute cases of trypanosomiasis such as are produced in rodents by *T. brucei, T. vivax* and *T. congolense*, the affected animals usually die within one week, the parasitaemia persisting without abatement (105, 202, 227). In animals that show acute or chronic illness, this phase lasts several weeks and is characterized by fluctuating parasitaemia and rapidly developing pancytopenia and marked alterations in the blood chemistry. Death occurs commonly at this phase in acutely affected animals because of the severe pancytopenia and other deleterious changes. Animals destined to suffer chronic trypanosomiasis also manifest an acute crisis but show less severe parasitaemia and pathology than observed in animals with peracute or acute infection. This phase appears to be a period when the yet inadequately organized defence mechanisms of the infected animal are shocked by a vicious foe which amongst its weapons, possesses the ability to circumvent the alerted defence mechanisms of the host by manifesting antigenic variation. The parasite has the round, so to say, and the host often succumbs unless the virulence of the parasite is not intense.

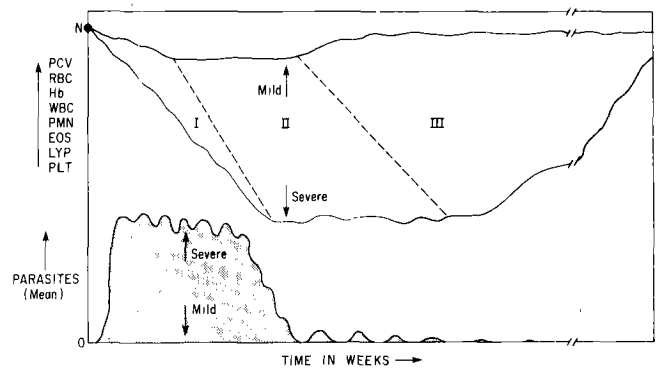

Fig. 1 : Hypothetical relationship between parasitaemia and cellular elements of blood during phases I, II and III of infection. N = normal level of each parameter ; PCV = packed cell volume ; RBC = red blood cell count ; Hb = haemoglobin concentration ; WBC = white blood cell count ; PMN = neutrophil ; EOS = eosinophil ; LYP = lymphocyte ; PL = platelet.

— Phase II : chronic crisis, follows imperceptibly after the « acute crisis ». It is characterized by low frequency and intensity of parasitaemia. The erythrocyte and leucocyte values persist, with minor fluctuations, at the low levels attained at the end of the phase I, for periods ranging from a few weeks to several months. It appears to be a period when the infected animal has fully mobilized its defence mechanisms to a level that is adequate to depress parasite multiplication but is not yet adequate to completely abort the infection or reverse the pathology that developed during phase I. With the tissue-invading trypanosomes, this is the

period when the parasites also establish extravascularly and are less numerous in the blood. Death could occur at this stage either as a result of the progressive pathology caused by the parasite *per se* and/or due to secondary infections since affected individuals are immunodepressed and cannot mobilize commensurate immunologic response to non-trypanosome viral, bacterial, protozoal and helminth infections.

— Phase III : recovery, or post-crisis is characterized by aparasitaemia or low very infrequent parasitaemia, symbolising the capitulation of the parasite to the host's defence mechanisms. The leucocyte, erythrocyte and thrombocyte levels begin to recover towards pre-infection levels, albeit slowly, and other pathological changes are also slowly reversed.

ERYTHROCYTE (RED BLOOD CELL, RBC) CHANGES

Occurrence and pattern of anaemia

Anaemia is a consistent finding in various trypanosome infections. It was reported in *T. brucei* infections of mice (2, 6, 16, 22, 91, 98, 114), rats (38, 100, 101, 114), rabbits (37, 71, 111, 112, 113, 118), monkeys (26), sheep and goats (61, 94, 96), cattle (49), dogs (154), and horses (148, 171). *T. gambiense* produced anaemia in rabbits (57) and man (168), while *T. rhodesiense* caused anaemia in rats (178, 179), monkeys (26, 182, 188), cattle (228), and man (29, 54, 232). *T. evansi* induced anaemia in rats (24, 109, 161), camels (109, 161, 175), horses (98, 172, 233), and dogs (161). *T. congolense* caused anaemia in rodents (38, 227), dogs and cats (45), sheep and goats (61, 116, 171), cattle (46, 48, 75, 76, 118, 124, 125, 163, 164, 171, 201, 210, 212, 222, 224), and horses (63). *T. vivax* also produced anaemia in rodents (93, 104, 202), sheep and goats (4, 9, 12, 61, 140), cattle (30, 66, 70, 72, 75, 76, 89, 105, 115, 129, 144, 160, 167, 185, 221), and horses (63, 81, 90, 197).

The anaemia of trypanosomiasis usually sets in during the first wave of parasitaemia (3, 6, 9, 48, 66, 91, 113), although some workers did not observe anaemia in early phase of human trypanosomiasis (81, 82, 189). Subsequent development of the anaemia is determined by the frequency and intensity of the parasitaemia. For instance, studies with bovine *T. congolense* infection showed that zebu cattle developed greater parasitaemia than the more tolerant N'Dama cattle ; the resultant anaemia was more severe and developed faster in the zebu than in N'Dama cattle (48). With each wave of parasitaemia, the rise of the parasitaemia during its growth phase is accompanied by a drop in the erythrocyte values while the decline phase and the period between the waves of parasitaemia are accompanied by a partial recovery of the erythrocyte values depressed during the growth phase ; the values drop further during the growth phase of the succeeding wave of parasitaemia. This relationship has been demonstrated in *T. brucei* infection of mice (2, 3, 6), in *T. congolense* infection of mice (227), and *T. vivax* infections of sheep and goats (4), and presumably exists in other trypanosome infections but has escaped notice because infected animals and man are rarely sampled daily for long periods for the measurement of parasitaemia and erythrocyte values. In peracute infection, such as those produced by *T. brucei, T. congolense* and *T. vivax* in rodents (104, 202, 227), the infected animals succumb to a single progressively more intense wave of parasitaemia and the erythrocyte values equally drop progressively until death. On the long term, the effect of these changes on erythrocyte values associated with successive waves of parasitaemia is to cause the erythrocyte values to fluctuate slightly in their decline, except in the peracute infections mentioned above. The animals with acute infection usually develop higher levels of parasitaemia and greater anaemia during the « acute crisis » than those with chronic infection (12, 185, 224).

Phase II is characterized by less severe parasitaemia, but the anaemia persists at the level attained during phase I. There may however be mild fluctuations in red cell values associated with the infrequent waves of parasitaemia. Generally, therefore, it is a stage in which accelerated erythrocliasis, which is less intense than occurred in phase I, is balanced by erythropoiesis. During « recovery » or phase III, erythropoiesis outstrips erythrocliasis.

Erythrocyte pathology associated with anaemia

Several erythrocyte morphological abnormalities including anisocytosis, poikilocytosis, polychromasia, punctuate basophilia, macrocytosis, microcytosis, spherocytosis and schistocytosis have been described, in various combinations, in *T. brucei* and *T. congolense* infections (18, 25, 37, 75, 112, 113, 164, 210, 224), and in human *T. rhodesiense* infection (29). Acantocytes, crenated erythrocytes, and Howell-Jolly bodies have also been reported in *T. brucei* infection of rabbits (113). Macrocytosis and extensive spherocytosis have been reported in *T. evansi* infection of camels (108). The existence of schistocytes, microspherocytes, keratocytes and doughnut-shaped erythrocytes has been confirmed by transmission and scanning electron microscopy (18). These red cell abnormalities became more apparent as the infection progressed (113). However, red cell abnormalities were not seen except for slight anisocytosis in pera-

cute, acute and chronic *T. congolense* infection of cattle (171) ; similarly, only anisocytosis due to appearance of microcytes and macrocytes was observed in *T. vivax* infections of sheep and goats (12) and cattle (167).

Erythrocyte osmotic fragility increased in *T. brucei* infections of mice (91, 98) and rats (102), *T. congolense* infections of mice (98) and cattle (164, 212), and in sheep infected with *T. vivax* (169). It was, however, not altered in *T. brucei* infection of rabbits (133). The increase in osmotic fragility was thought to be due to acidosis associated with anaemia and increased leakage of potassium (K+) from the red cells (102). RBC fragmentation and spherocytosis, which have been reported in several trypanosome infections, will also predispose to increased osmotic fragility.

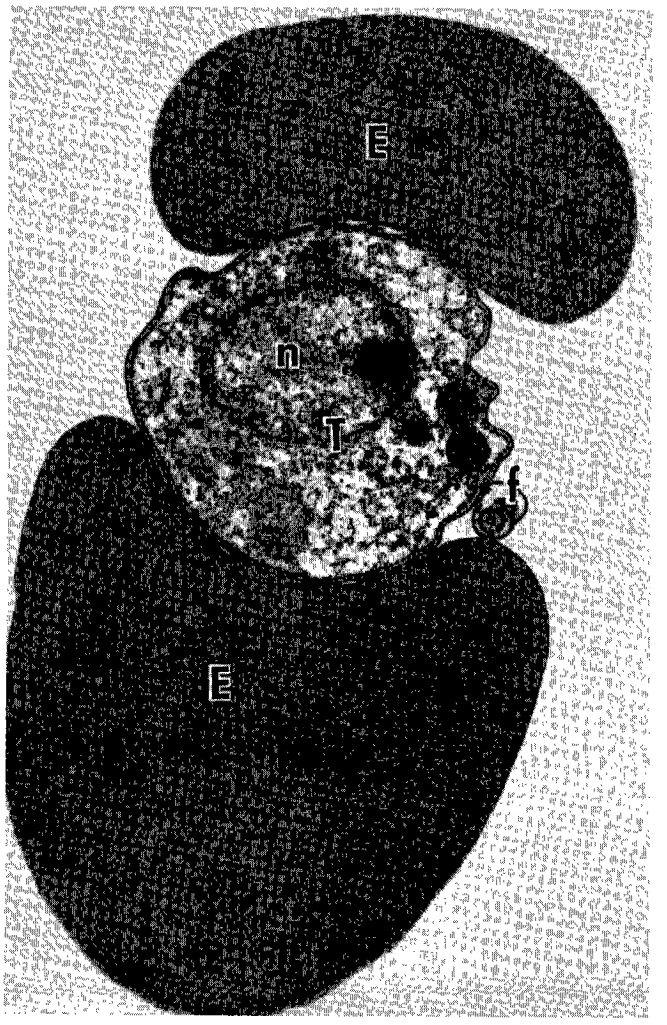

Fig. 2a : Transmission electron micrograph (TEM) showing trypanosome (T) and red blood cell (E) adhesion in mouse infected with T. brucei *; n = nucleus of trypanosome ; f = flagellum of trypanosome. (x 11,800).*

The erythrocyte sedimentation rate (ESR) increased in *T. brucei* infections of rats and sheep (1, 62, 94, 102), and in cattle infected with *T. vivax* (221) or *T. congolense* (201). ESR similarly increased during the first 40 days of *T. brucei* and *T. rhodesiense* infections of monkeys in association with decreased plasma protein concentration but subsequently declined to pre-infection levels (26). Increased ESR is thought to be of diagnostic significance in human trypanosomiasis (138). However, normal ESR was recorded in a single case of human trypanosomiasis (29). Increased ESR values are associated with presence of anaemia and increased serum fibrinogen and/or globulin (specifically a_2- and gamma-globulin) (73, 99, 187), and these changes occur in trypanosomiasis.

The adhesion of erythrocytes to trypanosomes has been demonstrated *in vitro* in *T. gambiense, T. rhodesiense, T. congolense, T. brucei, T. lewisi* and *T. evansi* infections (27, 28, 39, 58). This phenomenon has been shown to occur *in vivo* by transmission and scanning

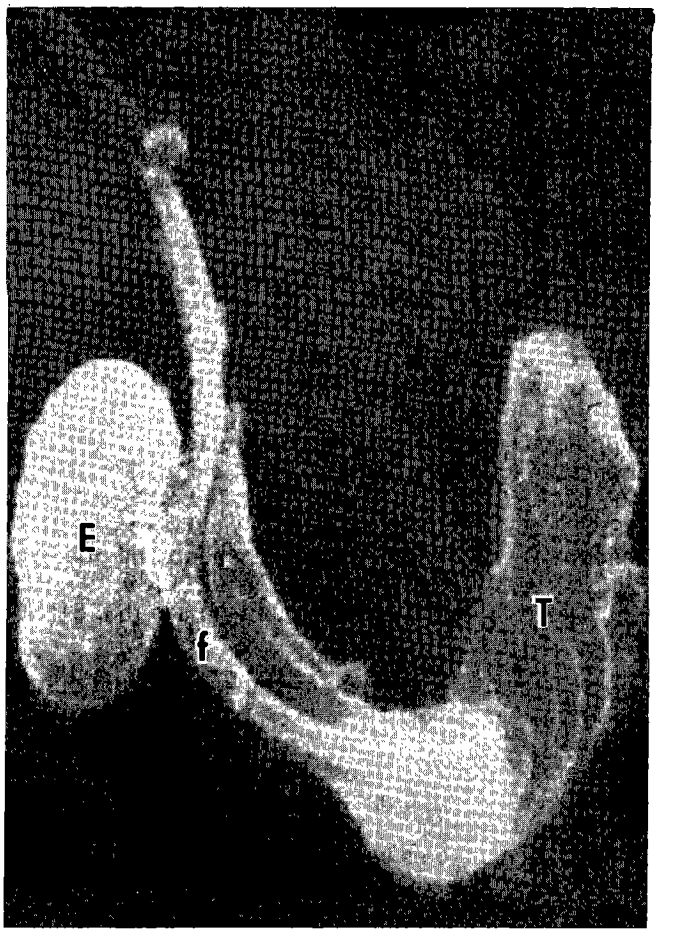

Fig. 2b : Scanning electron micrograph (SEM) of T. vivax *(T) adhered to goat red blood cell (E)* in vivo ; *f = flagellum of trypanosome. (x 16,300).*

electron microscopy (Fig. 2a, 2b), which also showed that affected erythrocytes may become cup-shaped and may develop tiny breaches on the red cell membranes which did not, however, appear to cause elution of haemoglobin (18). Adhesion has also been shown to cause haemolysis of red cells incubated with *T. congolense* (28). Treatment of erythrocytes with neuraminidase, poly-L-lysine, and sodium periodate, three compounds which cleave, bind, and oxidize sialic acid respectively, prevented the binding of *T. congolense* to bovine red cells, indicating that erythrocyte sialic acid was the binding site for the trypanosome ; further, the *T. congolense* bound to the trypanosomes with their anterior ends and binding was blocked by pre-treatment of the trypanosomes with trypsin and chymotrypsin (27). Erythrocyte membrane sialic acid levels were depressed in cattle infected with *T. vivax*, and this was most marked during the peaks of parasitaemia particularly the first ; since trypanosomes produced neuraminidase *in vitro*, this result was interpreted to mean that the trypanosomes produced neuraminidase *in vivo* resulting in cleavage of surface sialic acid and this may have been causaly related to the anaemia which incidentally developed most rapidly during the first parasitaemic peak (66).

Erythrocyte pyruvate kinase levels were elevated in *T. brucei* infections of mice (17) and rabbits (112, 113), while d-amino levulinic acid levels also increased in *T. vivax* infection of cattle (215). Similarly, RBC hexokinase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, and glutathione reductase increased in *T. brucei* infection of mice (17). These results were interpreted to reflect an increase in the population of circulating young RBC in response to the anaemia that developed (113, 215) and that anaemia was not precipitated by reductions in the activities of these enzymes in RBC (17).

Erythrocyte potassium levels markedly decreased in *T. brucei* and *T. equiperdum* infections of rats (101, 234) ; this was thought to be due to differential permeability of the RBC (234).

Immune haemolysis

Positive Coomb's antiglobulin tests have been recorded in *T. rhodesiense* infection of man (29, 133, 232), in *T. brucei* infection of mice (2, 98), and in *T. vivax* infection of cattle (71). In some of these as well as in other studies, IgM (*) and sometimes IgG directed against the trypanosomes were adsorbed to the host's erythrocytes (2, 125, 230). Auto-antibodies specifically directed against the host's erythrocytes were, however, lacking (24) but recent studies have shown that specific anti-RBC antibody was generated in *T. brucei* infection of mice (123) and in a haemorrhagic type of bovine *T. vivax* infection (25, 103). Complement has also been found to be adsorbed to erythrocytes (25, 178, 232). The development of anaemia was positively correlated with the percentage of IgM-positive RBC (2) and with the antibody response (24), and in the later stages of infection the abatement of parasitological crisis was accompanied by decreases in IgM-positive RBC and anaemia (2). Mouse RBC incubated with serum from 8-day murine *T. brucei* infection became IgM-positive (2). The most direct evidence that immunological mechanisms contribute to the genesis of anaemia is the observation that daily injection of soluble *T. evansi* antigen into rats resulted in antibody response beginning from the 6th day and this was accompanied by development of anaemia beginning from the 9th day with PCV (**) values dropping from 35 p. 100 to 20 p. 100 between days 10 to 20 of injection of antigen, the anaemia being accompanied by reticulocytosis (24). Nevertheless, haemolysis was not inhibited by injection of the immunosupressive agent cyclophosphamide into *T. brucei*-infected mice (91).

Haemolytic factors

In an early report, a haemolysin was demonstrated in the serum of a cow infected with *T. congolense* but the agent was not characterized (75). More recently, it was shown that living trypanosomes release a haemolytic factor, an a-globulin of molecular weight 10,000 daltons, which was demonstrated in the serum from day 2 after the onset of *T. brucei* infection of mice (91). Another study showed that free fatty acid (FFA), specifically linolenic acid, formed by the action of trypanosome phospholipases on trypanosome phosphotidylcholine, was released by living *T. congolense* and caused lysis of sheep RBC ; however, it was concluded that the quantity of FFA produced *in vivo* by *T. congolense* was far below the level capable of inducing haemolysis, and even that which is released is bound to plasma albumin (203, 204, 205, 206, 207). The role of these haemolytic factors *in vivo* in the induction of anaemia is therefore undefined. It is suggested that where heavy parasitaemias are produced, such as in *T. congolense* infection of mice, the amount of FFA may be sufficient to exceed the binding capacity of albumin and cause RBC lysis (204).

Erythrocyte survival and destruction

A consistent finding in infected animals and man is

(*) Ig : immunoglobulin, with subclasses IgA, IgG, IgM, IgE.

(**) PCV : packed cell volume.

accelerated erythrocliasis manifested by erythrophagocytosis (Fig. 3) in *T. brucei* infection of mice (2, 16, 98), *T. congolense* infections of mice (98), sheep (134) and cattle (75, 131, 210, 224), in *T.rhodesiense* infection of monkeys (182), and in *T. vivax* infections of sheep and goats (14, 64) and cattle (160). Histopathological and ^{51}Cr-RBC (*) studies have demonstrated that the spleen and liver, and to a less extent the lungs, ruminant haemolymph nodes and lymph nodes are the sites of RBC destruction (12, 14, 16, 18, 22, 75, 91, 111, 113, 147, 210, 232).

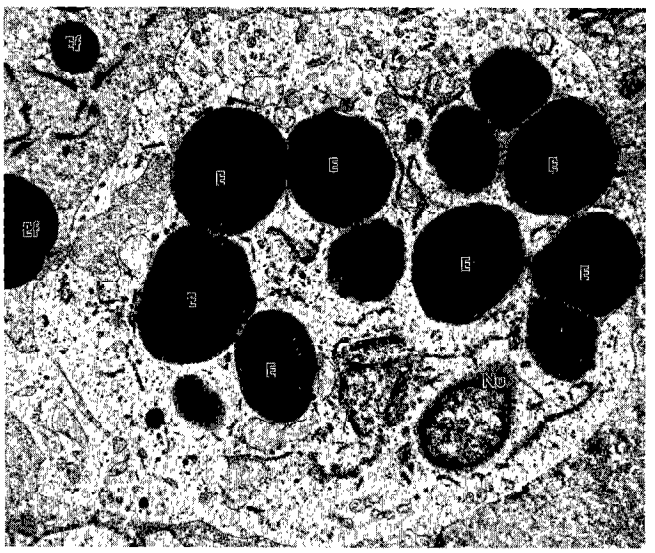

Fig. 3 : TEM of macrophage in the sinusoid of haemolymph node of a goat infected with T. vivax *showing phagocytosis of many red blood cells (E.). Nu = nucleus of macrophage ; Ef = non-phagocytized red blood cells. (x 8,800).*

Splenomegaly, which is presumably induced by erythrocyte and trypanosome phagocytosis, was reported in *T. brucei* infections of mice (2, 16, 17), rats (38) and rabbits (112, 113), in *T. congolense* infections of mice (152), rats (38) and cattle (75, 162, 211), in *T. evansi* infection of rats (24), and *T. vivax* infections of sheep, goats and cattle (4, 12, 140, 141). Hepatomegaly also occurred in *T. brucei* infection of mice (2, 17, 38), *T. congolense* infection of mice (153), *T. vivax* infections of sheep and goats (14), and *T. evansi* infection of rats (24). Splenomegaly and hepatomegaly were directly related to the severity of anaemia (17). Splenomegaly was accompanied by a marked increase in cellularity, a proliferation of macrophages, accumulation of RBC, and increased contact between cells (Fig. 4), (20, 152), changes which predispose to hypersplenism with RBC trapping and destruction (20).

(*) ^{51}Cr-RBC : erythrocytes labelled with radioactive sodium chromate, ^{51}Cr.

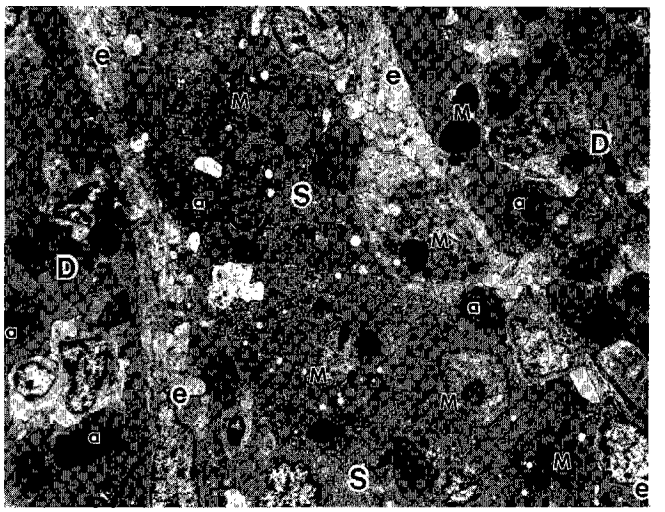

Fig. 4 : TEM of red pulp of spleen of mouse infected with T. brucei *showing marked cellularity, proliferation of macrophages (M) and intense erythropoiesis in the Billroth cord (D) and sinusoid (S) separated by sinus endothelium (e). Most other cells are normoblasts (a). (x 2,400).*

Anaemia was very severe in intact rabbits and very mild in splenectomized rabbits in *T. brucei* infection (111). Intravascular erythrophagocytosis by circulating monocytes has also been described in *T. congolense* infection of cattle (224) but is apparently a minor event.

In consequence of accelerated erythrocliasis, there is reduction in RBC survival, which has been reported in *T. brucei* infections of rodents (16, 91, 98, 111, 113, 114), *T. congolense* infections of mice (97, 227) and cattle (46, 47, 48, 137, 174, 210), *T. vivax* infections of sheep and goats (11), as well as in human *T. rhodesiense* infection (232). RBC survival was markedly reduced in intact rabbits infected with *T. brucei* but was normal in splenectomized infected rabbits (147). The speed of erythrocliasis varies from one period of the same infection to another, the faster coinciding with the period when erythrocyte values decrease most. Thus in calves infected with *T. congolense*, the T1/2 of ^{51}Cr-RBC was 128 ± 46 (SE)(*) hours at 4 to 6 weeks p.i. (*i.e.* phase I) when parasitaemias were at their highest level and PCV value was 25 p. 100, compared to a T1/2(**) of 321 ± 30 hours in controls ; later, by the 28th week post-infection when parasitaemias were low (*i.e.* phase II), the PCV values had risen to 27.5 ± 1.0 p. 100 compared to 34.0 ± 1.7 p. 100 for controls, while the T1/2 of ^{51}Cr-RBC was 243 ± 43 hours in infected animals compared to 304 ± 11 hours for controls (174).

(*) SE : standard error

(**) T1/2 : half life

Further, there is marked haemosiderosis in the spleen, liver and to a less extent the lungs, lymph nodes and in ruminant haemolymph nodes (14, 16, 18, 19, 29, 75, 76, 119, 131, 148, 164, 208), which further supports the occurrence of erythrocliasis.

While erythrocyte destruction occurs predominantly extravascularly, intravascular haemolysis has been described terminally in a few rats infected with *T. brucei* 667 (114, 158), and was also observed in a few mice infected with the same organism (ANOSA, personal observation). Two cows with mixed *T. congolense* and *T. vivax* infections had terminal redwater (*i.e.* haemoglobinuria) (92). While haemoglobinuria was not seen in another bovine *T. congolense* infection, haemosiderin was present in the renal glomeruli and interstitial tissue of the renal cortex suggesting that slight intravascular haemolysis had occurred (162, 164). Plasma haptoglobin was depressed in human gambian trypanosomiasis (40) and became too low to measure or absent in bovine *T. vivax* infection (68), which further suggest some degree of intravascular haemolysis.

Elevation of plasma bilirubin levels occurred in *T. congolense* infection of cattle (75, 76, 92, 164, 222, 224), *T. vivax* infections of cattle (75, 76, 92) and horses (197), and in *T. brucei* infections of rabbits (112) and horses (165), and further supports accelerated erythrocliasis. However, normal bilirubin levels were recorded in *T. brucei* infection of rabbits (113), and in *T. rhodesiense* infections of mice (150), monkeys (182) and man (29). While these normal values may appear inconsistent with the existence of increased erythrocliasis, it should be remembered that normal bilirubin levels could exist in haemolytic disease since the liver functional reserve is mobilized in such cases to conjugate and excrete the excess bilirubin produced by haemolysis (53). It is noteworthy that bilirubin was elevated in cattle with acute *T. congolense* infection but not in those with chronic infection (224), and was similarly elevated during crisis but not post-crisis except terminally (75).

Erythrocyte mass, blood and plasma volumes

Studies with Evans blue or ^{51}Cr labelled red cells (Table I) have shown that the erythrocyte mass decreased in *T. congolense* infections of mice (227) and cattle (46, 47, 48, 145, 174, 208, 210), in *T. brucei* infection of cattle (47, 49), and in *T. vivax* infections of sheep and goats (11, 44). RBC mass varied with severity and duration of infection. Thus, in *T. congolense* infection of mice, the RBC mass was more significantly depressed in CFLP mice which suffered more acute infection than in C57BL mice which were less severely anaemic as measured on day 8 post-infection ; further the RBC mass of the C57BL mice returned to normal 9 weeks post-infection despite the persistence of parasitaemia and anaemia, increased erythrocliasis (as shown by shortened ^{51}Cr-RBC half life), and increased blood and plasma volumes at this time (227). Similarly, RBC mass was reduced to 21.4 ± 2.16 ml per kg in mice infected with *T. brucei* for 7 days, was comparable to that of controls on weeks 3 and 5 and had risen to 36.3 ± 3.03 by the 8th week compared to 29.7 ± 1.78 recorded for control mice (2).

Elevated plasma volumes were reported in *T. brucei* infections of mice (2, 3, 6) and cattle (47, 49), in *T. congolense* infections of mice (227) and cattle (47, 48, 210, 212) and in *T. vivax* infections of sheep and goats (11, 44). The increases in plasma volumes tended to be more pronounced as the infection progressed in some studies (2, 227) but not in others (6, 209). Plasma volume estimates with ^{59}Fe-transferrin were greater than those measured with ^{125}I-albumin in both control cattle and those infected with *T. congolense* ; the difference was attributed to leakage of the ^{59}Fe-transferrin out of the plasma with incorporation into fresh RBC in the bone marrow while ^{125}I-albumin did not leak from plasma and was therefore considered a better material for determining plasma volume (49). The mean plasma volume of calves infected with *T. congolense* was 8.37 litres compared to 6.48 for control calves (209).

While increases in plasma volumes and decreases in RBC mass have consistently been recorded, total blood volume was found to be normal in *T. congolense* and *T. brucei* infections of cattle (47, 48, 210) and in *T. vivax* infection of sheep (44). On the other hand increased blood volumes were reported in *T. brucei* infection of mice (2, 3, 6), and in *T. congolense* infections of mice (227) and cattle (137, 210). The implication of a normal blood volume is that the expansion of the plasma volume was a mere compensatory pathophysiological event evoked by the need to maintain blood volume following a decrease in RBC mass. Increased blood volume on the other hand implies an over-compensation due to some other additional phenomenon such as the effect of increased gamma-globulin concentration (11, 44). Nevertheless, whether blood volumes remained normal in anaemic animals, indicating only compensatory increase in plasma volume, or is elevated, indicating over-compensatory increase in plasma volume, there exists a dilution of dissolved and cellular contents of plasma including RBC in either case, the only difference being a matter of degree, which is more pronounced when blood volume is increased.

Iron metabolism

Changes in serum iron levels are inconsistent. Thus

TABLE I Summary of published data on plasma and blood volumes, and red cell mass in trypanosomiasis.

Authors	Parasite species (Host)	Plasma volume (Technique)	Red cell volume (Technique)	Blood volume (Technique)
AMOLE *et al.* (1980)	*T. brucei* (mice)	Increased (^{125}I-albumin)*	Decreased/Increased (calculated from PCV)	Increased (Plasma + RBC volumes)
ANOSA (1980)	*T. brucei* (mice)	Increased (^{125}I-albumin)	ND**	Increased (^{125}I-albumin)
ANOSA & ISOUN (1976)	*T. vivax* (sheep, goats)	Increased (^{131}I-albumin)*	Decreased (^{51}Cr-RBC)	Increased (^{131}I-albumin)
CLARKSON (1968)	*T. vivax* (sheep)	Increased (Evans Blue)	Decreased (calculated using PCV)	Normal (calculated using PCV)
DARGIE (1978)	*T. congolense* (cattle)	Increased (^{125}I-albumin)	Decreased (^{51}Cr-RBC)	Normal (Plasma + RBC volumes)
DARGIE *et al.* (1979a)	*T. brucei* (cattle)	Increased (^{125}I-albumin)	Decreased (^{51}Cr-RBC)	Normal (Plasma + RBC volumes)
DARGIE *et al.* (1979b)	*T. congolense* (cattle)	Increased (^{125}I-albumin) (or ^{59}Fe***)	Decreased (^{51}Cr-RBC)	Normal (Plasma + RBC volumes)
MAXIE & VALLI (1978)	*T. congolense* (cattle)	Increased (^{125}I-albumin)	Decreased (^{51}Cr-RBC)	Normal (Plasma + RBC volumes)
NAYLOR (1971)	*T. congolense* (cattle)	Increased (Evans Blue)	ND	ND
VALLI *et al.* (1978)	*T. congolense* (cattle)	Increased (a) calculated from RBC mass and PCV (b) ^{59}Fe	Decreased (^{51}Cr-RBC)	Increased (calculated)
WHITELAW *et al.* (1980)	*T. congolense* (mice)	Increased (^{125}I-albumin)	Decreased (^{51}Cr-RBC)	Increased (Plasma + RBC volumes)

* Albumin labelled with radioactive iodine ^{125}I or ^{131}I.
** ND = No data provided.
*** ^{59}Fe = radioactive iron.

serum iron levels were depressed early in *T. congolense* infection of cattle but increased later (145) or terminally (201) ; increases were reported in *T. congolense* infection of cattle (48), while no significant alterations occurred in *T. brucei* infection of mice (17) and *T. congolense* infection of cattle (210). Plasma unbound iron binding capacity was decreased in one study of *T. congolense* infection of cattle (201) but was unchanged in another (144). Total iron binding capacity was depressed in cattle (201), normal in one group of calves and elevated in a second group (210).

Plasma iron turnover rate and erythrocyte iron uptake were always accelerated in these studies (48, 49, 114, 137, 212) ; however, the situation was slightly different in one of these studies, being age (of animal) and time (of infection) dependent (212). In this study, the disappearance of iron and its RBC uptake and turnover rate were slower in infected 6-month-old calves than normal, although not significantly in all cases, in the second week of infection and were only slightly accelerated in the 5th week, while iron disappearance and RBC turnover rate were faster and RBC iron

uptake normal in neonatal calves at both the 2nd and 6th weeks of *T. congolense* infection (212). These results indicate that RBC production is generally accelerated but may not always be so. A further point of note is that while the iron turnover and disappearance rates are often accelerated, the incorporation into circulating RBC may be normal, as shown by the results obtained in neonatal calves (212). This discrepancy indicates rapid haemolysis of young red blood cells (212) and/or may be due to ineffective erythropoiesis. It is noteworthy in this regard that macrophages engulfed both mature RBC, reticulocytes and occasional nucleated RBC in the spleen in mice infected with *T. brucei* (17, 18).

Studies with *T.brucei*-infected mice showed that the soluble iron content of the spleen and liver, which includes haeme iron and the easily-mobilizeable storage iron, ferritin, was markedly increased with infection, that the bone marrow iron was normal ; further, the level of superoxide dismutase, which catalyses the dissociation of superoxide radical into H_2O_2 (*) which in turn oxidises soluble ferritin into insoluble less mobilizeable haemosiderin, was depressed per unit weight of spleen and normal in liver, indicating that iron lack is unlikely to be a limiting factor to erythropoiesis (17). Normal marrow iron stores have also been reported in human *T. rhodesiense* infection (29).

BONE MARROW CHANGES

Many studies reported a gross expansion of red bone marrow (BM) in the long bones of infected animals, including in *T. brucei* infection of horses (148, 165), *T. congolense* infections of cattle, sheep and goats (144, 195, 211, 212), and *T. vivax* infections of sheep and goats (12). Other reports in bovine *T. congolense* infections showed that BM hyperplasia was evident in acute cases (76) or early part of infection (63) but in chronic cases the BM became hypoplastic (75, 76, 224) or normoplastic (163). During a period corresponding to phase II, it was reported that the red bone marrow rarely occupied more than 10 to 20 p. 100 of femoral marrow and in more advanced cases the marrow became yellow and gelatinous indicating unresponsiveness (48).

Enumeration of total nucleated cells (NC) and RBC in the femoral marrow of mice showed that NC and RBC were depressed in *T. brucei* 667 infection of CFLP mice (6). With the more tolerant deer mice infected with *T. brucei* EATRO 110, the NC and RBC of the femoral marrow from non-anaemic infected mice were normal, the NC of the anaemic infected mice was normal, while the RBC was halved (17). Bone marrow aspirates from cattle infected with *T. congolense* were significantly more cellular than those from the controls (128).

In most of these studies there was hyperplasia of the erythroid elements in the BM (12, 16, 112, 131, 134, 144, 148, 163, 182, 208, 210, 213), and this led to a drop in the myeloid : erythroid ratio (12, 16, 163, 208, 210). One study showed that erythroid hyperplasia was moderate in only a third of *T. brucei* infected rabbits, while another third showed slight hyperplasia, and a third showed no hyperplasia ; these reactions were quite inferior to the marked response shown by rabbits subjected to bleeding or given phenylhydrazine or aniline, indicating a depression of erythropoiesis by the trypanosome infection (37). Granulocyte hyperplasia was reported in a few studies (29, 144, 213, 224), while an increase in myeloblasts accompanied by maturation arrest at the metamyelocyte stage was reported in *T. congolense* infection of cattle (164). Bone marrow granulocyte reserve was depleted in *T. congolense* infection of cattle (209, 210), and in *T. vivax* infection of sheep (12). Reduction of eosinophil precursors and eosinophils, vacuolation of cytoplasm and nucleus of granulocytic cells, and disintegration of mature granulocytes also occurred (164), while plasma cells increased (22, 208).

In vitro studies have demonstrated that sera from cattle infected with *T. congolense* and *T. vivax* inhibited the development of marrow granulocyte/monocyte colonies (CFU-C) but not erythrocyte colonies ; further, the addition of sonicated *T. brucei, T. congolense* and *T. theileri* had no effect or granulocyte/monocyte colony formation but enhanced erythroid colony formation (117, 118). Depression of granulocyte/monocyte colony formation was most marked during the second and third weeks of infection when parasitaemia was very high and the PCV and WBC (*) values were decreasing progressively (118). The CFU-C inhibitor was found to be probably a trypanosome toxic product, a globulin precipitable by tricarboxylic acid (117). It was concluded from these studies that the inhibitor of leukopoiesis either destroys the granulocyte precursors directly or destroys the mature cells releasing a granulocyte inhibitor. The exact effect of this inhibitor *in vivo* was not defined, but the close relationship between the degree of depression of colony formation and the decrease in WBC values of the donor animals suggests an active *in vivo* involvement of the inhibitor. A further point of note is that monocytosis and proliferation of macrophages in the spleen, liver and other affected organs are commonly observed in trypanosomiasis (7, 8, 67, 112), suggesting that the inhibitor did not affect the monocyte precursor, and so probably acted after the divergence of the

(*) H_2O_2 = hydrogen peroxide

(*) WBC = white blood cell.

monocyte and granulocyte cell lines (7). The inability of the sera to depress erythroid colonies was probably due to the fact that the erythroid progenitor, CFU-E, is a more differentiated cell than the CFU-C and so was not susceptible to the inhibitor (118).

Megakaryocytes were numerous in the marrow of infected animals (131, 144, 182) and normal in human trypanosomiasis (29). Magakaryocytes were larger in cattle infected with *T. congolense* than in controls, and the cells showed an asynchrony of cyto-nuclear maturation, with the megakaryocyte volume being disproportionate to the nuclear volume, suggesting the existence of dysthrombopoiesis (208). In *T. vivax* infection of goats, transmission electron microscopy (TEM) demonstrated that megakaryocytes from infected goats were larger than those of control goats, showed thickening of surface marginal zone and emperipolesis of neutrophils and lymphocytes (9).

Reticulocyte counts and erythrocyte indices

Reticulocyte counts were considerably elevated in *T. brucei* infections of mice (2, 4, 16, 17, 91), rats (114) and rabbits (112, 113, 147), *T. rhodesiense* infection of monkeys (182), and *T. evansi* infections of rats (24) and camels (175). Normoblasts were numerous in the blood of rabbits infected with *T. brucei*, and like reticulocytes and RBC pyruvate kinase levels, were more numerous in rabbits with severe anaemia (113). Some normoblasts were seen in acute phase of bovine trypanosomiasis (75) while few normoblasts were present in the blood of *T. brucei* infected deer mice (17). Mild increases in reticulocytes occurred in *T. congolense* and *T. vivax* infections of sheep (134), but it is noteworthy that in this study some of the animals, particularly those with chronic infection, showed only insignificant reticulocytosis that was non-commensurate with the severe anaemia present. For instance, two sheep with PCV of 13 p. 100 showed reticulocyte counts of 6.4 p. 100 on day 13 post-infection and 0.1 p. 100 on day 72, respectively, while a third with a PCV of 11 p. 100 on day 49 had a count of 1.3 p. 100.

Reticulocytes were very rare, although macrocytes were seen (209) or were absent (212), in bovine *T. congolense* infection. In *T. vivax* infection, reticulocytes were seldom seen in sheep and goats with severe anaemia with PCV down to 8 to 12 p. 100 (12) and were lacking in rats infected for 5 days (202).

Reticulocytes dropped from between 5 and 7 p. 100 in control cattle to 0.1 p. 100 when parasitaemias were high but increased after treatment to 25 to 30 p. 100 in bovine *T. congolense* and *T. vivax* infections (75). In a horse infected with *T. brucei*, the reticulocyte counts ranged from 0.1 to 4.0 and remained between 0.2 to 2.8 except terminally when it rose to 4.1 p. 100 (63). The conclusion from these two studies was that haemopoiesis was depressed. However, in interpreting these two results, it is noteworthy that healthy horses and cattle do not have reticulocytes in circulation, and that while cattle release reticulocytes in anaemias due to severe sudden blood loss, horses never do (187).

The mean corpuscular volume (MCV) of RBC was elevated (*i.e.* macrocytic) in *T. brucei* infections of rats (114), rabbits (113, 147), *T. congolense* infection of cattle (75, 76, 144, 210, 212), and in *T. vivax* infections of sheep (12), horses (197) and cattle (185). These increases occurred during the early acute phase of infection, and in animals that developed chronic disease the MCV later became normal despite the persistence of the anaemia (113, 147, 163, 210, 212), or was reduced (*i.e.* microcytic) (12, 75, 76). Microcytosis was reported in a case of human *T. rhodesiense* infection (29). The MCV was only slightly elevated in one *T. brucei* infection of mice (17) and was unchanged in another (16) despite the existence of marked anaemia and considerable reticulocytosis ; the general observation in both infections was that most mouse polychromatophilic RBC, *i.e.* reticulocytes, were essentially normocytic. There was a highly significant correlation ($P < 0.001$) between MCV values and reticulocyte counts, and both parameters were higher in rabbits infected with a more acute strain (113). The mean corpuscular haemoglobin concentration (MCHC) was normal in *T. brucei* infections of mice and rabbits (16, 17, 114), in *T. congolense* infection of cattle (144, 163, 210, 212), and in *T. vivax* infection of horses (197), but was depressed by 15 to 25 p. 100 in *T. brucei* infection of rabbits (113) and in older calves infected with *T. congolense* (212). Mean corpuscular haemoglobin (MCH) was not altered in *T. brucei* infection of rabbits (113), but increased in *T. congolense* infection of calves (212).

Extramedullary haemopoiesis

Extramedullary haemopoiesis involving erythropoiesis, granulopoiesis and thrombopoiesis has been reported in the spleen, liver and less often the lymph nodes in *T. brucei* infections of mice and rats (20, 38, 114), *T. evansi* infection of rats (24), *T. congolense* infections of mice and rats (38, 152, 153) and cattle (162, 209), and in *T. vivax* infections of cattle and goats (105, 214, 215). Mouse spleen is normally active in haemopoiesis, but in these trypanosome infections the process, particularly erythropoiesis, became intensified in the cords, and spread into the sinusoids (20) and even the white pulp (152). The intensity of erythropoiesis was so great that erythroid cells constituted 57.5 p. 100 of splenic nucleated cells in mice infected with *T. congolense* for 60 days compared to only

6.5 p. 100 in control mice (152). Similarly, nucleated erythroid cells rose from 7.9 p. 100 in control mice to 38.5 p. 100 of all nucleated cells in the cords of the spleen, and from the 0 p. 100 in the sinusoids of control mice to 25.7 p. 100, in mice infected with *T. brucei* for 7 to 10 weeks (20) ; in the same study no evidence of erythropoiesis was seen in the liver of mice infected for the same period.

However, many descriptions of the histopathology of the spleen, liver and lymph node in trypanosomiasis failed to report the existence of haemopoiesis. TEM studies of spleen, liver and haemolymph nodes of severely anaemic goats infected with *T. vivax* did not reveal extramedullary erythropoiesis (22). While this may imply that the phenomenon did not exist in these studies, it is probable that it existed in some infections but was not detected. It is pertinent that while splenic erythropoiesis was not easily detected in mouse spleens examined as conventional H & E sections with light microscopy (4, 156), it became very conspicuous with electron microscopy (20). Smears and histologic sections demonstrated splenic normoblastic hyperplasia in *T. brucei* infection of rats (114, 158) but smears were superior to histological sections in detecting extramedullary haemopoiesis in bovine *T. congolense* infection (208). Presumably, the reason for the failures to detect extramedullary haemopoiesis with histological sections is the fact that erythroid cells too often resemble small lymphocytes.

MECHANISMS OF ANAEMIA IN TRYPANOSOMIASIS

Because of the extra-erythrocytic location of trypanosomes, the mechanisms by which they induce anaemia are not immediately obvious as appear those of intraerythrocytic parasites such as *Babesia* and *Plasmodium*. Consequently the mechanisms of anaemia in trypanosomiasis have been studied extensively particularly in the last two decades, but without complete elucidation. Nevertheless, considerable information is now available on various aspects of the anaemia. Attention has been drawn by several investigators to the close relationship between trypanosome parasitaemia and the development of anaemia. Several mechanisms have been implicated in the causation of anaemia, and these include haemolysis, dyshaemopoiesis, haemodilution, and haemorrhage.

Haemolysis

There is a general concensus amongst investigators that haemolysis is a central factor in trypanosome anaemia, and that it generally sets in during the first wave of parasitaemia. DE GRUCHY (53) enunciated that the general features of haemolytic anaemias include evidence of accelerated RBC destruction (jaundice, hyperbilirubinaemia, haemoglobinaemia, haemoglobinuria and haemosidenuria, the last 3 being associated with intravascular haemolysis only), evidence of compensatory erythropoietic hyperplasia (reticulocytosis, normoblastaemia, macrocytosis, erythroid hyperplasia of bone marrow), evidence of RBC damage (spherocytosis, increased osmotic fragility, fragmentation of RBC), and demonstration of shortened RBC survival. As presented in the foregoing sections, these criteria are satisfied by most trypanosome infections, except that haemoglobinaemia and haemoglobinuria are seldom reported, indicating that haemolysis is essentially extravascular. Additional evidence in support of haemolysis include erythrophagocytosis and haemosiderosis seen in spleen, liver and other organs. Most authors report that haemolysis was most marked during the early acute crisis (phase I) when parasitaemias were high, and it is also at this period that most of the criteria cited above are satisfied. Haemolysis becomes less marked during the succeeding chronic crisis when parasitaemia has waned.

While the existence of haemolysis is generally accepted, there is less concordance on the mechanisms that precipitate it. The data presented in the foregoing sections indicate that the aetiology is complex, involving many factors which are related directly or indirectly to the trypanosomes (Fig. 5).

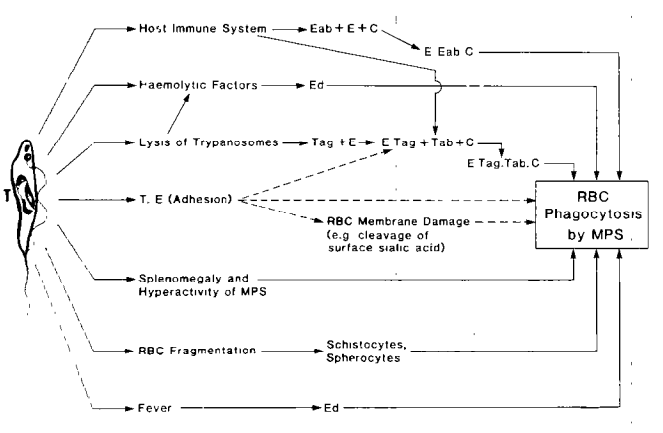

Fig. 5 : Factors involved in RBC destruction by the mononuclear phagocyte system (MPS) in trypanosomiasis. Solid lines ( ) represent reasonably established phenomena while broken lines (----) represent possible phenomena. E = erythrocyte, Ed = damaged erythrocyte ; Eab = anti-erythrocyte antibody (autoantibody) ; C = complement ; T = trypanosome ; Tag = trypanosome antigen ; Tab = anti-trypanosome antibody. (Tag + E = E.Tag means that trypanosome antigen plus eryhtrocyte yield erythrocyte yield erythrocyte-trypanosome antigen complex).

Firstly, the RBC acquire trypanosome antigen released by live trypanosomes or following trypanolysis, or presumably as a result of adhesion to trypanosomes, are subsequently coated with anti-trypanosome antibody (IgM, IgG), and are then phagocytized with or without complement. It has also been suggested that preformed trypanosome antigen-antibody complexes may attach to RBC leading to phagocytosis (25, 125, 230). Further, autoantibodies specific against RBC have been demonstrated (25, 103, 123) and these will predispose the RBC to phagocytosis. Several workers consider these immune mechanisms to be important causes of anaemia particularly in the early phase of infection (2, 24, 25, 72, 103, 125, 232), based on the observations that IgM binding of RBC was marked in acute phase of *T. brucei* infection in mice but was no longer demonstrated in the chronic phase when parasitaemia was low and RBC values had improved slightly (2), and that direct antiglobulin tests (DAT) were positive when Hb (*) and PCV values were low but DAT was negative when the red cell values had recovered slightly (72). Further, IgM- and IgG-bound RBC were first detected 7 to 10 days post-infection when severe anaemia developed but were less consistently positive thereafter until euthanasia 15 to 18 weeks post-infection in cattle infected with *T. congolense* (125), and maximal immune response in rabbits infected with *T. evansi* was highest 10 to 12 days after infection at which time RBC destruction was maximal, while the injection of *T. evansi* eluates into rats induced antibody response, caused moderate anaemia with reticulocytosis (24). The observation that the injection of the immunodepressive agent, cyclophosphamide, did not alleviate the anaemia of *T. brucei* infection in mice, although the dose and time of injection were not indicated (91), suggests that factors other than immune haemolysis are also important in the causation of early anaemia.

Secondly, haemolytic factors or haemolysins secreted by live trypanosomes or released following trypanolysis have been shown to cause haemolysis *in vitro* (75, 91, 204), and may play a role in *in vivo* haemolysis. This role is doubted because the FFA which are released are bound to plasma albumin and could only induce haemolysis if the albumin binding capacity is exceeded as could occur in infections producing heavy parasitaemias (204).

Thirdly, RBC adhere to trypanosomes *in vivo* and this could lead to acquisition of trypanosome antigen by the RBC, damage to the RBC membrane such as development of minute pores (17) or cleavage of RBC surface sialic acid by trypanosomal neuraminidase (66), which predispose the RBC to phagocytosis. Another possibility is that the adherent RBC-trypanosome complex can be phagocytized by macrophages. Since trypanosomes are only numerous during acute crisis, this mechanism can only be important at this time.

Fourthly, RBC fragmentation, which may be induced by microthrombi (14, 104, 105, 214, 215), vascular damage (210), splenomegaly (25) or glomerulonephritis (18), results in the production of schistocytes, microspherocytes, and spherocytes which are phagocytized, inducing microangiopathic haemolytic anaemia.

Fifthly, fever which occurs consistently in trypanosome infections, has been suggested as possibly inducing RBC damage and phagocytosis (48, 113), based on the studies on its effects on RBC (120, 121).

Sixthly, expansion with hyperactivity of the mononuclear phagocyte system (MPS) associated with hepatomegaly and splenomegaly has been implicated as a factor in the anaemia. This is based on the fact that the spleen is markedly enlarged with proliferation and activation of macrophages (17, 19, 156, 159), accompanied by markedly increased cellularity which inevitably interfere with RBC motility (20). Extrapolation of these changes suggests that such enlarged hypercellular spleens apparently increase the transit time of erythrocytes in the spleen, exposing them to deleterious intrinsic factors of the splenic environment including low pH, low glucose level and low cholesterol levels which interfere with Na+ pump, induce spherocytosis, and increase osmotic fragility which altimately lead to their premature phagocytosis (155, 225). That these phenomena, collectively termed hypersplenism, operate in trypanosomiasis is supported by the observation that splenectomy considerably alleviated the anaemia of *T. brucei* infection of rabbits (111, 147), and that RBC survival in these rabbits was comparable to control rabbits (147). RBC destruction due to splenomegaly and overactivity of the MPS was thought to be the main factor responsible for erythrocliasis during the « steady state » (chronic crisis), when the parasitaemia disappears but anaemia persists (49). These authors based their conclusion on the report that after repeated stimulation, the MPS remains active for a long time after removal of the stimulus (110). In our experience and based on other reports, the parasites do not actually disappear completely from the circulation during chronic crisis but appear less frequently and in smaller numbers than during acute crisis ; such parasites will serve to sustain the stimulus on the MPS. The role of hypersplenism in inducing anaemia is less marked in some infections such as *T. vivax* infection of sheep and goats in which the spleen was enlarged only 3 times compared to 25.9-fold enlargement observed in *T. brucei* infection of mice (12, 14, 20) ; it is significant that splenectomy did not alleviate the *T. vivax*-induced anaemia in sheep (12). It is also noteworthy that in

(*) Hb : haemoglobin concentration

some chronic infections the spleen size returned to normal or the organ became atrophic (75, 141) ; in such circumstances hyperactivity of the spleen cannot be important.

Finally, it is apparent that since most of these haemolytic mechanisms are trypanosome-dependent (even hypersplenism requires the stimulus of trypanosome and RBC clearance to develop), haemolysis as a mechanism of induction of anaemia can only be important during those periods when trypanosomes are numerous in the blood, a view which is supported by the observation by many workers that accelerated RBC destruction occurs when parasitaemia is marked. The only mechanisms which possess the potential to persist after trypanosomes have decreased as in chronic crisis are hypersplenism and RBC fragmentation due to glomerulonephritis and splenomegaly. However, it is plausible that the other mechanisms may not completely cease but will persist at very reduced undetectable levels during chronic crisis. The role of each mechanism will vary from one host-parasite combination to another due to variations of host and parasite factors.

Dyshaemopoiesis

Considerable evidence has emerged that during acute crisis (phase I) of infection there is accelerated erythropoiesis. This evidence includes reticulocytosis, which is present in some but not in other infections, macrocytosis, marrow erythroid hyperplasia, extramedullary erythropoiesis, increased iron uptake in bone marrow, and increases in RBC enzyme concentration including pyruvate kinase, d-aminolevulinic acid, hexokinase, glutathione reductase, and glutathione peroxidase which indicate the presence of many young circulating RBC. The available evidence from reticulocyte counts indicate, however, that while response is marked in rodent and monkey infections with *T. brucei* and *T. rhodesiense*, and in *T. evansi* infection of camels, it was very mild in *T. congolense* infection of sheep (134) and non-existent in *T. vivax* infections of sheep and goats (12) and in *T. congolense* infection of cattle (212), even though sheep showed considerable reticulocytosis to comparable severe anaemia induced by haemonchosis (5), and anaplasmosis and babesiosis (15). In these trypanosome infections lacking reticulocytosis, macrocytosis was, however, consistently present, and additionally red cell d-aminolevulinic acid was elevated in one study indicating that many young RBC which were fully haemoglobinated were entering the circulation. In such animals, it is obvious that even though erythropoiesis was accelerated, it was not commensurate with the level of anaemia. In rodent trypanosomiasis, which appears to stimulate more effective erythropoiesis with marked reticulocytosis, the observations that only 1/3 of rabbits infected with *T. brucei* showed moderate erythroblastic activity while another 1/3 showed slight response and 1/3 showed no response, and that the reaction to superimposed phenylhydrazine and aniline administration to infected animals was decidedly less marked than was observed with similar doses administered to normal mice (37), suggest that even in rodent trypanosomiasis the response is also not maximal.

During chronic crisis, the situation appears different. Thus microcytosis was commonly reported, iron uptake, although greater than in control animals, lagged behind the level recorded during acute crisis, marrow hypoplasia also occurred (48, 74, 224), while reticulocytes virtually disappeared despite persistence of severe anaemia (134).

The factors responsible for dyshaemopoiesis are probably complex. Stem cell injury with reduction in numbers due to trypanosome toxins and competition between different stem cell lines for space and nutrition has been suggested (118, 128, 206). Phagocytosis of erythroid cells has been demonstrated in the spleen of mice infected with *T. brucei* (19, 20) and this may represent an intrinsic erythroid cell abnormality or may be due to immunological mechanisms similar to those responsible for phagocytosis of mature RBC and reticulocytes. Another possible mechanism of dyshaemopoiesis is the trapping of iron in macrophages of the MPS with resultant reduced availability for erythropoiesis, akin to the anaemia of chronic disorders (48). Is is noteworthy that although erythroid colony formation was not depressed by serum from *T. congolense* infected cattle (118) and that marrow cultures from calves infected with *T. congolense* formed as many colonies as those of control calves (128), there was less haemoglobinization in the erythroid colonies from infected calves than those from control calves (128). A third possibility is a failure of adequate release of young RBC, which is supported by the fact that in spite of erythroid hyperplasia and gross expansion is either very low or non-existent.

In conclusion, there is ample evidence that dyshaemopoiesis contributes to the anaemia of trypanosomiasis, and its effect is more marked during chronic crisis than in acute crisis, and is more operative in ruminants than in rodents. It is noteworthy that while *T. brucei* and *T. rhodesiense* infections of rodents and monkeys and *T. evansi* infection of camels, which are parasites belonging to the *T. brucei* or tissue invading subgroup, have so far been reported to stimulate considerable reticulocytosis, *T. vivax* and *T. congolense* infections induce scanty or no reticulocyte response in ruminants and mice, although the mouse infection lasted only 5 days (202) raising the possibility that the duration was too short to allow development of reticulocytosis. It is not clear from these results whether responsiveness as determined by reticulocytosis is predetermined by the species of parasites or

host as these results seem to suggest. This question cannot be resolved presently because marrow response has not been investigated in ruminants infected with the tissue invading trypanosomes, while similarly there are no studies in rodents infected with haematinic trypanosomes.

Haemodilution

While increases in plasma volumes have consistently been reported, there is a divergence of opinion on the status of the total blood volume, some workers reporting that it remained normal while others reported that it increased (Table I). Nevertheless, it is noteworthy that trypanosome anaemia and indeed most anaemias have a haemodilutionary component because whether the blood volume remains normal so that the increase in plasma volume is only a pathophysiological compensatory mechanism to maintain total blood volume, or the total blood volume is increased amounting to over-compensation, the erythrocyte values are depressed below a level they should attain had the plasma volume remained at the « normal » level existing prior to the destruction of RBC.

Haemorrhage

Haemorrhages have been reported in human sleeping sickness caused by *T. rhodesiense* (29, 181), and in acute *T. vivax* infections of cattle, sheep and goats (14, 89, 92, 103, 160, 213, 214, 221, 222). Generally, however, these haemorrhages were minor and most likely do not contribute significantly to the anaemia. However, it is possible that the haemorrhages produced by the haemorrhagic *T. vivax* infections recently described in Kenya (103, 160, 222) may be sufficient to account for a small component of the anaemia. The extravasated RBC, except those lost into the lumena of the digestive, respiratory, and female reproductive tracts, are engulfed and lysed by macrophages in the affected areas, as was demonstrated in the lymph nodes of goats and sheep infected with *T. vivax* (14).

ANOSA (V. O.). Haematological and biochemical changes in human and animal trypanosomiasis. *Revue Elev. Méd. vét. Pays trop.*, 1988, **41** (1) : 65-78.

Trypanosome infections are generally characterized by anaemia, leucopenia, thrombocytopenia, as well as biochemical aberrations such as hypoglycaemia, elevated BUN, hypoalbuminaemia, and hypogammaglobulinaemia primarily due to elevated IgM levels. Despite the variations in hosts (man, domestic and experimental animals) and trypanosomes (*T. brucei, T. gambiense, T. rhodesiense, T. evansi, T. vivax, T. congolense*), the severity of the haematological and biochemical changes associated with various host-parasite combinations is determined by the level of parasitaemia which develops during the early phase of infection. Three phases of trypanosome infections are recognizeable including the « acute crisis » characterized by high parasitaemia and accelerated destruction of erythrocytes, development of thrombocytopenia and leucopenia, and of marked biochemical pertubations. A « chronic crisis » supervenes in surviving animals and is characterized by lower levels of parasitaemia but with persistence of the haematological changes, reversal of some biochemical changes such as hypoglycaemia and persistence of others such as the plasma protein changes. A third phase, « recovery », occurs in animals that survive the two previous phases, and is characterized by abatement of parasitaemia or even sterilization, accompanied by gradual reversal of the abnormalities previously developed. Whether a host passes through these three phases depends on the severity of the lesions that develop during acute and chronic crisis, the existence of secondary infections, and the level of host's nutrition. The haematological and biochemical abnormalities induced by trypanosomes arise from their direct and indirect effects via their products, on host cells such as RBC, WBC, platelets, and tissues such as liver, kidney, bone marrow and lymphoid organs, resulting in cell destruction and organ malfunction, as well as from extractions from and additions to host chemistry associated with parasite metabolism. *Key words* : Man - Animal - Trypanosomiasis - Haematological change - Biochemistry.

The references are printed aside and will be sent to readers who will ask for them (Write to the journal).

ANOSA (V. O.). Modificaciones hematologica y bioquímica en la tripanosomosis humana y animal. *Revue Elev. Méd. vét. Pays trop.*, 1988, **41** (1) : 65-78.

Generalmente, se caracterizan las tripanosomosis por anemia, leucopenia, trombocitopenia y trastornos del metabolismo como la hipoglucemia, un aumento de la tasa de nitrogeno ureico de la sangre, la hipoalbuminemia y la hipogammaglobulinemia causada por un aumento del nivel de las IgM. Aunque se observan variaciones según el huespéd (hombre, animales domésticos y animales de experimento) y las especies de tripanosomas (*T. brucei, T. gambiense, T. evansi, T. vivax, T. congolense*), se determina la gravedad de las alteraciones hematologica y bioquimica, asociadas con varias combinaciones huésped-parásito, por el nivel de parasitemia que aparece durante la primera fase de la infección. En efecto, se puede notar tres fases sucesivas durante estas infecciones :

— una crisis aguda con una parasitemia elevada, una destrucción muy rápida de los eritrocitos, trombocitopenia, leucopenia y perturbaciones bioquimicas acentuadas ;

— una crisis crónica que se nota en los animales supervivientes. Se caracteriza por una parasitemia más reducida, sin embargo con persistencia de las alteraciones hematologicas, atenuación de algunos trastornos del metabolismo como la hipoglucemia ; pero persistencia de otros como las modificaciones de las proteinas plasmáticas ;

— la curación ocurre en los animales que superviven a las dos fases precedentes : la parasitemia disminuye y aún desaparece con una normalización del metabolismo. La resistencia, durante estas fases, de un huésped infestado depende del estado nutricional, de la gravedad de las lesiones que se desarrollan durante las crisis aguda y crónica y de la existencia de infecciones secundarias. Los trastornos hematologico y bioquímico causados por los tripanosomas provienen de efectos directos o indirectos a través de sus productos de degradación sobre las células del huésped (hematíes, leucocitos, plaquetas) y sus tejidos (hígado, riñones, médula osea, órganos linfoideos), de dónde destrucción celular y funcionamiento orgánico defectuoso, sustracción y adición de productos bioquimicos en el huésped ligadas con el metabolismo del parásito. *Palabras claves* : Hombre - Animal - Tripanosomosis - Modificación hematologica - Bioquímia.